Critical Issues in Head and Neck Oncology

Key Concepts from the Seventh THNO Meeting

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C. René Leemans
Jean-Pascal Machiels
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Editors
Jan B. Vermorken
Department of Medical Oncology
Antwerp University Hospital, Edegem
Belgium and Faculty of Medicine and
Health Sciences, University of Antwerp
Antwerp
Belgium

C. René Leemans
Department of Otolaryngology – Head and
Neck Surgery
Amsterdam University Medical Centers
Vrije Universiteit
Amsterdam
The Netherlands

Piero Nicolai
Section of Otorhinolaryngology – Head and
Neck Surgery, Department of Neurosciences
University of Padua
Padua
Italy

Volker Budach
Departments for Radiation Oncology and
Radiotherapy
Charite University Medicine Berlin
Berlin
Germany

Jean-Pascal Machiels
Oncology
Cliniques Universitaires Saint-Luc
Woluwe-Saint-Lambert
Belgium

Brian O'Sullivan
Department of Radiation Oncology
The Princess Margaret Cancer Centre
University of Toronto
Toronto
ON
Canada

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Preface

The seventh Trends in Head and Neck Oncology (THNO-7) took place in the Crowne Plaza City Center in Athens, Greece, November 7–9, 2019, and was organized by the same coordinating team as the fifth and the sixth version with support from Pharma and practical logistical support from Congress Care. The conference was endorsed by the European Head and Neck Society (EHNS) and the European Organization for Research and Treatment of Cancer (EORTC). As on previous occasions, the setup was educational, with a multidisciplinary focus. Case presentations, organized by our colleagues in Athens (Dr. Amanda Psyrri and Dr. Athanassios Argiris) and some members of the coordinating team, induced a lively interaction between faculty and audience and underlined the importance of individualized patient care. Thanks to the dedication of all the faculty members this book will be available soon after the actual meeting, guaranteeing the most up-to-date information in this rapidly evolving field. We are most grateful to all the faculty members for their efforts in realizing this important goal.

Edegem, Belgium — Jan B. Vermorken
Berlin, Germany — Volker Budach
Amsterdam, The Netherlands — C. René Leemans
Louvain-la-Neuve, Belgium — Jean-Pascal Machiels
Padua, Italy — Piero Nicolai
Toronto, Canada — Brian O'Sullivan

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Part I
Biomarkers in Head and Neck Squamous Cell Cancer (HNSCC)

Chapter 1
Promising Biomarkers for Early Diagnosis and Prognosis Prediction

Philip Sloan and Max Robinson

Introduction

There have been several recent reviews of biomarkers in relation to head and neck cancer [1–4] and although many markers show a degree of utility, none have so far translated into routine practice, apart from p16 testing for oro-pharyngeal squamous cell carcinoma [5] and PDL-1 prior to the administration of nivolumab or pembrolizumab in recurrent/metastatic squamous cell carcinoma [6]. Modern cellular pathology laboratories do routinely use a wide range of diagnostic biomarkers for immunohistochemical and molecular testing of biopsy material, however. Quality assurance is important, both for the laboratory processes and the interpretative diagnostic skills of the pathologist when using biomarkers [7]. For many types of cancer, accredited testing is routinely performed to guide therapy, but in head and neck cancer, such testing is only slowly finding applications. To achieve accreditation for such companion biomarkers, not only must the clinical utility be demonstrated by robust evidence but a health economic case also needs to be established. In essence the introduction of companion biomarkers into a pathology service depends on the drug therapies and practices being used by head and neck oncologists. Only if the biomarker can be used to select those who will benefit from a therapy or exclude those who will not benefit, can it find routine application. Increasingly, it is likely that tumour agnostic therapies based on molecular pathology will be used in clinical practice. An interesting example is the use of neurotrophic tropomyosin receptor kinase (NTRK) inhibitors which are licenced for use in a variety of tumours that carry the molecular signature. In head and neck cancer these include paediatric tumours and secretory carcinoma of the salivary glands [8]. The challenge for pathologists and oncologists is to identify which tumours to test and determine the

P. Sloan (✉) · M. Robinson
Department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Trust and Newcastle University, Newcastle upon Tyne, UK
e-mail: philip.sloan@newcastle.ac.uk; max.robinson@newcastle.ac.uk

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best testing method. Although genomic panel testing is attractive in that a wide spectrum of molecular signatures not identified by histology can be detected, turnaround time and cost are currently barriers, although these are likely to improve with time [9]. Immunohistochemistry is currently the most favoured option for NTRK detection because there are antibodies with high sensitivity and specificity ranging from 95–100% and 92–100%, respectively [10, 11]. Tumours with NTRK1/2 fusions demonstrate cytoplasmic expression and rarely perinuclear and nuclear membrane staining whereas those with NTRK3 fusions demonstrate cytoplasmic or nuclear expression [10–12]. Immunohistochemistry is relatively inexpensive and can offer a rapid turnaround time. However, as new drugs linked to molecular targets are developed, strategies for screening tumours to identify those that rarely contain a signature abnormality will have to be developed, so that patients can benefit [13].

Biomarkers that are used as companions to therapeutic drugs are often first identified as purely prognostic markers, and with accrual of further knowledge and drug development, they may become molecular targets or used to identify tumours that are likely to respond to a particular therapy. In this short review, a number of selected biomarkers that show promise for head and neck cancer therapies will be described.

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs, matrixins) are members of the metzincin protease superfamily of zinc-endopeptidases. It is five decades since the first MMP (MMP-1, collagenase) was identified from amphibian tissue. Currently a family with 28 members are classified as MMPs in vertebrates. The classification of human MMPs is based on their substrate specificities and the common structural domain architecture. Diverse biological functions are known within the subfamilies: collagenases, gelatinases, stromelysins, matrilysins, MMP membrane-type (MT)-MMPs and other MMPs are known. Numerous studies have found that MMP genes are frequently upregulated in cancer (reviewed in [14]). MMPs are thought to play significant roles in cancer progression, functional promotion of angiogenesis, invasion, metastasis and avoidance of immune surveillance. Indeed, much of the focus of research into MMPs in cancer has been related to tumour stroma and in particular angiogenesis, with a view to developing inhibitory drugs for MMPs and their natural inhibitors [15]. Drugs developed that targeted the MMP system and its inhibitors decades ago did not translate into practice. However, more specific targeting of small engineered molecules that can deliver payloads makes MMPs attractive targets [16, 17]. Recently, there has been interest in the membrane type MMPs and a systematic review of MT1-MMP (MMP-14) has demonstrated its potential as a

prognostic biomarker for cancer [16]. Over-expression of MMP-14 was significantly correlated with a poor overall survival in multiple cancers (HR: 2.22; 95% CI: 1.72–2.87). Also, high levels of MMP-14 were strongly associated with tumour progression and metastasis (HR: 1.83; 95% CI: 1.36–2.46) [16]. In ongoing studies, we are seeking to use MMP-14 as a target to guide entry of drugs to the cancer cells using novel circularised peptide molecules [17]. Various tumour types are known to have high MMP-14 expression including breast, ovarian, lung, and bladder cancer (reviewed in [16]) and MMP-14 has low expression in normal tissues. An immunohistochemical assay was developed on the Ventana platform using a Millipore MT1-MMP (MMP-14) primary antibody (MAB3328) at 1:6000 using Optiview chemistry as the detection system. When this was applied to tissue microarrays covering multiple cancer indications we found frequent overexpression in the malignant cells (membranous and cytoplasmic staining) and also in the stromal compartments. Expression levels were estimated by consensus review by two pathologists using an H-score which is the product of staining intensity (0–3) and percent positivity (0–100). H-scores (0–300) were derived separately for tumour membrane, cytoplasm and stroma in each case. Data modelling was used to identify a threshold for the identification of significant expression and the data could be used to define groups suitable for recruitment into a clinical trial. Interesting, MMP14 was consistently overexpressed in squamous cell carcinoma, enabling head and cancer patients to enter into a phase1 trial (manuscript in preparation). Quality assurance is an important part of using an immunohistochemical test for entry into a clinical trial [7]. Cell lines showing a range of MMP-14 expression (0–3) were developed and used alongside tumour tissue as positive controls on each slide to ensure consistency of staining and stability (Figs. 1.1 and 1.2).

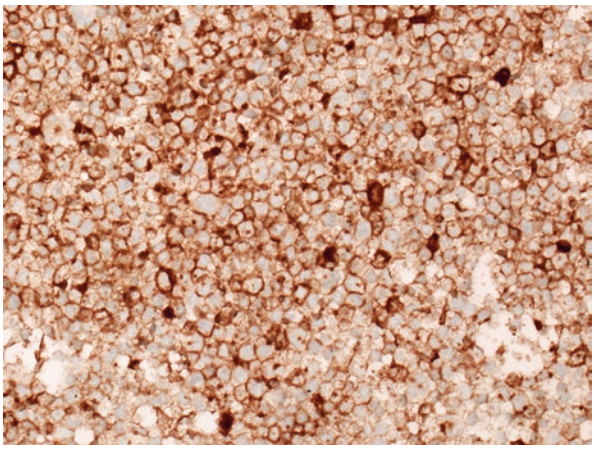

Fig. 1.1 Immunohistochemistry for MMP14 showing membranous staining intensity grade 3 in a cell line

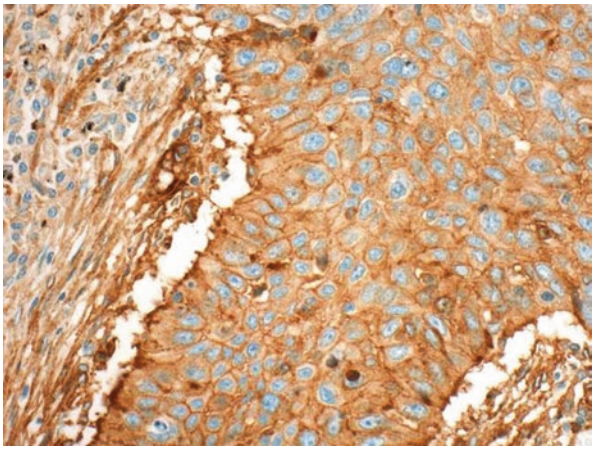

Fig. 1.2 Immunohistochemistry for MMP14 in a squamous carcinoma showing membranous and stromal overexpression

Autophagy Biomarkers

Autophagy is a self-degradative process that plays a role in removing misfolded or aggregated proteins and clearing damaged organelles. In cancer, autophagy is generally thought of as a promoting mechanism improving the survival of cancer cells by recycling nutrients, although its deregulation has been linked to non-apoptotic cell death. Strategies for both inhibiting and promoting apoptosis were therefore developed as potential cancer therapies. In this chapter, an interesting translational ongoing study in early stage cutaneous melanoma, including those arising in the head and neck, will be described (Fig. 1.3). The combination of an autophagy marker, Epidermal Autophagy and Beclin 1 Regulator 1 (AMBRA 1) and a cornified envelope differentiation marker, loricrin was used, where the biomarker expression was studied not in the tumour but in the overlying epidermis [18]. Most cases of cutaneous melanoma, including those in the head and neck, are diagnosed at an early stage. Detection and surgical excision results in high cure rates. Nevertheless, a small subset of patients with AJCC stage I disease progress and die from their disease. Initially three cohorts comprising a total of 455 AJCC stage I melanomas from the north east of England were studied. Immunohistochemistry for AMBRA1 and loricrin expression was validated and used to assess loss or downregulation of the markers in the epidermis overlying the melanoma, using adjacent non-tumour epidermis as an inbuilt control. The data indicate that the use of both markers in combination can stratify stage I patients at high and low risk of progression. In multivariate analysis of combined validation cohorts, the high-risk AMBRA1/loricrin (AMLo) expression pattern carried a HR of 3.89 (95% CI 1.8–8.41, $P < 0.001$) of melanoma recurrence [18]. The aim of our ongoing study is to validate AMLo in

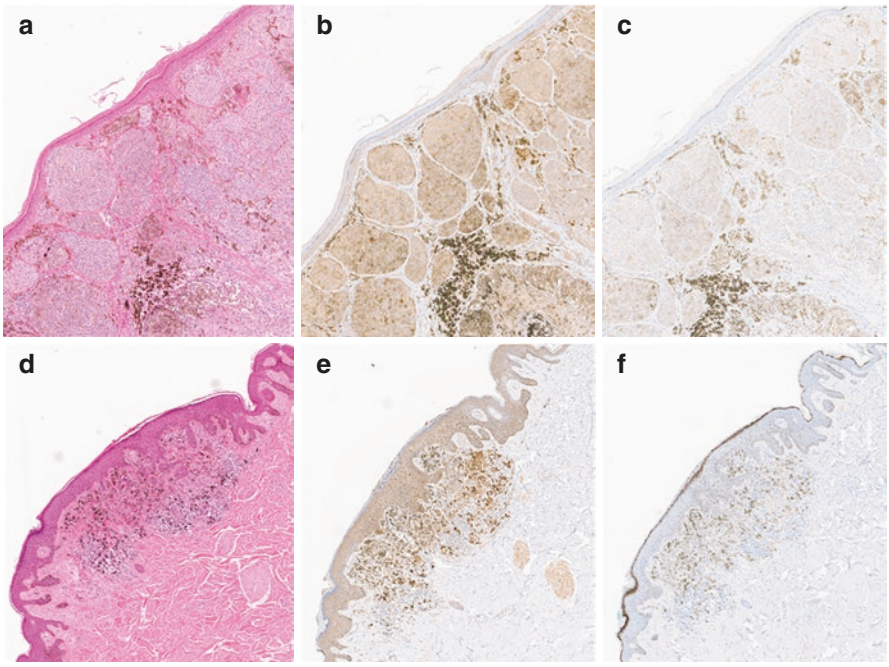

Fig. 1.3 Expression of AMBRA1 and Loricrin in cutaneous malignant melanoma. Top row: high risk melanoma, (**a**) haematoxylin and eosin, (**b**) loss of expression of AMBRA1 in the epidermis, (**c**) interrupted Loricrin staining in the upper epidermis. Bottom row: low risk melanoma, (**d**) Haematoxylin and eosin, (**e**) maintenance of AMBRA1 expression in the overlying epidermis, (**f**) intact band of Loricrin staining over the melanoma

several large international cohorts using digital pathology and consensus scoring in order to develop an accredited test. Preliminary data suggest that such a test may be able to replace sentinel node biopsy in early stage melanoma, avoiding an invasive and expensive investigation.

The study is of particular interest because the biomarkers used reflect changes not in the melanoma cells but in the tumour microenvironment. It may be that epidermal keratinocytes play an important role in melanoma switching between radial and vertical growth-phase and developing invasive growth as suggested by in-vitro studies [19]. The epidermis and stroma may not be simple bystanders and play crucial roles in early melanoma progression.

It is also possible that autophagy biomarkers could be used for head and neck squamous cell carcinoma and this is currently under investigation.

Intra-tumoural Immune Cells as Biomarkers

As in many human cancers, the presence of tumour infiltrating lymphocytes (TILs) is a prognostic biomarker in head and neck cancer [20, 21]. Currently, TILs are not quantified routinely in pathology services and do not form part of datasets for reporting head and neck cancer [21]. Increasingly, pathologists describe their presence or absence along with recognised histological prognostic biomarker features including peri-neural spread, lympho-vascular space invasion and pattern of invasive front in their reports. The presence of high levels of TILs is at least as powerfully prognostic as HPV status in oro-pharyngeal cancer [22]. The International Immuno-Oncology Biomarkers Working Group has published guidelines for pathologists to enable some standardisation of methods and facilitate consistency for TILs evaluation in cancer, including head and neck squamous carcinoma [23]. Two principal methods of quantification can be used. Classically, stromal TILs can be assessed over the whole tumour, recording the average percentage of TILs per stromal area at 200× magnification. An alternative approach is to assess maximum lymphocytic infiltration ('TIL hotspots') in a single field at 200× magnification where TILs are most dense. The values between average and hotspot counts can be dramatically different and there is a further limitation imposed by the biopsy size. Intra-tumoural heterogeneity is well described and small core biopsies may not be representative of the whole tumour volume. Further research is needed to clarify which is the most prognostic method, or if combined with immunotherapy, which is the most predictive method. Digital platforms are increasingly being used and when validated algorithms become available it may be possible to use artificial intelligence (AI) systems to provide both types of TIL count to the oncologist.

The success of CAR-T cell therapy for haematological malignancy has accelerated interest in using adoptive T cell therapies for solid tumours, though responses are more limited [24]. The wide use of immunotherapies has also driven interest in adoptive T cell therapy strategies [25]. Over decades, TIL therapy has demonstrated consistent success in treating metastatic malignant melanoma. Response rates greater than 50% and complete lasting response rates of over 20% were reported almost a decade ago [26]. Such findings have promoted interest in the development of similar adoptive T cell strategies in other cancers including head and neck squamous cell carcinoma [25].

Non-acral melanoma has a high mutational burden presumably due to years of exposure to ultra violet light. Mutations give rise to neo-antigens on the neoplastic cells that serve as potent stimulators of T cell–mediated anti-tumour responses within the host immune system. Squamous cell carcinoma of the head and neck also arises after years of exposure to mutagens in the form of tobacco and alcohol, and has been found to have a relatively high mutational burden [27, 28]. Both lung cancer and head and neck cancer show responses to PD-1 blockade adding further support to the concept that mutational burden is an important component of immunogenicity.

Table 1.1 Clinical trials using adoptive T-cell therapies for head and neck cancer

Therapy	Disease	Phase	Estimated dates[a]	Status[a]	Trial number
TIL	HPV associated cancer	II	2012–2016	Completed	NCT01585428
TIL	NPC	II	2015–2020	Unknown	NCT02421640
TIL	HNSCC	II	2017–2022	Active, recruiting	NCT03083873
TIL	HNSCC	II	2019–2024	Active, recruiting	NCT03645928
TIL	HNSCC	I	2020–2023	In set up	NCT03991741
Adoptive	HNSCC, solid tumours	I	2017–2025	Active, recruiting	EudraCT2017–002323-25
TCR	NPC (EBV+)	I	2001–2012	Completed	NCT00609219
TCR	EBV LMPs	I	2007–2011	Active	ACTRN12607000191493
TCR	HPV associated cancer	I/II	2014–2016	Completed	NCT02280811
TCR	NPC (EBV+)	III	2014–2023	Active, not recruiting	NCT02578641
TCR	HNSCC, all solid tumours	I	2015–2018	Active, not recruiting	NCT02366546
TCR	HPV associated cancer	I	2015–2033	Active, recruiting	NCT02379520
TCR	NPC (EBV+)	I	2015–2033	Active, not recruiting	NCT02065362
TCR	HNSCC	I	2016–2034	Active, not recruiting	NCT02989064
TCR	HPV associated cancer	I/II	2017–2026	Active, recruiting	NCT02858310
TCR	HPV associated cancer	I	2018–2021	Active, recruiting	NCT03578406
TCR	HNSCC	I	2018–2023	Active, recruiting	NCT03247309
CAR-T	HN cancer	I/II	2015–2020	Active, recruiting	NCT01818323

Data from ClinicalTrials.gov, anxctr.org.au and clinicaltrialsregister.eu
[a]Accessed June 11th 2020
TIL Autologous tumour infiltrating lymphocytes, *TCR* T cell receptor engineered T cells, *CAR-T* Chimeric antigen receptor T cells, *HNSCC* Head and neck squamous cell carcinoma, *NPC* Nasopharyngeal carcinoma

Currently, clinical trials are underway in head and neck cancer that employ conventional TIL therapy, T cell receptor engineered T cells and chimeric antigen receptor T cell therapy, many with promising responses (Table 1.1). One Phase III clinical trial is being conducted to assess if combined gemcitabine-carboplatin (GC) followed by adoptive T-cell therapy would improve clinical outcome for patients with advanced nasopharyngeal carcinoma. It follows a successful Phase II trial involving 38 patients at the National Cancer Centre, Singapore [29]. Thirty-eight

patients were enrolled, and 35 received GC and EBV-Cytotoxic T lymphocytes. A response rate of 71.4% with 3 complete responses and 22 partial responses was achieved. The 2-year and 3-year overall survival rates were 62.9% and 37.1%, respectively (median follow up of 29.9 months).

Much remains to be done in the field of T cell engineering for infusion therapies [30]. In the era of predictive, preventative, personalized, participatory (P4) medicine, advances in technology make identification of an individual profile of biomarkers a realistic possibility and in time precision profiles may supplant the use of single molecule predictive biomarkers [31]. During the early development of many cancers it is known that a series of mutations occur, that may be described as founder mutations [32, 33]. With time, a complex pattern of mutations occurs resulting in separate clones with differing mutation patterns. It is often one of these clones that leads to relapse or recurrence after oncological therapy. Key to the future of adoptive T cell therapy is the identification of founder mutations, present in all clones of the neoplasm, and the targeting of the engineered T cells to these neo-antigens. Head and neck cancer is a good candidate for adoptive T cell therapy and the presence of virus in both oropharyngeal (HPV) and nasopharyngeal carcinoma (EBV) offers additional non-host proteins that may be exploited for cell therapies.

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Chapter 2
Biomarkers for Hypoxia, HPVness, and Proliferation from Imaging Perspective

Sebastian Sanduleanu, Simon Keek, Lars Hoezen, and Philippe Lambin

Introduction

Advances in imaging and treatment technology over the last few decades have brought an improvement in locoregional control among head and neck squamous cell carcinoma (HNSCC). Despite advances in treatment, from robotic surgery to new systemic therapies such as immuno-radiation and programmed cell death protein-1/programmed cell death ligand-1 (PD-1/PD-L1) blockers for metastatic disease, the overall survival rates are still poor with around 50% 5-year survival. This is mainly caused by treatment resistance, recurrence and distant metastasis, which in turn can be caused by hypoxia, resistance due to clonogenic cell populations, and inadequate immune response [1, 2].

Adequate staging and tumor delineation through molecular imaging and imaging biomarkers based on routine clinical images could improve the precision of radiotherapy and surgery, which may lead to a reduction of recurrences.

Radiomics and deep learning are machine learning techniques that have the potential to infer quantitative information from routine medical images in HNSCC [3] (Fig. 2.1). Imaging biomarkers derived from such techniques can be predictive

S. Sanduleanu (✉) · S. Keek · L. Hoezen
The D-Lab and the M-Lab, Department of Precision Medicine, GROW—School for Oncology, Maastricht University, Maastricht, The Netherlands
e-mail: s.sanduleanu@maastrichtuniversity.nl; s.keek@maastrichtuniversity.nl; l.hoezen@maastrichtuniversity.nl

P. Lambin
The D-Lab and the M-Lab, Department of Precision Medicine, GROW—School for Oncology, Maastricht University, Maastricht, The Netherlands

Department of Radiology and Nuclear Medicine, GROW—School for Oncology and Developmental Biology, Maastricht University Medical Centre+, Maastricht, The Netherlands
e-mail: philippe.lambin@maastrichtuniversity.nl

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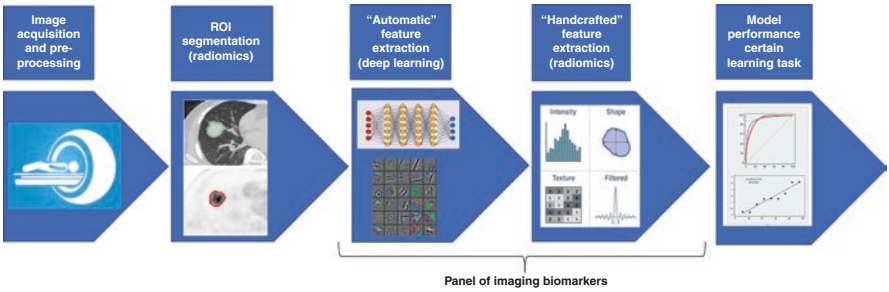

Fig. 2.1 The radiomics and deep learning workflow. Medical images are acquired, pre-processed, and are provided to the deep learning/radiomics workflow. Region of interest (ROI) segmentation is required for radiomics analysis and can be done manually or with automatic segmentation (deep learning). The radiomic features and deep features can be combined using a feature merge layer on which predictions are based. The feature merge layer can comprise a neural network layer but also a machine learning model in which only the most salient features from both pipelines are fed. Eventually the model performance for a specific learning task is assessed

and/or prognostic. A prognostic biomarker provides information about the trajectory/outcome of a patient with cancer, regardless of therapy. Meanwhile, a predictive biomarker is a biomarker that can represent a subgroup of patients who are most likely to respond to the therapy in question. In order to distinguish these two terms, the biomarker-positive and -negative subgroups and experimental and control subgroups are needed. So, when the experimental group shows a difference in survival when tested positive and negative and the survival of the negative response is higher than the control group, this is a prognostic biomarker. If the control group shows no differences in survival when tested positive and negative this is a predictive biomarker. These can also be combined; this means a biomarker can be both prognostic and predictive.

The aim in this chapter is to discuss current trends in head and neck oncology imaging, from imaging biomarkers for HPV-status and hypoxia to recent advances in artificial intelligence (AI) in head and neck oncology.

Imaging Biomarkers for the Assessment of HPV-ness

Human papilloma virus (HPV) positive oropharyngeal squamous cell carcinoma (OSCC) is a rapidly increasing group of patients worldwide (from 16% to 73% in the last 20 years) which responds much better to therapy, whether this is surgery, radiation, or chemotherapy [4, 5]. HPV positive patients have therefore been considered extensively for de-escalation trials [6] in order to decrease toxicity while achieving similar control rates. In 2018, the HPV status of the patient was implemented in the 8th edition of the American Joint Committee on Cancer (AJCC) staging of OSCC [7]. In this staging method, p16 immunohistochemistry (IHC) is used

as a surrogate marker for high-risk HPV [8]. However, p16 IHC is not a perfect surrogate marker for HPV, and consensus on the best way to determine HPV status has currently not been reached [9]. An example of a standard method to determine HPV is polymerase chain reaction (PCR) on paraffin-embedded tissue. However, this method is expensive and time-consuming, and requires the invasive procedure to acquire a biopsy. A study by Molony et al. [10] shows tumor morphology, classified as keratinizing or non-keratinizing, is a significant predictor of HPV status and performs better in determining HPV status in combination with p16 IHC compared to p16 IHC alone. Previous studies have suggested computed tomography (CT) readouts of the tumor showed phenotypical differences between HPV-positive and -negative tumors [11], suggesting an alternative method to determine HPV-status. Indeed, Leijenaar et al. [12] developed a signature based on radiomic features to predict HPV status on routine clinical CT images, showing potential for the determination of HPVness through different methods.

Imaging Biomarkers for Tumor Hypoxia

Tumor hypoxia, also known as the occurrence of oxygen-deficient areas within the tumor, is a known prognostic factor in head and neck cancer. One way to look at both diffusion and perfusion-limited hypoxia is to look at vascular density, vascular permeability, blood volume, and blood flow within the tumor with dynamic contrast enhanced (DCE)-magnetic resonance imaging (MRI)/CT. Although perfusion CT has a dedicated FDA-cleared analysis software and displays greater resolution when compared to DCE-MRI, the required dose of ionizing radiation limits its ability to be used in trials with repeated scanning. In DCE-MRI, moving artifacts from breathing and swallowing and the susceptibility artifacts from interface air-tissue are frequent when scanning the head and neck region with this method, which could substantially affect the tumor-segmentation accuracy and the quantitative imaging biomarker (radiomics) feature extraction. Therefore, at this moment, the data obtained from pre-treatment DCE-MRI seems to be insufficient to allow translation to clinical practice. To our knowledge there is not a single DCE-MRI imaging biomarker study in head and neck looking specifically into association with (histopathologically confirmed) tumor hypoxia, though there are e.g. multiparametric MRI-based prognostic signatures for e.g. advanced nasopharyngeal carcinoma.

Hypoxia imaging PET radiotracers such as ^{18}F-FMISO and ^{18}F-HX4 are promising but not widely available. Hypoxia PET imaging is nevertheless difficult to implement in clinical practice since these PET-agents generally tend to generate smaller signal-to-background ratios compared to e.g. [^{18}F]-FDG (and consequently lower target-background image contrast), imaging is labor intensive (instructions of multiple bed positions and acquisitions at multiple time points), costly (chemical process to produce the radioligand is slightly more expensive), and lacking standard

calibration procedures and inconvenient for the patient due to the time-consuming acquisition protocols. Another way would be to infer quantitative imaging biomarkers from routine ^{18}F-FDG PET and (contrast enhanced) CT images using hypoxia PET tracers as gold standard for training these models.

The aim of the study by Crispin-Ortuzar et al. [13] for instance was to design a surrogate biomarker for ^{18}F-FMISO maximum tumor-to-blood uptake ratio (TBR_{max}) based on pre-treatment ^{18}F-FDG PET and contrast-enhanced CT imaging features. The level of hypoxia of a lesion was defined in terms of its TBR_{max} on the last static scan. In particular, in this study a lesion was considered to be hypoxic if $TBR_{max} > 1.4$. The further aim was to study its performance in the context of hypoxia-based patient stratification. In her study, 121 lesions from 75 head and neck cancer patients were used in the analysis. Patients received both pre-treatment ^{18}F-FDG and ^{18}F-FMISO PET/CT scans. In total, 79 lesions were used to train a cross-validated least absolute shrinkage and selection operator (LASSO) regression model based on quantitative imaging features, while the remaining 42 were held out for internal testing. The best performance on the unseen test subset in this study was obtained from the combined CT and ^{18}F-FDG PET signature, with an area under the receiver operating characteristic curve (AUC) of 0.833, while the model based on the 90th percentile of ^{18}F-FDG uptake alone had a test AUC of 0.756.

Such imaging biomarkers, when improved to accurately detect hypoxia, could be used to stratify patients for hypoxia-modifying therapy.

Evaluation Treatment Response with RECIST 1.1

Objective assessment of both tumor shrinkage as well as time to development of disease progression after (non-)cytotoxic systemic therapy are important endpoints both in clinical trials as well as on patient-level. The revised response evaluation criteria in solid tumors (RECIST) 1.1 in 2009 [14] sought to improve the accuracy and efficacy of this assessment by (1) reducing the maximum of lesions for longest diameter measurement from 10 to 5 (in maximum two organs) (2) disease progression (PD) not only requires 20% increase in the sum of measurements, but also a 5 mm absolute increase (to guard against over calling PD when the total sum is very small) (3) inclusion of FDG-PET response assessment as an adjunct to determination of progression.

One of the key questions for debate by the RECIST Working group developing RECIST 1.1 was whether it is appropriate to move from anatomic unidimensional assessment of tumor burden to either a volumetric assessment or to a more functional assessment with MRI and/or PET. At that point the Working Group concluded that there is not sufficient standardization or evidence to abandon the current unidimensional anatomical assessment of tumor burden and functional imaging with FDG-PET was only to be used as an adjunct for the determination of progression.

The Potential for Automatic Head and Neck Segmentation and Volumetric RECIST Assessment as Surrogate Imaging Marker for Tumor Proliferation

Treatment planning for high precision radiotherapy of head and neck cancer patients requires accurate delineation of many organs at risk for radiation induced injury as well as gross tumor volumes and (elective) lymph node regions. Manual contouring is a laborious task which suffers from large inter- and intra-rater variability. To reduce manual labor, several fully automated, atlas-based [15] as well as deep learning based [16] methods for head and neck CT image segmentation have been developed. Although these methods save a considerable amount of time as they do not require human input, they are also prone to errors [17].

Significant challenges arise currently using RECIST 1.1 endpoints, which could be mitigated by volumetric methods. First and most foremost, during treatment the longest diameter of the tumors may remain unchanged, while the irregularly shaped and morphologically complex tumors may still shrink in terms of absolute volume (Fig. 2.2).

Secondly, while the RECIST criteria were developed traditionally to assess the efficacy of cytostatic drugs, while other systemic therapies may not shrink tumor size but rather trigger a cytostatic response or alter the physiological properties of a tumor such as metabolism, cell proliferation, and angiogenesis. In the case of immunotherapy, initial tumor enlargement is common, which according to RECIST would be classified as progressive disease.

One of the ways to deal with these challenges is to quantify volumetric measures on CT, MRI, and PET as biomarkers for systemic treatment response is as addressed in the Quantitative Imaging Biomarker Alliance (QIBA) profile initiative in 2007 by the Radiological Society of North America (RSNA). The main purpose of this initiative was to unite researchers, healthcare professionals, and industry to advance quantitative imaging and the use of imaging biomarkers in clinical trials and clinical

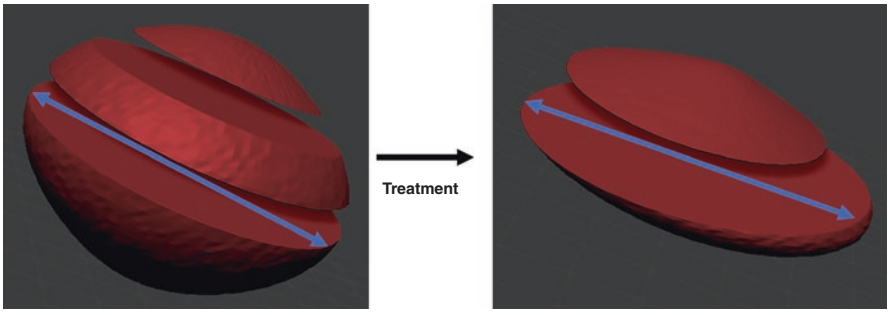

Fig. 2.2 Traditional longest diameter measurement according to RECIST versus volume: longest diameter remains unchanged while overall volume shrinks

practice. One of the QIBA committees is involved in establishing a process map (measurement accuracy, technical feasibility, and comparison with standard RECIST measurements) for qualifying volumetric measures on CT as a biomarker for treatment response as well as determining whether changes in volume are medically meaningful or just add to overall costs and complexity of care [18].

Early treatment response assessment allows the physician to stop an ineffective treatment sooner and enable a transition to a more effective alternative. Generally, volumetric tumor assessment is more costly and time-consuming to perform. Nevertheless, the greater sensitivity [18] associated with volumetric measurement can increase the statistical power per subject, resulting in fewer patient inclusions in clinical trials followed up over shorter periods of time and subsequently decreasing overall time and cost. Additionally, the question is whether volumetric imaging adds value to a clinical trial, in other words if it significantly impacts clinical decision-making. Although this issue still remains to be determined and validated, some preliminary findings find a role for volumetric imaging. In one retrospective study by Hayes et al. [19] on 42 lung cancer patients participating in an open-label phase 2 study, volumetric measurements (semi-automatic segmentation algorithm on CT) on first follow-up (4 weeks after start of treatment) were better able to predict overall survival than RECIST measurements. A second study by Kim et al. [20] found in a cohort of 135 non-small cell lung cancer patients that hyperprogressive disease treated with immune checkpoint inhibitors on the basis of volumetric measurement is more precise than is defining it on the basis of one-dimensional analysis in terms of overall survival. To our knowledge at the moment this chapter has been written there were no such volumetric versus RECIST comparison initiatives in head and neck cancer.

Conclusions and Future Directions

In recent years, explainable AI (XAI), the implementation of transparency and traceability of statistical black-box machine learning methods [21], has been attracting much interest in medicine. The reenactment of the machine decision-making process is necessary not only to comprehend and reproduce the learning and extraction process, but also because for medical decision support it is necessary to understand the causality of learned representations [22–24]. Furthermore, the implementation of explainable AI would help to enhance the trust of medical professionals in future AI-systems. Nevertheless, currently there is still an inherent tension between machine learning performance (predictive accuracy) and explainability, as often the best-performing methods such as deep learning are the least transparent, and the ones providing a clear explanation (e.g. decision trees) are less accurate [25]. This still makes this very much an active area of research. Advanced functional imaging techniques to address the inherent limitations of the current RECIST, such as perfusion CT, dynamic contrast-enhanced MRI, and diffusion-weighted MRI are currently only considered to be experimental endpoints because they have not yet completed the rigorous validation process needed to qualify as

true surrogate endpoints. With the advent and improvement of explainable automatic segmentation algorithms, volumetric endpoints (perhaps with the aid of advanced functional imaging techniques) in the near future will offer increased sensitivity to anatomical measurements and provide the necessary physiological information to interpret response to highly selective, patient tailored therapies, particularly in the cases where RECIST falls short.

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Chapter 3
Mechanisms of Cetuximab Resistance and How to Overcome It

Ines De Pauw, Carolien Boeckx, and An Wouters

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer type worldwide and accounts for a standardised worldwide incidence of roughly 600,000 individuals/year [1]. HNSCC remains one of the most challenging malignancies to treat. For example, the overall 5-year relative survival proportion for the Belgian 2013–2017 cohort was about 52% in males and 59% in females [2]. Unfortunately, patients with multiple metastases typically have very poor prognosis with a 5-year overall survival of only 4% [3]. Therefore, innovative therapeutic strategies are a necessity to increase the survival outcomes.

The introduction of targeted therapies that inhibit oncogenic signalling pathways is now at the forefront of personalised medicine in cancer treatment. As the epidermal growth factor receptor (EGFR) initiates important signalling pathways and is overexpressed and/or deregulated in a wide range of malignancies, this receptor is considered as an excellent drug target. Improved understanding of EGFR signalling in cancer has led to the development of two main categories of EGFR-targeting agents: the monoclonal antibodies (mAbs, such as cetuximab and panitumumab) and the tyrosine kinase inhibitors (TKIs, such as erlotinib and gefitinib) [4].

I. De Pauw · C. Boeckx · A. Wouters (✉)
Center for Oncological Research (CORE), University of Antwerp, Wilrijk, Belgium

Integrated Personalized and Precision Oncology Network (IPPON), University of Antwerp, Wilrijk, Belgium
e-mail: An.Wouters@uantwerpen.be

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The Epidermal Growth Factor Receptor

The physiological function of EGFR is to regulate epithelial tissue development and homeostasis through cellular processes such as proliferation, maturation and apoptosis [5]. These cellular processes play an important role in the transformation of healthy cells into malignant tumour cells. The activity of the EGFR signal transduction pathway is tightly controlled in healthy cells, but deregulation of EGFR signalling plays a crucial role in carcinogenesis [6]. Accordingly, EGFR signalling has been studied intensively in order to understand its importance in cancer biology.

EGFR is a cell surface receptor that belongs to the HER or ErbB tyrosine kinases family. Besides EGFR, also known as HER1 or ErbB1, other members of the HER family include HER2 (ErbB2 or Neu), HER3 (ErbB3), and HER4 (ErbB4). The structure among these receptors is very similar: they consist of an extracellular ligand-binding domain, a single membrane-spanning region, a juxtamembrane nuclear localisation signal and a cytoplasmic tyrosine-kinase domain [7]. HER receptors are activated by a range of growth factors that belong to the EGF-family and can be divided into three groups (Fig. 3.1). The first group includes EGF, transforming growth factor-α (TGF-α) and amphiregulin, which all bind to EGFR. The second group includes betacellulin, heparin-binding EGF (HB-EGF) and epiregulin which bind to both EGFR and HER4. The third group is composed of the neuregulins (NRG1-4), which is further subdivided based on their ability to bind HER3 and HER4 (NRG1 and NRG2), or only to HER4 (NRG3 and NRG4) [8]. Until now, no known ligand exists for HER2 [7, 9].

Binding of a ligand to the extracellular domain of these receptors leads to a conformational change that allows for receptor homo- and hetero-dimerisation and activation of intrinsic tyrosine kinase activity resulting in transphosphorylation of specific tyrosine residues within the intracellular domain. Autophosphorylation triggers a series of intracellular pathways that may result in cancer-cell proliferation, blocking apoptosis, activating invasion and metastasis, and stimulating tumour-induced neovascularisation. Figure 3.2 gives an overview of the HER receptor

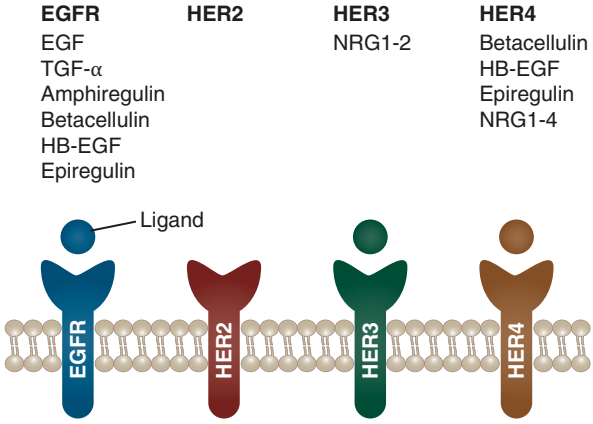

Fig. 3.1 The HER family receptors and ligands. Binding of a ligand to the extracellular domain of the receptor leads to receptor homo- and hetero-dimerisation and activation. TGF-α, transforming growth factor-α; HB-EGF, heparin-binding EGF

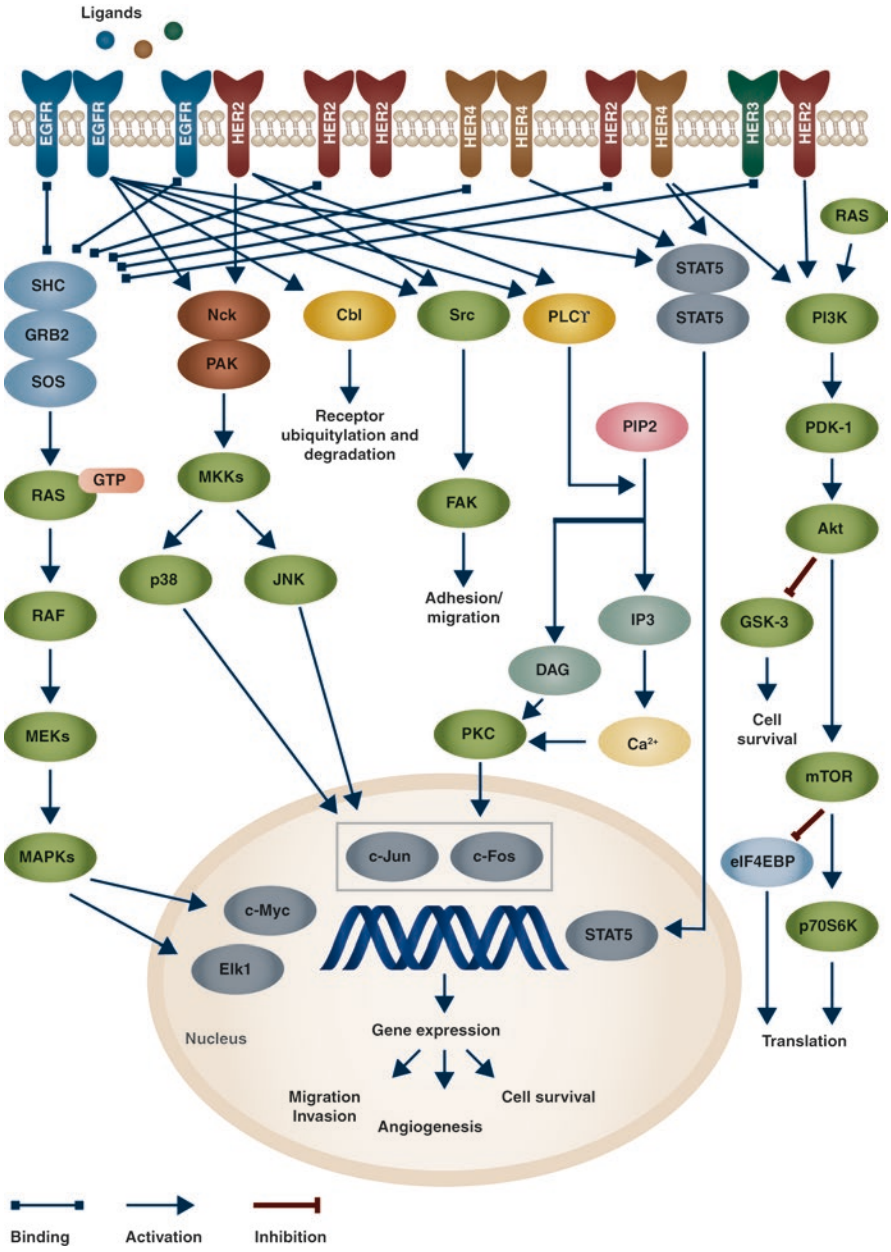

Fig. 3.2 HER receptor signalling pathways. Ligand binding to HER receptors is followed by receptor homo- and hetero-dimerisation and the activation of several downstream signalling pathways

signalling pathways, including the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, the signal transducers and activators of transcription (STAT) pathway, Phospholipase Cγ, Src kinase pathways, the NcK/PAK signalling cascade and Cbl-mediated endocytosis.

During the 1980s, several studies described the overexpression of EGFR in a variety of epithelial tumours, which supported the hypothesis that dysregulated EGFR expression and signalling play an important role in the development of cancer [7, 10–14]. Over the last years, the oncogenic role of EGFR has been characterized in more detail and several alterations have been described [15]. Firstly, gene amplification leading to EGFR overexpression is often observed in human cancers [16, 17]. Secondly, point mutations and deletions in the EGFR gene can result in increased catalytic tyrosine kinase activity or a truncated form of the receptor, resulting in ligand-independent activity [18]. The most common tyrosine kinase EGFR mutations include the deletion of four conserved amino acids residues in exon 19 and the point mutation L858R in exon 21 [19, 20]. In addition, the EGFR variant III (EGFRvIII) is also frequently detected and constitutes a truncated form of EGFR caused by an in-frame deletion of 801 base pairs (exon 2–7) in the coding sequence of the extracellular domain [21, 22]. Next, increased ligand expression leads to constitutive stimulation of EGFR. EGF-related growth factors can be produced either by the tumour cells themselves or by surrounding stromal cells [23]. Finally, impaired receptor downregulation also results in sustained EGFR signalling [24]. All these alterations, consequently, result in increased EGFR activation and/or deregulation of EGFR signal transduction pathways. As EGFR stimulates many complex intracellular signalling pathways that are involved in proliferation, differentiation, apoptosis, angiogenesis and metastasis, activation of EGFR drives the malignant behaviour of the tumour [25].

Cetuximab as Anti-EGFR Targeting Agent

For over a decade, the EGFR monoclonal antibody cetuximab is approved by the European Medicines Agency (EMA) and Food and Drug Administration (FDA) for HNSCC treatment in three specific settings, i.e. (1) in combination with radiation therapy for locoregionally advanced HNSCC; (2) in combination with platinum-based therapy and 5-fluorouracil for first-line treatment for recurrent or metastatic (R/M) HNSCC; and (3) as a single agent for R/M HNSCC patients who failed on prior platinum-based therapy [26–28].

The therapeutic effect of the monoclonal antibodies, such as cetuximab and panitumumab, is exerted by binding to the extracellular domain of EGFR, thereby hindering ligands to bind and activate EGFR, preventing receptor dimerisation and promoting EGFR internalisation [15]. Importantly, as a chimeric human:mouse immunoglobulin G1 (IgG1), cetuximab can also elicit host anti-tumour immune responses. Through its IgG1 backbone, cetuximab can bind CD16 fragment

crystallisable (Fc) receptors located on natural killer (NK) cells, macrophages and granulocytes, of which NK cells have been proven to be the most potent effectors [29]. Binding of the IgG1-Fc part of cetuximab to CD16 on NK cells triggers cytolytic activity called antibody-dependent cellular cytotoxicity (ADCC), which is predominantly mediated by perforin and granzymes [30]. Furthermore, cetuximab has been shown to enhance cross-priming of cytotoxic T-lymphocytes via professional antigen-presenting cells, such as dendritic cells [31], mainly through induction of immunogenic cell death of tumour cells [32]. These results confirmed the important immune-related mechanism of action of cetuximab, in addition to its receptor blocking effects.

Mechanisms of Cetuximab Resistance

Personalised medicine using targeted therapies, based on the molecular profile of the tumour, may achieve the much-needed progress in cancer treatment. After the initially promising results of EGFR-targeted therapies such as cetuximab, therapeutic resistance poses a challenging problem and limits the success of effective cancer therapies in the clinic [33]. If resistance to therapy is present at baseline, this is defined as intrinsic (primary) resistance and can be explained by resistance-conferring factors pre-existing in the bulk of tumour cells. Moreover, nearly all patients whose tumours initially respond inevitably become acquired (secondary) resistant. Acquired resistance refers to disease progression in the face of ongoing treatment that was initially effective [34].

Indeed, despite the enhanced EGFR expression in the majority of HNSCC tumours, therapeutic resistance remains a major roadblock in the search to effective HNSCC therapies and only a small subset of HNSCC patients benefit from cetuximab as a single agent (<15%) or combined with chemotherapy (36%). The addition of cetuximab to either radiotherapy in the locoregionally advanced disease setting, or to platinum and 5-fluorouracil chemotherapy (EXTREME regimen) for treatment of recurrent or metastatic HNSCC improved median overall survival from 29.3 to 49 months and from 7.4 to 10.1 months, respectively. Nevertheless, time-to-treatment failure in patients treated with the EXTREME regimen ranges only around 5 months, despite cetuximab maintenance [28, 35, 36].

Therapeutic resistance to anti-EGFR therapy may arise from mechanisms that can compensate for reduced EGFR signalling and/or mechanisms that can modulate EGFR-dependent signalling. Over the last years, a wide range of potential molecular mechanisms of resistance to EGFR-targeting agents has been described [15].

Altered Response Elicited at the Level of EGFR

Drug resistance can arise from sustained EGFR signalling that is elicited at the level of EGFR itself by ligand or receptor overexpression, amplification or mutation [15, 19, 20, 37–41]. Furthermore, EGFR can escape the internalisation and lysosomal degradation route and function as a transcription factor in the nucleus, inducing sustained EGFR signalling [42].

Binding of ligands to EGFR drives homodimerisation or heterodimerisation with ErbB family members, resulting in the initiation of downstream signalling pathways. Therefore, overexpression of its ligands may contribute to cetuximab resistance. A correlation with enhanced response to cetuximab therapy and overexpression of the EGFR ligands amphiregulin and epiregulin in K-Ras wild type metastatic colorectal tumours has been reported [40]. In HNSCC patients receiving cetuximab-docetaxel treatment, high amphiregulin levels were detected in 45% of the patients. A significant correlation was found between high amphiregulin levels and shortened overall survival and progression free survival compared with patients with low amphiregulin expression [41].

Neither the expression level of the EGFR protein, nor the amplification status of the *EGFR* gene could be linked to therapeutic response [43, 44]. Activating mutations have been observed in the tyrosine kinase domain or in the extracellular ligand-binding domain of EGFR [18]. The most common tyrosine kinase EGFR mutations include deletion of four conserved amino acids residues (leucine-arginine-glutamic acid-alanine) in exon 19 and a point mutation, L858R, in exon 21, which account for 90% of all EGFR tyrosine kinase mutations in non-small cell lung cancer (NSCLC) [45–47]. These EGFR tyrosine kinase mutations are associated with an improved clinical response to TKIs (gefitinib or erlotinib) in NSCLC patients but they are rarely found in HNSCC. Literature data suggest that the incidence of such activating mutations in HNSCC patients range from 0 to 15.7% [45–60]. In these studies, a total of 889 HNSCC samples were screened for EGFR tyrosine kinase mutations, of which 34 (3.8%) contained a mutation. Interestingly, the missense mutation T790M in exon 20, which is associated with acquired resistance to EGFR TKIs in about half of all patients with NSCLC, was found in 7.5% of all EGFR mutations in HNSCC [61]. Given that in HNSCC, the overall prevalence of mutations in the EGFR tyrosine kinase domain is only 2.8%, it is challenging to identify specific EGFR mutations related to response or resistance to anti-EGFR therapy [62].

Next to the above-discussed mutations, the EGFR variant III (EGFRvIII) is a truncated form of EGFR. The causing mutation consists of an in-frame deletion of 801 base pairs (exon 2–7) in the coding sequence of the extracellular domain, resulting in ligand-independent tyrosine kinase activity [21, 63]. The mutant EGFRvIII form is associated with increased proliferation, tumour growth, cell motility and invasion in vitro and resistance to anti-EGFR therapy [21, 64]. The mutation frequency of EGFRvIII in HNSCC ranges from 0 till up to 48% [41, 49, 51, 64–67]. It has been suggested that the EGFRvIII might be more available in the recurrent/

metastatic disease setting and might be responsible for the lack of response to EGFR-targeted therapies [67].

Occasionally, a part of the EGFR receptor escapes the internalisation and lysosomal degradation route and translocates to the nucleus [42, 68]. In oral squamous cancers, nuclear EGFR was observed in 24.3% of patients [69]. This nuclear EGFR functions either as a transcription factor of cyclin D1, inducible Nitric Oxide Synthase (iNOS), B-Myb and cyclo-oxygenase-2 (COX-2), or as a tyrosine kinase phosphorylating and stabilizing proliferating cell nuclear antigen (PCNA), resulting in an activation of the nitric oxide pathway and increased G_1/S progression of the cell cycle [70–74]. Consequently, the proliferative potential of the cancer cells is thereby enhanced. The presence of nuclear EGFR is not only associated with poor prognosis, but also with treatment resistance [69, 75, 76]. Besides its potential involvement in resistance mechanisms, nuclear EGFR is also associated with local recurrence [75].

Molecular Alterations in Effectors Downstream of EGFR

Resistance to EGFR inhibitors can also be the result of molecular alterations in effectors downstream of EGFR [15]. In particular, the RAS, PI3K, Akt, STAT and Src proteins have been suggested to contribute to drug resistance [77–88]. We previously showed that proteins related to the Ras-MAPK pathway are involved in mechanisms of resistance towards cetuximab in HNSCC [89]. This is confirmed in other studies, highlighting the significance of persistent activation or reactivation of the Ras-MAPK pathway in EGFR targeting drug resistance [90–92].

Firstly, K-Ras is a protein located downstream of EGFR in the Ras-MAPK pathway. Somatic point mutations in *K-Ras* occur in a variety of human malignancies, most frequently in pancreatic cancer, NSCLC and colon cancers [93, 94]. A mutation in codon 12 or 13 in this gene leads to constitutive activation of the protein, regardless of upstream activating signals. In colorectal tumours, these mutations confer resistance to therapy with the EGFR targeting monoclonal antibodies cetuximab and/or panitumumab [77, 78, 95, 96]. Approximately 30–40% of colorectal tumours harbour a *K-Ras* mutation [97, 98]. In contrast, in HNSCC, these *K-Ras* mutations are infrequent; in different reports the frequency of *K-Ras* mutations in HNSCC is ranging from 0 to 9.1% [45, 51, 99].

Another family member of the Ras proto-oncogenes is H-Ras. Mutations in *H-Ras* have been reported in literature and vary between 0 and 22% [100–104]. A very recent study demonstrated that *KRAS/HRAS* mutations were associated with poor progression-free survival among HNSCC patients treated with cetuximab in the first-line recurrent setting, but not among patients treated with cetuximab in combination with radiotherapy [105], thus suggesting that not only *K-Ras* but also *H-Ras* mutations might play a role in cetuximab resistance.

Secondly, further downstream of K-Ras in the MAPK signalling pathway, a member of the dual-specificity phosphatase (DUSP) family is located. DUSP

proteins are involved in a negative feedback mechanism of the MAPK signalling pathway by dephosphorylation of the threonine-glutamic acid-tyrosine motif on MAP kinases [106]. Therefore, DUSP proteins can be seen as tumour suppressor proteins, and loss of their expression may promote constitutive activation of ERK and uncontrolled cell growth. Moreover, inhibition of the MAPK pathway can be compensated by suppression of the DUSP enzymes [92]. Both the cytoplasmic DUSP5 and the nuclear DUSP6 can dephosphorylate ERK1/2, thereby blocking the MAPK signal transduction cascade [107].

Low DUSP6 expression has proven to be clinically significant as it was observed in 40% of patients with oesophageal squamous cell carcinomas and 75% of nasopharyngeal patients [108]. This might occur through hypermethylation of CpG islands in intron 1 or loss of heterozygosity of the DUSP6 locus [109, 110]. As DUSP6 is a critical negative regulator of Erk1/2 [111], we previously evaluated the level of Erk1/2 phosphorylation and demonstrated that significantly more Erk1/2 phosphorylation was present in cetuximab resistant HNSCC cells after cetuximab treatment compared with cetuximab sensitive HNSCC cells. Furthermore, apigenin, an Erk1/2 inhibitor, dose-dependently inhibited survival of cetuximab resistant cells and a significant decrease in cell survival was observed when these cells were treated with a combination of apigenin and cetuximab [89]. Additionally, sustained or reactivated Erk, caused by downregulation of *DUSP6*, has been observed in lung cancer cells with acquired erlotinib resistance [91]. This highlights the significance of our findings and indicates that the exact function of the DUSP family proteins in relation to cetuximab resistance in HNSCC should be further elucidated.

Thirdly, besides activation of the Ras/Raf/MAPK signalling pathway, EGFR can also mediate activation of the PI3K/Akt pathway. This pathway is involved in various biological processes essential for normal cellular functionality, including survival, proliferation, differentiation, angiogenesis, protein synthesis and glucose metabolism. Besides these physiological functions, the pathway is also associated with a number of oncogenic processes and is one of the most frequently dysregulated pathways in cancer, including HNSCC [112, 113]. As such, aberrant signalling can lead to the stimulation of cell growth, inhibition of cell death and the promotion of invasion and migration [114–116], which is all in favour of cancer cells. Furthermore, increasing evidence indicates that the PI3K/Akt pathway frequently remains activated, despite anti-EGFR treatment and therefore plays an important role in resistance to EGFR-targeting therapies [117–120].

Fourthly, Src kinases are upstream as well as downstream activators of EGFR and other receptor tyrosine kinases. Upon EGFR stimulation, Src kinases are activated and associate with EGFR. As such, they can affect cellular proliferation and survival by activation of STAT family of transcription factors, especially STAT3 and STAT5 [121, 122]. In vitro studies showed reduced activity of Src kinases following EGFR inhibition [123]. Elevated Src levels and/or kinase activity have been shown in HNSCC and other malignancies [122, 124]. Therefore, activation of Src kinases by EGFR upstream or downstream signalling might result in resistance to anti-EGFR therapy.

As mentioned earlier, nuclear translocation of EGFR is a possible mechanism of resistance to therapy and this has been observed in patients treated with cetuximab and radiotherapy. Phosphorylation of EGFR on tyrosine 845 by the Src kinases enhances EGFR-mediated mitogenesis by binding and phosphorylating the STAT5b transcription factor and this has been described as the underlying mechanism responsible for nuclear translocation of EGFR [88, 125]. Indeed, dasatinib, a Src inhibitor, blocks EGFR translocation to the nucleus in HNSCC cell lines and, therefore, might be a potential way to evade resistance to anti-EGFR therapy [126]. In addition, in oral squamous cell carcinoma, it was shown that the combination of cetuximab and a Src inhibitor may provide more effective therapy than either inhibitor alone [127]. Collectively, these results indicate that Src inhibitors may be useful in overcoming anti-EGFR resistance by decreasing activated STAT3 and STAT5.

Finally, when considering resistance to anti-EGFR therapy, the signal transducer and activator of transcription (STAT) family proteins are also important downstream EGFR effectors. This family plays an important role in transmitting survival signals and anti-apoptotic signals that are initiated through activation of EGFR; especially activation of STAT3 and STAT5 has been linked to phosphorylation of EGFR [122, 128, 129]. Therefore, dysregulation of the STAT signalling pathway has been proposed to be implicated in malignant transformation.

Activation of STAT3 leads to the activation of several survival proteins, including Bcl-xl, Bcl-2 and survivin [84]. In HNSCC, STAT3 activation can be mediated by JAK and Src signalling, and partially by EGFR signalling [126, 130]. As such, STAT3 can be inhibited via EGFR blocking and this has been demonstrated in vitro and in vivo [131]. It has been shown that the anti-proliferative effects of cetuximab, as well as cetuximab-induced apoptosis, are more pronounced in STAT3 knockdown cells compared to control cells [84]. Recently, increased STAT3 expression was found in two acquired cetuximab-resistant HNSCC cell lines, compared to their parental lines. Moreover, STAT3 knockdown promoted increased cytotoxicity both in the presence and absence of cetuximab in the resistant lines [132], suggesting that STAT3 may be a common target in cetuximab resistance.

Cross-talk with Other Receptor Tyrosine Kinases

Selective stress of EGFR-targeting agents can lead to activation of alternative signalling pathways to compensate for the reduced EGFR signalling, thereby promoting cell survival [15]. Examples of alternative receptor pathways include other HER receptor family members, insulin growth factor type 1 receptor (IGF-1R) and MET [133–139].

Firstly, as mentioned earlier, EGFR is a family member of the ErbB receptor family, and activation of other members of this family might result in resistance to anti-EGFR therapy. In the literature, activation of HER2 signalling has been associated with cetuximab resistance, as its signalling occurs through many of the same downstream effectors of EGFR. Using an in vitro model of acquired cetuximab

resistance, a marked increase in the phosphorylation status of the C-terminal fragment of HER2, 611-CTF, was observed. Combination therapy of afatinib (an irreversible EGFR/HER2/HER4 inhibitor) with cetuximab resulted in a dramatic reduction in cetuximab resistant tumour volumes compared to either agent alone in monotherapy [133]. Therefore, it was suggested that dual inhibition of EGFR and HER2 could be an effective approach to enhance the efficacy of cetuximab, in order to prevent and/or overcome cetuximab resistance. Likewise, a study by Yonesaka et al. has shown that cetuximab resistance could be induced by activation of ErbB2 signalling. The underlying mechanism involved amplification of ErbB2 or upregulation of heregulin, both leading to persistent ERK1/2 activation. Moreover, restoring cetuximab sensitivity was accomplished by inhibition of ErbB2 or by disruption of ErbB2/ErbB3 heterodimerisation in vitro as well as in vivo [134]. More studies are warranted in order to determine the frequency of HER2 mutations in HNSCC and their role in the response to TKIs.

Secondly, activation of the insulin growth factor type 1 receptor (IGF-1R) leads to downstream activation of the Ras/Raf/MAPK and PI3K/Akt pathway and enhances survivin expression, all contributing to cell proliferation, altered cell adhesion, enhanced motility properties and impaired apoptosis [140, 141]. Analysis of the HNSCC subsets of the Cancer Genome Atlas has identified 4% amplification and mutation of IGF-1R gene in human papillomavirus (HPV) negative HNSCC patients [142]. Furthermore, activation of IGF-1R has been reported to induce resistance to EGFR TKIs [143]. It was shown that heterodimerization of EGFR with IGF-1R was increased in cetuximab resistant HNSCC cancer cells [144]. This heterodimerization of EGFR with IGF-1R lead to increased activity of EGFR and might be an important platform for cetuximab-mediated signalling in head and neck tumours that have become resistant to anti-EGFR therapy. As such, dual targeting of EGFR and IGF-1R could be a promising therapeutic strategy.

Thirdly, the *MET* proto-oncogene encodes a transmembrane receptor tyrosine kinase MET, also known as c-MET or hepatocyte growth factor receptor (HGFR). The MET pathway can be deregulated in two different ways: on the one hand by mutation and/or amplification of MET, and on the other hand by increased ligand expression and/or activity, both resulting in persistent activation of the PI3K/Akt signalling pathway [138]. Circa 80% of primary HNSCC tumours express the ligand hepatocyte growth factor (HGF), MET, or both, thus activating important downstream signals, which overlap with EGFR signalling [65, 145]. Moreover, MET mutations or amplifications have been observed in 13.5% and 13% of HNSCC tumours, respectively [146]. As high MET expression could be observed in 58% of patients with recurrent/metastatic HNSCC [65], the role of MET in resistance to anti-EGFR therapy has been investigated in a number of studies. Chau et al. did not detect any association between response to erlotinib and time to progression or overall survival, in recurrent/metastatic HNSCC patients with high MET expression [65]. Experiments in vitro and in vivo showed that MET confers resistance to cetuximab via activation of the MAPK pathway. In addition to the direct role of MET in reactivation of the MAPK pathway, MET stimulation also abrogated the well-known cetuximab-induced compensatory feedback loop of HER2/HER3 expression

[147]. In a HNSCC xenograft model, a delay in tumour growth was observed after administration of crizotinib, a MET TKI [145]. Collectively, these data suggest that high MET expression might play a role in cetuximab resistance.

Alterations in Proteins Outside the EGFR Pathway

Not only alterations in proteins involved in EGFR signalling but also proteins such as cyclin D1 and p53, linked to more general characteristics of cancer (such as proliferation, apoptosis, invasion and metastasis) can confer resistance to EGFR inhibitors [148–151].

The Aurora kinases A and B are highly conserved serine/threonine kinases that play an essential and distinct role in mitosis [152, 153]. Overexpression of both kinases is frequently present in many types of malignant tumours, and in the case of HNSCC, overexpression of Aurora kinase A is found in up to 90% of tumours [153–155]. Overexpression of Aurora kinase A is correlated with tumour progression, a metastatic phenotype and shortened survival, and is therefore regarded as a negative prognostic marker [152, 154, 155]. High expression levels of Aurora kinase B are found in glioblastoma, ovarian carcinoma and hepatocellular carcinoma and are associated with poor prognosis [156].

The EGFR pathway can elicit overexpression of Aurora kinase A at two different levels, i.e. (i) EGF increases the translational efficiency of Aurora kinase A; and (ii) translocation of EGFR to the nucleus results in binding to the Aurora kinase A promoter and thereby increasing its transcription. Both ultimately result in chromosome instability and tumourigenesis [73, 157].

Next to its role as a prognostic factor, studies indicated evidence for a role of Aurora kinase A in the response to therapy. Overexpression of Aurora kinase A triggered the activation of two important molecules involved in the regulation of drug resistance, Akt and NF-κB [158]. Interestingly, knockdown of Aurora kinase A in HeLa cells resulted in sensitisation to cisplatin, and Aurora kinase A overexpression could overcome cell death induced by paclitaxel [158]. Furthermore, treatment of HNSCC cells with cetuximab and a pan-Aurora kinase inhibitor R763 resulted in a rapid and efficient decrease in the level of the Aurora kinase substrate S10HH3. These results could not be confirmed by using a specific Aurora kinase A inhibitor and, therefore, it was concluded that the effects of the pan-Aurora kinase inhibitor were most likely mediated by its blockage of Aurora kinase B activity [152]. Similarly, we previously showed that cell growth of cetuximab resistant cells could be inhibited by blocking Aurora kinase B [89]. Collectively, these results indicate that the Aurora kinases may be an interesting target for HNSCC tumours resistant to anti-EGFR therapy.

The G_1/S-specific cyclin D1 forms a complex with CDK4 and CDK6 and functions as a regulatory subunit of CDK4 and CDK6, the activity of which is required for cell cycle G_1/S transition. As previously mentioned, nuclear EGFR functions as a transcription factor for cyclin D1. Moreover, constitutive activation of STAT3 is

required for EGFR-mediated cell growth and results in elevated levels of STAT3 target genes, including cyclin D1 [129, 159].

HNSCCs that are unrelated to the human papillomavirus (HPV), are often driven by $p16^{INK4A}$ inactivation and cyclin D1 overexpression that cause hyperactivation of cyclin-dependent kinase 4/6 (CDK4/6), which drives the cell cycle and tumour growth. Deregulated cyclin D1 expression also causes resistance to EGFR inhibitors. These somatic genomic alterations pointed to inhibition ofCDK4/6 as a potential targeted therapeutic strategy in HPV-unrelated HNSCC. The CDK4/6 inhibitor palbociclib arrests cell cycle progression by selective CDK4/6 inhibition and might also reverse intrinsic resistance to cetuximab by countering the actions of deregulated cyclin D1. The antiproliferative and antitumour effects of selective CDK4/6 inhibition have indeed been demonstrated in HNSCC cell lines and xenografts. In HPV-unrelated HNSCC cell lines, the combination of palbociclib and an EGFR inhibitor synergistically reduced cell viability andERK1/2 phosphorylation. Importantly, a recent multicentre, phase 2 trial, showed that the combination of palbociclib and cetuximab exhibited substantial antitumour activity in platinum-resistant and in cetuximab-resistant HPV-unrelated HNSCC [160]. As such, further investigation of selective CDK4/6 inhibition as a therapeutic strategy in HPV-unrelated HNSCC is certainly warranted.

The tumour suppressor protein p53 has a critical role in controlling cell cycle progression and, consequently, loss of its function is linked to the carcinogenic process. In response to a variety of cellular stimuli, p53 can induce cell cycle arrest, apoptosis or senescence.

A study investigating the difference between cetuximab resistant and their sensitive parental lung cancer cells, identified p53 as the most downregulated and pERK1/2 as the most upregulated cellular signalling protein. Downregulation of p53 was also observed in erlotinib resistant cells. Furthermore, silencing of p53 in cetuximab sensitive cells resulted in reduced sensitivity to the drug, whereas restoring p53 function in resistant cells resulted in enhanced cetuximab sensitivity [149]. In vivo experiments, using a stable cetuximab resistant clone with tetracycline-inducible p53 showed that repair of p53 restored cetuximab sensitivity in tumour xenografts resistant to cetuximab [149]. In addition, cetuximab was able to inhibit cell growth in p53 wild type cells, but not in p53 mutated cells [151]. In general, there is insufficient experimental evidence to unequivocally state that loss of functional p53 can be predictive of resistance to anti-EGFR therapy.

Epithelial-to-Mesenchymal Transition

We and others have proposed epithelial-to-mesenchymal transition (EMT) as a mechanism of resistance towards EGFR targeting therapeutics [161–169]. EMT is characterized by loss of epithelial cell characteristics and acquisition of mesenchymal phenotypic traits, causing tumour cells to detach from neighbouring cells and to migrate into adjacent tissue [170–172]. However, it has been reported that EGFR

inhibition can promote an infiltrative front composed of mesenchymal-like cells, which made up a small subpopulation of the tumour before therapy [173]. Increased expression of IL8 and HB-EGF have been linked with EMT [37, 174–180] and we showed that both genes were upregulated in our cetuximab resistant HNSCC cells and these cells shows traits of EMT, including higher migratory and invasive capacity. Moreover, our microarray profile revealed upregulation of several epithelial markers in cetuximab sensitive HNSCC cells, whereas cetuximab resistant cells were characterized by upregulation of protease urokinase (*PLAU*), transgelin (*TAGLN*), *ADAM19* and thrombospondin (*TSP-1*), all of which have functions associated with features of EMT [181–185]. Similarly, it has been reported that HNSCC cells with a mesenchymal-like morphology and elevated migratory potential were found to be less sensitive to irradiation and cetuximab [186]. Overall, these findings clearly indicate that cetuximab resistant cells show enhanced characteristics of EMT.

Hypoxia and Angiogenesis

Regions within solid tumours often experience mild to severe oxygen deprivation (hypoxia) and it has been well documented that poor oxygenation is a pathophysiological property of the majority of human solid tumours, including HNSCC [187]. Importantly, oxygen deficiency has a major impact on clinical responses to cancer treatment, and it was shown that hypoxic tumour regions often contain viable cells that are intrinsically more resistant to treatment with radiotherapy and/or chemotherapy [188, 189]. Both preclinical and clinical studies support an important link between hypoxia and upregulation of EGFR in cancers that do not display genetic alterations of the receptor [190]. Subsequent EGFR signalling stimulates hypoxia-inducible factor (HIF) signalling and thus augments induction of proteins that promote cellular survival in a hostile microenvironment. As the HIF transcription factors play a pivotal role in the cellular adaptation to hypoxic stress, EGFR-induced HIF signalling thus augments the induction of proteins that promote cellular survival in a hostile microenvironment. As a consequence, the presence of tumour hypoxia may contribute to resistance to EGFR inhibitors [191]. HNSCC patients with high levels of hypoxia-associated factors indeed were more likely to relapse, following induction therapy that included cetuximab [192], suggesting that the role of tumour hypoxia in therapeutic resistance might be particularly relevant for regimens containing EGFR-targeting monoclonal antibodies [192].

Lee et al. reported only minimal distribution of cetuximab to hypoxic tumour regions [193]. As monoclonal antibodies are large molecules, which are consumed by binding to receptors on the cell surface, this might indeed lead to poor penetration within solid tumours. However, cetuximab has a long half-life in the circulation, so that a more uniform distribution in tissues might be established, even if penetration of tissue is relatively slow. Indeed, in contradiction to the observation by Lee et al., Santiago et al. reported that cetuximab was homogeneously distributed within FaDu HNSCC xenografts, with no difference between hypoxic and

non-hypoxic tumour cells [194]. These findings were in line with clinical data on the distribution of anti-EGFR antibodies in HNSCC [195] and indicate that cetuximab accesses not only (oxygenated) cells in proximity to the tumour blood vessels, but indiscriminately reaches all tumour cells.

Only few papers illustrate hypoxia-induced treatment resistance and most studies on EGFR-targeting antibodies supported a markedly increased anti-tumour potency of cetuximab in vivo (over that observed in vitro), suggesting that factors of the tumour microenvironment might influence the in vivo response.

The first reports on this topic addressed the association between the EGFR pathway and tumour angiogenesis. Together with the demonstrated antiproliferative and pro-apoptotic effects, the anti-angiogenic activity of cetuximab is now believed to contribute to its overall anti-tumour activity in vivo. With regard to this anti-angiogenic effect, numerous studies have shown that treatment of human cancer cells in vitro and in vivo with cetuximab reduced the production of VEGF [196–198]. Luwor et al. found that cetuximab reduced the levels of HIF-1α, leading to transcriptional inhibition of VEGF expression [199]. Immunohistochemical analysis of HNSCC tumour xenografts after systemic administration of cetuximab demonstrated inhibition of the in vivo expression of tumour angiogenesis markers, including VEGF and Factor VIII [200].

Apart from the observed anti-angiogenic effects, it has also been speculated that hypoxia enhances the sensitivity to the cytotoxic effect of EGFR-targeted monoclonal antibodies [201]. For example, cetuximab was more cytotoxic against hypoxic than well-oxygenated A431 epidermoid cancer cells grown in vitro and it reduced the overexpression of hypoxia markers (HIF-1α, CA9, VEGF) [198]. Likewise, we observed that both EGFR-inhibitors cetuximab and erlotinib maintained their growth inhibitory effect under hypoxia in vitro in three cetuximab-sensitive HNSCC cell lines [201]. Whether this was a direct interaction between hypoxia- and EGFR-mediated signalling pathways or indirectly via reoxygenation as a consequence of cell loss due to the cytotoxic effect of cetuximab [202] was not elucidated in these studies, but both mechanisms might be involved.

Therefore, several studies have focused on the molecular mechanisms behind the cross-talk between hypoxia and EGFR inhibition and on the role of HIF-1α in this process [191]. Importantly, it was observed that cetuximab could clearly downregulate HIF-1α levels in cancer cell lines that were sensitive to EGFR inhibition and it was shown that inhibition of HIF-1α was required, although it might not be sufficient, to mediate the response of cancer cells to EGFR-targeted monoclonal antibodies [199, 203–205]. In contrast, overexpression of HIF-1α in cancer cells that were originally sensitive to treatment with cetuximab conferred substantial resistance to this anti-EGFR therapy [204]. It was also reported that cetuximab sensitised HNSCC cells to radiation in part through inhibition of the radiation-induced upregulation of HIF-1α [206]. Overall, further in-depth studies are needed to fully understand these observations.

As inhibition of proteasomal degradation did not alter the rate of HIF-1α reduction by cetuximab treatment, it was suggested that cetuximab mainly acts by inhibiting HIF-1α protein synthesis [199, 207]. In hypoxic gastric cancer cells, it was

shown that cetuximab reduced HIF-1α expression via inhibition of both MAPK and PI3K/AKT signalling downstream of EGFR [208]. However, most other studies suggested that the exact mechanism of reducing HIF-1α synthesis by cetuximab involved only inhibition of the PI3K/AKT pathway. The inhibition was shown to be prevented in cancer cells transfected with constitutively active PI3K or constitutively active AKT, but not in cells with a constitutively active MEK [204].

Overall, despite their individual key roles in promoting cancer progression and treatment resistance, our knowledge about the impact of intratumoural hypoxia on the activity of the EGFR signalling pathway in cancer and vice versa remains rather limited. As such, further studies are warranted to define the precise mechanistic and therapeutic implications of the hypoxic response relative to the EGFR signalling pathway in cancer.

Strategies to Overcome Cetuximab Resistance

Despite the reported intrinsic and acquired resistance to EGFR-targeting agents, interest in targeting EGFR for the treatment of HNSCC remains high, with new strategies, such as inhibitor combinations and novel irreversible or multi-targeting inhibitors, currently being evaluated.

Irreversible and Multiple HER Receptor Inhibition to Overcome Resistance

The ongoing challenge of therapy resistance has prompted a new approach to treat cancer patients, notably multiple inhibition of HER receptors simultaneously or irreversible inhibition. As mentioned above, the HER family of receptor tyrosine kinases comprises four members, i.e. EGFR (HER1, ErbB1), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). The particular mode of activation of the HER network involving ligand-induced homo- and hetero-dimerisation of the four HER receptors has prompted a new approach to inhibit this complex network and prevent premature emergence of resistance [15, 209]. The simultaneous inhibition of both partners in a HER dimer, using covalent binders that confer irreversible inhibition, represents one of these new paradigms. In this light, we will discuss two multitargeted compounds, being MEHD7945A (duligotuzumab) and afatinib.

For MEHD7945A (duligotuzumab), a monoclonal antibody with dual EGFR/HER3 specificity, we demonstrated that this compound has only a limited potential to establish a clear concentration-dependent cytotoxic effect in intrinsically and acquired cetuximab resistant HNSCC cell lines [210]. An additive but not synergistic interaction between MEHD7945A and cisplatin was observed. As the cytotoxic effect of MEHD7945A was not dependent on the expression of EGFR and HER3 in

HNSCC cell lines, other mechanisms besides HER3 expression and signalling seem to play a pivotal role in resistance to cetuximab. This finding was supported by clinical data from the MEHGAN study, a randomized phase II study comparing MEHD7945A with cetuximab in platinum-pretreated but cetuximab-naïve HNSCC patients. This study demonstrated no benefit for MEHD7945A over cetuximab in neither all randomized patients, nor in patients whose tumours expressed high levels of HER3 or neuregulin (NRG1), a ligand of HER3. In addition, MEHD7945A also demonstrated disappointing results in a clinical study with *RAS* wild type CRC patients [211]. In this study, MEHD7945A plus FOLFIRI (leucovorin, 5-fluorouracil and irinotecan) did not appear to improve the outcomes of *RAS* wild type CRC patients compared with cetuximab plus FOLFIRI. Similarly, no association was found between progression free survival or objective response rate and HER3 or NRG1 expression. Due to the lack of survival benefit reported in several clinical trials, no additional clinical studies have recently been initiated with MEHD7945A (ClinicalTrials.gov), indicating the need to further investigate the potential of other multiple HER receptor inhibitors, such as for example afatinib.

In contrast to the first-generation EGFR inhibitors, afatinib is an irreversible HER family blocker that inhibits the enzymatic activity of EGFR, HER2 and HER4 [212–215]. As HER3 is kinase-inactive and requires obligate heterodimerization with other HER-family receptors, afatinib also inhibits HER3-mediated signal transduction. The increased inhibition scope of HER receptors by afatinib most likely leads to a more robust blockade of the HER network [216]. Previous preclinical research demonstrated effective cytotoxic activity of afatinib in HNSCC cell lines and xenograft models [217]. Consequently, treatment with afatinib might result in a distinct and more pronounced therapeutic benefit.

In this light, we demonstrated that afatinib was able to establish cytotoxicity in cetuximab sensitive, intrinsically and acquired resistant HNSCC cell lines, independent of the HPV status [218]. Neither cetuximab resistance nor HPV status had a significant impact on the efficacy of afatinib. Nevertheless, we noticed that intrinsically and acquired cetuximab resistant HNSCC cell lines tended to show higher IC_{50} values compared to their isogenic cetuximab sensitive counterparts, thus suggesting the possibility of cross-resistance between cetuximab and afatinib.

In HNSCC patients, the randomized phase II study of afatinib monotherapy versus cetuximab in R/M HNSCC patients reported that afatinib showed antitumor activity comparable to cetuximab with lack of cross-resistance [219]. In contrast, however, subgroup analysis of the phase III LUX-Head and Neck 1 trial with R/M HNSCC patients progressing on or after platinum-based therapy, suggested in 2017 that afatinib is more effective in patients whose tumours are cetuximab naïve [220, 221]. Nevertheless, a phase Ib study in patients with non-small cell lung cancer and HNSCC demonstrated promising results when afatinib was given in combination with standard-dose cetuximab [222].

The above-mentioned subgroup analysis of the LUX-Head and Neck 1 trial also suggested, based on prespecified biomarker assessment, increased benefit in patients whose tumours were, HPV-negative, had EGFR amplification, low HER3 expression and high PTEN expression [221]. However, our preclinical data suggested that

the efficacy of afatinib was not significantly influenced by the HPV status of the cell line. Furthermore, in 2018, Machiels et al. reported that none of these biomarkers were significantly predictive of response for afatinib in a window of opportunity study of the European Organization for Research and Treatment of Cancer (EORTC) study in treatment-naïve HNSCC patients selected for primary curative surgery [223]. Possible explanations for these differences include the low number of patients resulting in low statistical power and the different clinical settings (curative versus palliative). Although these data were exploratory, Machiels et al. reported that the hypoxic gene signature and TP53 status needed to be further investigated as a predictive biomarker of afatinib activity. Our preclinical data support this finding, as the cytotoxic effect of afatinib was increased under hypoxic conditions in HNSCC cell lines. Consequently, further preclinical and clinical research are required to draw final conclusions upon the possible predictive role of cetuximab sensitivity, HPV status, hypoxia and TP53 status for the treatment with afatinib

Overall, the extended inhibition scope of HER receptors by afatinib leads to a more robust blockade of the HER network than MEHD7945A.Nevertheless, optimisation of combination treatment regimens with afatinib and conventional as well as other targeted therapies is necessary. Furthermore, identifying predictive biomarkers in order to select the patients that benefit most from these particular combination strategies is of crucial importance.

Identification of Drug Resistance Mechanisms and Predictive Biomarkers

In addition to optimising therapy strategies, optimal patient selection for anti-EGFR-based therapy remains a major challenge. As such, efforts at identifying predictive biomarkers to select HNSCC patients most likely to benefit from EGFR-targeted therapy have yet to succeed [15, 224, 225]. Unravelling the molecular pathways underlying resistance to EGFR inhibitors could have important implications, not only regarding patient selection, but also regarding the identification of novel drug targets for the treatment of HNSCC patients. In the paragraphs above, we discussed which mechanisms of cetuximab resistance are already known and which ones deserve further investigation. This enhanced knowledge will guide us to rationally design and test novel combination therapies that overcome resistance to EGFR-targeting agents in cancer treatment.

Conclusion and Future Perspectives

In conclusion, we hypothesize that the anti-tumour effects of cetuximab will be synergistic with agents targeting oncogenic bypass pathways responsible for therapeutic resistance towards cetuximab in HNSCC. Of particular interest and complexity are regimens combining immunotherapy with EGFR-targeted therapy. Indeed, the integration of immunotherapeutic approaches is now considered as a new perspective for the treatment of HNSCC patients. In this regard, the anti-PD-1 immune checkpoint inhibitor pembrolizumab has recently been approved by the American FDA and the European EMA for first-line treatment of R/M HNSCC [226]. As discussed previously, the working mechanism of cetuximab has largely been attributed to the direct effects of EGFR inhibition, but cetuximab also demonstrates additional immune-based mechanisms of activity through stimulation of antibody-dependent cellular cytotoxicity and enhancement of cytotoxic T-lymphocyte cross priming by dendritic cells [227–229]. As such, the immune system of the patient is involved in the anti-tumour effect of cetuximab and combinations with immunotherapeutic approaches also look highly promising for the treatment of HNSCC. We are hopeful that, with these novel combination strategies, cetuximab resistance can be prevented and a more pronounced therapeutic benefit can be achieved, ultimately resulting in improved survival and quality of life for HNSCC patients.

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Chapter 4
The Role of Liquid Biopsies for Monitoring Disease Evolution

Ingeborg Tinhofer

Introduction

Tissue biopsies have been used by clinicians to diagnose and manage disease for more than 1000 years [1]. The first report on the use of needles for puncturing a thyroid gland cancer came from a court physician to the Andalusian caliph Al-Hakim II [1]. From then until the modern era of precision oncology, tissue biopsies have remained the most widely used tool not only for cancer detection and staging but also for molecular tumor profiling to guide targeted therapy for the individual patient. However, tumor biopsies generally involve invasive medical procedures that can be difficult and risky, especially in cancer patients with advanced disease. Moreover, even if a fresh tumor biopsy can be safely taken, the material for the molecular analysis might be limited as a relevant amount of the tissue is reserved for routine pathology. Due to the restrictions of solid tissue sampling, it is often necessary to resort for molecular profiling to archival tumor samples that were collected long time prior to the planned molecular analysis, typically at the time of initial biopsy or surgical resection. Changes in the mutational pattern and/or subclonal spectrum of tumors occurring during disease progression can decrease the diagnostic accuracy of the molecular test in this situation. The invasive nature of solid tissue collection

I. Tinhofer (✉)
Department of Radiooncology and Radiotherapy, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK) Partner Site Berlin, Berlin, Germany

German Cancer Research Center (DKFZ), Heidelberg, Germany

German Cancer Consortium (DKTK), Berlin, Germany
e-mail: ingeborg.tinhofer@charite.de

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Table 4.1 Cell-free tumor DNA versus solid tissue biopsies: pros and cons

Consideration		Cell-free tumor DNA		Solid tissue
Sampling	+ +	Non or minimally invasive + Serial monitoring easy	– –	Invasive, more challenging to obtain Serial testing more difficult
Biology	–	No direct correlation with tumor histology or cellular phenotype possible	+	Can correlate with histology and cellular phenotype
	+	Allows global view on intratumoral heterogeneity	–	Represents one small tumor region
Pre-analytical	+	Easy to standardize across centers	+	Uses existing, validated tissue processing and handling approaches
	–	Need for specific blood stabilization tubes		
	–	Confounding patient-related factors poorly characterized (clonal hematopoiesis)		
Clinical utility	–	Limited evidence for treatment selection and screening	+	Substantial evidence for treatment selection in multiple entities for early and advanced cancers

makes it also very difficult to accomplish serial sampling under treatment, which would be required for the analysis of clonal evolution and acquired drug resistance.

The above-mentioned hurdles can be overcome by liquid biopsies which can harvest cancer-related biomarkers from blood, saliva or urine. Advantages and disadvantages of liquid and solid biopsies are summarized in Table 4.1. Liquid biopsies represent a non- or minimally invasive, inexpensive source for tumor material, including circulating tumor cells (CTCs) and tumor-derived cellular components like extracellular vesicles, miRNA, protein, and cell-free (cf) circulating tumor (ct) DNA. Liquid biopsies allow 'real time' assessment of the tumor status, thereby providing a global view on spatial and temporal intratumoral heterogeneity both in primary and metastatic disease. Liquid biopsies have successfully been used for selection of molecular treatment [2] as well as in-depth analysis of the molecular changes associated with acquired drug resistance [3]. While serial tissue biopsies for monitoring depth and duration of treatment responses are rarely possible, liquid biopsies can be applied for this purpose, allowing detection of tumor progression up to several months before clinical relapse [4]. High sensitivity at stages of very low tumor burden would also give the chance to use liquid biopsies for early cancer screening. In fact, laboratories around the world are currently competing by developing cancer screening tests based on a simple blood sample.

In this article, I will summarize current evidence of the diagnostic value of liquid biopsies for disease monitoring in HNSCC, with special emphasis on the potential clinical value in cancer screening, post-treatment surveillance, molecular profiling for molecularly guided treatment selection, monitoring of treatment efficacy and the analysis of acquired drug resistance.

Liquid Biopsies for Cancer Screening and Disease Monitoring

The concept of using a blood test for cancer screening is not new. Blood tests for prostate specific antigen (PSA) or cancer antigen 125 (CA-125) have been broadly used for early detection of prostate or ovarian cancer. However, these previous methods can be extremely nonspecific and result in high rates of false positive tests. A blood test for large-scale population screening would require high specificity, clinically useful sensitivity, and highly accurate identification of the tissue of origin, in order to limit costs and the complexity of evaluating asymptomatic patients. A fundamental question for recent DNA-based approaches is whether small tumors would release sufficient amounts of tumor DNA into the circulation to allow sensitive detection of the cancer-associated changes.

Early Detection of Virally Associated HNSCC Based on Plasma DNA

First evidence for a potential application of analysis of circulating tumor-related DNA for screening for HNSCC came from Epstein-Barr virus (EBV)-associated undifferentiated nasopharyngeal cancer. Earlier observations of short EBV DNA fragments in blood samples from nasopharyngeal carcinoma patients [5], which were released by carcinoma cells and not associated with viral particles [6], had suggested that plasma EBV DNA might represent a useful biomarker for identifying early-stage nasopharyngeal carcinoma among asymptomatic individuals. This hypothesis was successfully tested in a large Asian screening study enrolling more than 20,000 participants [7]. The investigators could confirm that detection of viral DNA in plasma by real-time polymerase-chain reaction (PCR) can identify individuals with early-stage disease with sensitivity and negative predictive values of 97% and 99.995%, respectively. Circulating viral DNA in human papilloma virus (HPV)-driven oropharyngeal carcinoma has emerged as further promising biomarker for screening and disease monitoring in HNSCC because approximately 90% of patients have detectable plasma HPV DNA at the time of diagnosis [8, 9]. It was also shown that kinetics analysis of HPV DNA can be used to predict the likelihood of disease control after definitive chemoradiation [10]. In the latter study, having high baseline copy number (>200 copies/mL) and >95% clearance of HPV DNA by day 28 of chemoradiation was established as a favorable clearance profile associated with improved outcome [10]. Future clinical trials are certainly necessary to explore whether earlier detection of cancer relapse also improves post-recurrence survival outcomes. If so, then integration of HPV DNA-based monitoring might support the current worldwide efforts of developing de-escalated treatment strategies for HPV-positive oropharyngeal carcinoma patients.

Cell-free DNA Analysis in Non-virally Related HNSCC

In non-virally related HNSCC, most attempts of developing a non-invasive test for screening and disease monitoring have failed so far. The large interpatient heterogeneity observed in genomic profiles from these tumors and the absence of recurrent hotspot driver alterations have hampered the development of a broadly applicable screening tool based on a single biomarker. Point mutations in the tumor suppressor gene *TP53* represent the most frequent genetic alteration in HNSCC [11], suggesting that *TP53* mutant variants might represent a promising biomarker for screening and disease monitoring in HPV-negative carcinomas. In line with this assumption, previous molecular studies have identified the presence of mutant *TP53* variants in histologically clear surgical margins as potential marker of residual disease identifying patients at high risk of tumor recurrence [12, 13]. Detection of tumor specific *TP53* mutations in plasma cfDNA from HNSCC patients using digital droplet PCR was shown to be technically feasible, providing further support for the use of *TP53* alterations as diagnostic biomarker in post-treatment surveillance of HNSCC patients [14]. However, the use of cfDNA-based approaches interrogating single-nucleotide variants that focus on key gene alterations such as *TP53* might be less useful for blood-based cancer screening, as this approach may be hampered by confounding signals from clonal hematopoiesis associated with blood-specific mutations in cancer-associated genes like *TP53* [15]. Similarly, approaches based on detecting copy number alterations e.g. in genes at the chromosome 11q13 locus displaying amplifications in approximately one third of HPV-negative HNSCC patients [11] may be limited by smaller relative differences between cases and controls, resulting in a need for increased sequencing depth as well as technical variation restricting the signal-to-noise ratio [16].

DNA Methylation Analysis for Cancer Screening

Recently, the Circulating Cell-free Genome Atlas (CCGA) consortium [17] has launched a large prospective, observational, longitudinal, case-control study for discovery, training, and validation of a multi-cancer screening test. Based on bisulfite sequencing of plasma cfDNA and using machine learning algorithms, a classifier was developed and validated for cancer detection and tissue of origin localization [17]. Recently, very promising results were reported from a pre-specified CCGA sub-study including 6689 participants with previously untreated cancer (n = 2482) or without cancer (n = 4207) [18]. cfDNA sequencing of informative methylation patterns detected a broad range of cancer types at metastatic and non-metastatic stages with specificity and sensitivity performance approaching the goal for population-level screening [18]. Although good sensitivity (i.e. >85% over all stages) at a fixed test specificity of >99.8% was observed in the subset of HNSCC cases, results have to be interpreted cautiously due to low HNSCC patient numbers

both in the training (n = 65) and validation set (n = 18) of this study [18]. Interestingly, the investigators found that incorrect tissue of origin identification by the methylation classifier often occurred among HPV-driven cancers (e.g. cervix, anus, head and neck cancers), suggesting that test accuracy might be further improved by leveraging this information.

Cell-Free Circulating Tumor DNA for Mutation Profiling

A mutational load ranking in the upper third of all tumor entities [19] and large interpatient genetic heterogeneity [11] are key features of HNSCC. Signs of high genetic instability are primarily detected in cases with a history of heavy smoking and alcohol consumption, most likely resulting from the extensive DNA damage that has been caused by tobacco carcinogen exposure for years. Exacerbating the complexity of the genetic landscape in HNSCC, intratumoral heterogeneity in terms of spatial and temporal differences in the mutational patterns of key driver genes can occur [20–22]. First evidence of ctDNA being a suitable source for studying the mutational landscape of tumors was provided by the landmark study of Bettegowda and colleagues in which 640 patients with various cancers were included [23]. The investigators were able to demonstrate that mutant DNA fragments can be found at relatively high concentrations in the blood circulation of most patients with metastatic cancer and at lower but detectable concentrations in a substantial fraction of patients with localized disease [23]. In the small subgroup of HNSCC patients (n = 12) included in this study, mutant ctDNA was detected in 70% of cases [23]. One of the largest subsequent studies so far including 25,578 blood specimens from 21,807 patients with over 50 different cancer types confirmed that mutations in genes associated with cancer can be identified in circulating plasma DNA in the vast majority of patients with advanced cancer [24]. Schwaederle et al. examined the frequency of genetic mutations of ctDNA in 670 cancer patients, of whom 25 had HNSCC, and reported that HNSCC harbors the highest frequency of ctDNA mutations in plasma when compared to lung, gastrointestinal, brain, and breast cancers [25].

The preliminary results from the small HNSCC cohorts included in these histology-agnostic studies were corroborated by a study specifically focusing on HNSCC patients (n = 93) in whom mutations (mainly affecting *TP53* in HPV-negative and *PIK3CA* in HPV-positive cases) were detected in 81% and 85% of plasma and saliva samples, respectively [26]. Recently, Galot and coworkers specifically explored the relevance of plasma ctDNA to characterize the mutational landscape in recurrent/metastatic HNSCC [27]. Using a panel of 604 cancer-related genes they reported mutant variant detection in 20/39 patients (51%). In line with the above mentioned studies across different histologies, a significantly higher probability for ctDNA detection was observed in patients with metastatic disease compared to patients with only locoregional recurrence (70% vs. 30%) [27]. This finding suggests a potential limitation of panel NGS-based ctDNA analysis in R/M

HNSCC given that around one third of recurrent HNSCC patients will have locoregional relapse without distant metastases. However, the lower detection rate of mutant variants in locoregional recurrence in the study of Galot et al. could also have technical rather than biological reasons. Indeed, by applying the more sensitive digital droplet PCR the detection rates could be significantly increased [27].

Concordance Between Liquid and Solid Tissue-Based Mutational Analysis

In the large observational study of Zill et al. the commercially available Guardant360 assay (GuardantHealth Inc., Redwood City, CA) covering approximately 70 actionable tumor mutations was used [24]. It was shown that ctDNA mutation patterns were highly consistent with the distribution reported for tumor tissue in the publicly available The Cancer Genome Atlas (TCGA), with correlations ranging from 0.90 to 0.99 [24]. Comparative analysis using matched archival tissue in a subset of 386 patients confirmed the overall high concordance in sequencing results of liquid and solid tumor biopsies [24]. Of note, test accuracy of ctDNA sequencing increased to 98% when blood and tumor tissue were collected less than 6 months apart. In contrast to these promising results, a remarkably poor overall concordance between molecular profiles established from liquid and solid tumor biopsies was reported by Galot and colleagues in R/M HNSCC [27]. Considering the 18 patients from whom blood and tissue samples were available, only 19% of the mutant variants (40/209) identified in solid tumors were also detected in plasma. A similar observation was made in a small study of HNSCC cases (n = 36) harboring mutations in either *TP53*, *NOTCH1*, *CDKN2A*, *CASP8* or *PTEN* in tumor tissue, of which only 28% could be detected in plasma cfDNA [28].

Currently used NGS panels for ctDNA analysis range from small panels of 20 genes to large comprehensive panels of up to several hundred genes. It is very likely that the above-described differences in variant detection between tumor tissue sequencing and cfDNA sequencing depend on the used NGS technology and platform. Most targeted NGS panels originally developed for tissue sequencing have an average sequencing depth of 500×. This coverage has shown to be sufficient to give consistent results in the detection of single nucleotide variants (SNVs) and small insertion/deletions (indels) in tumor tissues [29]. Given the low allele fractions of mutant variants (median: 0.41%) in plasma samples in the majority of cancer patients [24], a higher sequencing depth will be required for sensitive mutation detection in plasma cfDNA. Since coverage is usually inversely proportional to the number of genes to be sequenced, an increase in sensitivity of ctDNA-based mutational profiling might thus be realized by using small sets of genes harboring known actionable alterations rather than comprehensive panels of several hundred genes. Indeed, ultra-high sequencing depths (i.e. 50,000–100,000× coverage) combined with a molecular barcoding strategy and *in silico* elimination of highly stereotypical

background artifacts were shown to significantly improve recovery of ctDNA molecules, allowing detection of mutant variants down to allele frequencies of 0.004% [30–32]. This high sensitivity however comes at the price of significantly higher costs per single analysis, raising concerns about affordability in clinical routine, especially if serial liquid biopsy analyses might become part of the routine follow-up scheme for cancer patients.

ctDNA Versus CTCs: Which Is the Better Source for Mutation Analysis?

To our knowledge, a comparative analysis of whether ctDNA or CTCs might represent the better source for genomic molecular profiling of HNSCC tumors, at situations when tumor tissue collection is not feasible, is missing until now. First evidence from lung cancer suggested superiority of CTC-over plasma ctDNA-based analysis, since EGFR activating mutation were detected in CTCs from 11 of 12 patients (92%) but only in matched plasma ctDNA from 4 of 12 patients (33%) (P = 0.009) [33]. In contrast, mutation detection at comparable frequencies was reported for CTC-derived genomic material and paired plasma ctDNA from studies in breast [34] and colon cancer [35]. In a relevant number of cases though, CTCs exhibited a mutation that was not detected in ctDNA, and vice versa [35]. Mutation detection in CTCs and plasma-ctDNA might thus provide complementary information suggesting the use of an integrated liquid biopsy approach [34, 35].

Liquid Biopsies for Treatment Selection and the Analysis of Resistance Mechanisms

Perhaps most importantly, evidence is accumulating that liquid biopsies can be used to predict drug response and drug resistance in patients initiating a targeted therapy, pointing to their potential in precision medicine. Of clinical relevance, taking into account FDA-approved agents and eligibility for clinical trials, the ctDNA assay used in the study of Schwaederle et al. identified a possible treatment option for approximately one half of all patients [25]. Furthermore, nearly 1 in 4 ctDNA alteration-positive patients (23%) across 6 cancer indications in the study of Zill et al. [24] had one or more alterations previously suggested to confer resistance to an FDA-approved on-label therapy, which would also inform clinical decision-making.

Studies in HNSCC specifically evaluating the value of ctDNA for personalized treatment selection are lacking so far. The largest genetic landscape analysis of ctDNA was performed within the framework of the randomized multicenter phase II trial BERIL-1 in which the efficacy of buparlisib (BKM120), an oral pan-PI3K

inhibitor plus paclitaxel or placebo plus paclitaxel was evaluated in patients with R/M HNSCC progressing on/after one previous platinum-based chemotherapy regimen for R/M disease [36]. In the accompanying biomarker study, ctDNA mutation profiles could be established in 112/158 patients (71%) using targeted NGS [37]. The percentage of actionable alterations detected in liquid biopsies and the overall concordance with tumor tissue were not directly reported by the investigators. However, as derivable from the presented overview of the most frequent gene alterations in ctDNA at screening [37], alterations in genes of the PI3K/AKT/mTOR pathway (*PIK3CA, PIK3CG, PIK3C2G, PIK3R1, PIK3R4, PIK3R5, AKT3, PTEN, RICTOR, RPTOR, TSC1, TSC2, MTOR*) were found in 29/112 patients (26%). This suggest that ctDNA mutation profiling could indeed be used to select patients with gene alterations druggable by inhibitors in clinical development for HNSCC. Per BERIL-1 protocol, the PI3K activation status was defined as the presence of a *PIK3CA* mutation and/or a loss of PTEN expression [37]. Statistical analyses did not suggest a difference in OS between the buparlisib and placebo arms in the PI3K-activated subgroup, however, the low number of patients in this subgroup (n = 18) weakened the statistical power to evaluate a possible relationship between genotype and clinical outcome [37].

Braig et al. were the first group to study processes of clonal tumor evolution occurring in HNSCC tumors under pressure of molecular therapy. Patients receiving cetuximab/platinum/5-fluorouracil treatment for R/M HNSCC were included in this prospective biomarker study. Targeted NGS was used for detection of mutations in four genes (*EGFR*, *KRAS*, *NRAS* and *HRAS)* in diagnostic tumor tissue as well as blood samples taken under and after completion of combination therapy/maintenance [38]. Mutations in the four genes were not detected in tumor tissue of cetuximab-naïve patients, except for *HRAS* mutations in 4.3% of patients. Interestingly, 46% of patients with on-treatment disease progression showed acquired *RAS* mutations in ctDNA, while no *RAS* mutations were found in the non-progressive subset of patients, indicating that acquisition of *RAS* mutant clones correlated significantly with clinical resistance [38]. Of note, the emergence of mutations preceded clinical progression in half of the patients, with a maximum time from mutation detection to clinical progression of 16 weeks [38]. These findings corroborate previous results from colon cancer where *KRAS* mutations were identified as frequent drivers of acquired resistance to cetuximab, and could be detected in blood of cetuximab-treated patients as early as 10 months before radiographic progression [39].

Circulating Tumor Cells for Prognosis of Outcome in HNSCC

Evidence of a potential role of CTCs in disease progression of HNSCC has been provided by numerous independent studies over the last 30 years. A review of these studies would be beyond the scope of this article. I would therefore like to refer the

reader to two recent reviews on this topic [40, 41]. In our own study in locally advanced HNSCC patients treated with surgery and adjuvant chemoradiation [42], a significant negative association between the persistence of CTCs after surgery and outcome was observed. The use of different cut-offs for definition of CTC-positive blood samples and the inclusion of heterogeneously treated patient cohorts in the majority of previous studies at least call for caution with regard to a definite conclusion on the prognostic value of CTCs.

The availability of robust, easy-to-handle CTC detection devices such as the CellSearch® platform has opened the door for the integration of CTC analysis into clinical routine. However, the mere enumeration of CTCs has proven insufficiently informative to prompt widespread clinical adoption. There is accumulating evidence that more extended phenotyping of CTCs might be necessary for improving their diagnostic value. Identification of the true metastasis-inducing subclones within the bulk CTC population remains a challenge but is imperative in order to improve the diagnostic potential of CTCs. Genome-wide single-cell RNA-seq and DNA-seq performed in CTCs have already provided crucial new insights into CTC heterogeneity and mechanisms of therapeutic resistance in other cancer types [43, 44], but such analyses still have to be done in HNSCC. These analyses combined with multiparametric CTC phenotyping by imaging flow cytometry or automated immunofluorescence microscopy setups [41] will certainly increase our understanding on the relevant biological mechanisms endowing CTCs with the potential to emigrate from the primary site to blood circulation, to survive their journey and to re-seed at distant organs, thereby supporting the development of CTCs as liquid biomarker in HNSCC.

Conclusions

Liquid biopsies have been successfully used to guide treatment decisions in patients with lung cancer harboring EGFR and ALK mutations. This review summarized the current evidence from the literature pointing to a clinical potential in HNSCC as well (Table 4.2). Prospective randomized clinical studies are needed to firmly establish the usefulness of liquid biopsy for detecting molecular markers in clinical practice, by demonstrating that treatment decisions based on liquid biopsies result in better outcome. In addition, the persistence of CTCs after surgery should be studied further to determine whether they indicate the need for adjuvant therapy regardless of the tumor size or nodal status. Currently, one of the most promising use of liquid biopsies is in the detection of cancer progression and development of drug resistance. Liquid biopsies may help in elucidating the molecular resistance mechanisms in cetuximab-containing regimens. However, prospective evidence on the usefulness of liquid biopsies in the assessment of drug responses to achieve the best benefit for HNSCC patients is still needed.

Table 4.2 Promising clinical applications for liquid biopsies in HNSCC: Evidence from the literature

Aim	Biomarker	Technique	Tumor localisation	References
Early detection	cfDNA methylation pattern	Targeted bisulfite sequencing	All sites	[17, 18]
	EBV DNA	Quantitative real-time PCR	NPC	[7]
	HPV DNA	Droplet PCR	HPV+ OPC	[8]
Posttreatment surveillance	HPV DNA	Droplet PCR	HPV+ OPC	[9, 10]
	TP53 mutations	Targeted NGS	HPV– HNSCC	[14]
	CTCs	Imaging flow cytometry, qPCR	All sites	[40–42]
Molecular treatment selection	Actionable alterations	Targeted NGS	All sites	[24, 27, 36]
Response monitoring	*HRAS, KRAS, NRAS* mutations	Targeted NGS	R/M HNSCC	[38]

cfDNA cell-free deoxynucleic acid, *CTCs* circulating tumor cells, *EBV* Epstein–Barr virus, *HNSCC* head neck squamous cell carcinoma, *HPV* human papillomavirus, *NGS* next-generation sequencing, *NPC* nasopharyngeal carcinomas, *PCR* polymerase chain reaction, *R/M* recurrent/metastatis

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Chapter 5
NK Cells in Immunotherapy: How Important Are They?

Denaro Nerina and Marco Carlo Merlano

Introduction

Treatment of head and neck squamous cell carcinomas (SCCHN) rapidly evolved during the last decade, mainly due to the inclusion of immune-checkpoints inhibitors (ICIs) in the routine therapy of relapsed metastatic disease (R/M-SCCHN). However, head and neck cancers remain a major clinical problem and most R/M--SCCHN patients ultimately die of their disease. Nonetheless, the experience matured with ICIs demonstrating that the immune system and their components play a crucial role in the control of R/M-SCCHN.

Natural killer (NK) cells are key-player in cancer immunosurveillance, cancer control and prevention of metastatization. Indeed, in the 1980s, several studies reported a higher incidence of cancers in individuals with defective NK cell function supporting the role of NK cells in immunosurveillance [1, 2].

In human solid tumors, NK cell infiltration is poor in non-small cell lung cancer, colorectal cancer and melanoma, but it is high in breast cancer, kidney cancer and SCCHN. The latter show the highest infiltration of NK cells [3]. The density of infiltrating NK cells correlates with the patient's prognosis in many solid tumors, including oropharyngeal squamous cell carcinoma (OSCC) [4]. Indeed, Wagner et al. showed a relationship between the prognosis of OSCC patients and NK levels, regardless of HPV status, although higher numbers of CD56 positive (CD56+) cells were found in HPV-positive patients compared to HPV-negative patients. The elevated abundance and activity of cytotoxic NK cells in OSCC patients with HPV driven carcinogenesis might contribute to the favorable outcome in HPV-related OSCC [5].

D. Nerina
Oncology Department, AO Santa Croce e Carle Cuneo, Cuneo, Italy

M. C. Merlano (✉)
Candiolo Cancer Institute, FPO-IRCCS Candiolo, Turin, Italy

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There is evidence that NK cells are involved in the metastatic spreading: the number of circulating NK cells inversely correlate with circulating tumor cells, and the decline of cytotoxicity and of cytokine production of NK cells after major surgery correlates with the risk of metastases [6].

Indeed, the epithelial-mesenchymal transition (EMT) that is a central step during metastatization, leads to expression of new antigens that reactivate the cytolytic effect of NK cells [7].

Chockley et al. showed that EMT leads to NK cell mediated metastasis specific immuno-surveillance. Indeed, EMT modulates the adhesion molecule CADM1 on the surface of tumor cells, increasing the susceptibility to NK cytotoxicity in lung and breast cancer [8].

NK Cells, Antitumor Effects and Antibody Dependent Cell Cytotoxicity

In general, NK cells are extraordinary effective war machines able to kill stressed (infected) or mutated (tumoral) cells through multiple mechanisms (Fig. 5.1). NK cells are divided into two major subsets according to their cell surface expression levels of CD56 and CD16. $CD56_{dim}/CD16_{bright}$ NK cells predominantly mediate natural cytotoxicity, whereas the $CD56_{bright}/CD16_{dim}$ subset plays a role in immune regulation through a high cytokine secretion potential [9].

A series of activating and inhibitory receptors on the membrane of NK cells may sense inducible stress molecules and self-proteins. The prevalence of one signal over the other, results in aggression or tolerance. NK cells also bear ligands for death signal receptors expressed on the membrane of the target cells, such as FAS or TRAIL.

Finally, they secrete a high number of cytokines with antitumor activity such as IFN-ɣ.

In addition, NK cells are also the most powerful inducer of antibody dependent cell cytotoxicity (ADCC).

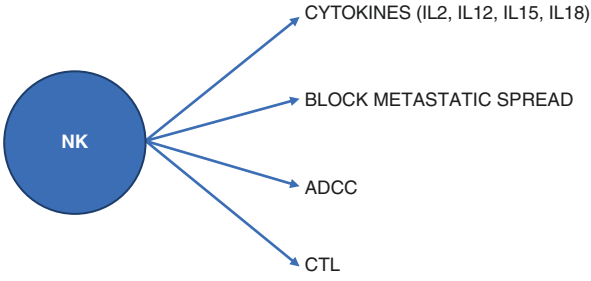

Fig. 5.1 NK functions

Indeed, FC-gamma receptors (FCɣR), the receptor family linked to ADCC, include both activating and inhibiting receptors, and are expressed on a number of different immune cell lineages. NK cells host only FCɣRIII and FCɣRIIc (CD16 and CD 32c), both activating receptors, which make NK cells the most important lineage able to trigger ADCC.

ADCC is a complex but highly efficient mechanism leading to the elimination of damaged, infected or mutated cells. It involves five main actors: (1) the effector cell, (2) the Fcγ Receptor (FcγR), (3) the antibody, (4) the target antigen on the surface of the target cell and (5) the target cell itself [10]. Figure 5.2 reports the five ADCC players.

As reported above, when we speak about ADCC we consider primarily NK cells.

1. The effector cells.

   Cancer cells and many immune cells can damp NK cells, such as tumor associated fibroblast (TAFs), tumor associated macrophages (TAM), T regulatory cells (Tregs) and myeloid derived suppressor cells (MDSC).

   They all reduce NK function following NKp44, NKp30 and DNAM1 down-regulation, reduce NK degranulation and IFN-γ production, and inhibit NKG2D expression [11].

   Among the many mechanisms that tumor cells use to impair NK cells, the release of inhibitory soluble ligands such as MIC-A and MIC-B into the tumor microenvironment (TME), the high levels of TGF-β and of other immune-suppressive cytokines are among the best known [12]. Overall, inhibition of NK cells follows the same mechanisms of CD8+ T cell inhibition.

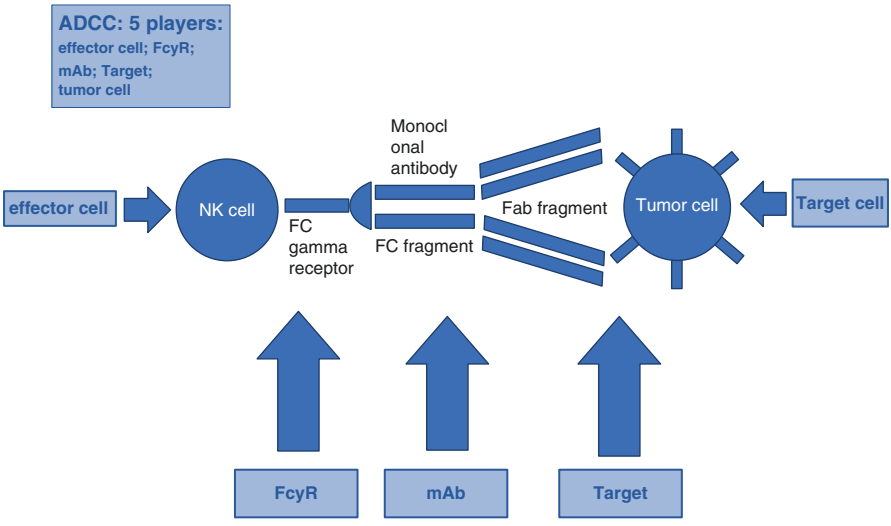

Fig. 5.2 Main actors of ADCC

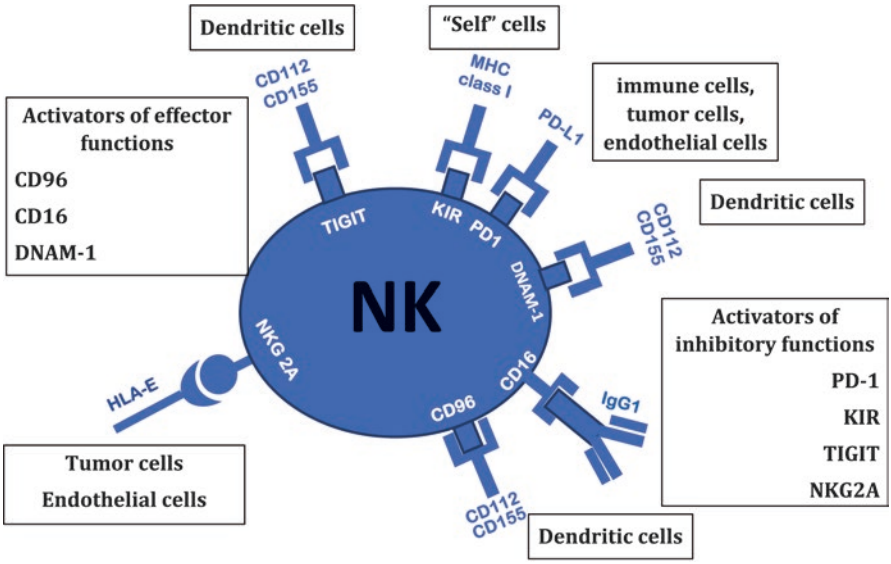

Fig. 5.3 Major mechanisms of NK cells stimulation/inhibition

Figure 5.3 summarizes the most important interactions between tumor cells and NK cells and other inhibitory mechanisms.

However, a residual activity of NK cells still exists in the TME, since high NK cell infiltration usually correlates with favourable prognosis in many tumors [4]. In particular, Taylor et al. [13] observed that inducible ADCC was the most predictive marker for clinical outcome in SCCHN. In support of this observation, Lattanzio et al. [14] observed in a series of SCCHN patients treated with cetuximab and radiotherapy, that patients with ADCC activity above the median had a statistic significant benefit in overall survival. Similarly, patients with metastatic, wild-type, colon cancer treated with cetuximab and with ADCC activity above the median had a significant gain in overall survival [15].

2. The FcγR.

The Fc receptor for IgG (FcγR) belongs to the immune globulin superfamily and includes many families (FcγRIa [CD64a], FcγRIIa [CD32a], FcγRIIb [CD32b], FcγRIIc [CD32c], FcγRIIIa [CD16a], FcγRIIIb [CD16b]) which are expressed by many immune cell lineages and link to the Fc fragment of the antibody with different affinity. FcγRIIb is the only inhibitory receptor, links to any IgG subclass with high affinity and is represented in all the immune cells harbouring FcγRs, but not in NK cells.

Therefore, NK cells are the sole immune cells expressing only activator receptors (FcγRIIIa and FcγRIIc) [16]. However, FcγRIIc is expressed only in about 40% of healthy human subjects [17], and is not yet completely understood [18]. Therefore, most attention is devoted to FcγRIIIa and in particular to its

polymorphisms. Indeed, experimental models support the positive role of the Valine homozygosis at FcγRIIIa-158 toward an increased ADCC activity [19], although its real impact in clinic is not yet clear [20, 21]. Moreover, Rooney et al. suggested that elevated ADCC activity observed in cancer patients may exceed the value of FcγRIIIa polymorphism as prognosticator [13].

3. The antibody.

   IgG includes four classes: 1, 2, 3 and 4. NK-cell dependent ADCC is a Fc segment mediated effector function triggered mainly by IgG1 and IgG3. However, whilst IgG1 seems to be independent from subclasses, IgG3 function is largely allotype-dependent [22]. IgG2 and IgG4 weakly link to FcγRIIIa, but they do not induce ADCC [22, 23]. In conclusion, IgG1 is the most effective IgG class able to trigger ADCC, regardless of allotype.

4. The target antigen (TA).

   The TA density on the target cell surface is the fourth actor of ADCC. There is in vitro evidence that the expression of the TA on tumor cell surface is a key factor influencing cytotoxicity [24, 25]. However, the importance of the expression of the TA is not evident in the clinic. For instance, cetuximab has shown clinical activity regardless of EGFR expression. There are many factors that may explain this discrepancy. First, the target effect of cetuximab may mask the immunological effect of the antibody. Second, ADCC depends on the efficiency of additional immunologic variables, such as those we are discussing here.

   However, some clinical data supporting the role of the TA density in clinic do exist [26]. Our group observed that in patients treated with cetuximab and radiotherapy, ADCC activity directly correlates with the outcome, but patients with high ADCC and high EGR density (EGFR+++) showed the best outcome [14].

5. The target cells.

   The mutational status of the target cell may affect ADCC. For instance, mutation of KRAS leads to constitutive activation of the PI3K/AKT pathway, resulting in direct inhibition of BAD and caspase 9, inhibition of p53 via MDM2 and upregulation of antiapoptotic proteins such as BCL-X, BCL-2 and COX-2. All together these effects confer resistance to apoptosis induced by granzyme B [27, 28].

   Therefore, even if all the described actors of ADCC are efficient, the mutational status of the target cell may prevent ADCC induced apoptosis.

Strategies to Enhance Antitumor NK Cell Function

Since activation or inhibition of immune functions depends on the balance between positive and negative regulators of signaling, the activation, or re-activation, of antitumor NK activity depends upon the upregulation of the former and/or the downregulation of the latter.

A third factor to enhance NK cell activity, is improving their homing into tumor nests, because they are often detained within the stroma surrounding cancer cells.

Reinforcement of NK Cell Activity

Preactivation with cytokines (IL12, IL15, IL18) induces memory-like (cytokine-induced memory-like, CIML) NK cells that show enhanced effector functions lasting for weeks [29].

It is generally accepted that IL-12, IL-15, and IL-18 preactivation induces in NK cells a rapid and prolonged expression of CD25, resulting in a functional up-regulation of high-affinity IL-2 receptor (IL-2Rαβγ) that confers responsiveness to picomolar concentrations of IL-2, favouring NK cells expansion [30].

Cytokine preactivation also induces expression of markers such as the chemotactic receptor CXCR4 necessary for homing of NK cells [31].

Terrèn et al. demonstrated that IL-15 might contribute more than IL12/18 to CIML NK cell-mediated cytotoxicity against target cells, although all the three cytokines are needed to improve activity of NK [32].

Moreover, JAK inhibitors (JAK/STAT pathway, responsible for cytokine regulations) are able to modify NK cell biology in vitro and in vivo.

Schönberg et al. reported that the JAK 1–2 inhibitor ruxolitinib, impairs IL-2 preactivated NK killing ability. Reduced NK cell numbers in ruxolitinib-exposed patients may depend on the inhibition of various important cytokine signals essential for NK maturation (i.e. IL-2 and IL-15) [33].

Ewen et al. found that the activation of NK cells with IL-12/15/18 led to a decreased expression of the inhibitory receptors of the KIR family reinforcing NK effector potential [34].

Moreover IL15-stimulates DCs to activate NK cells in an IL15 dependent manner, indeed, IL-15 DCs, but not IL-4 DCs, promoted NK cell tumoricidal activity towards both NK-sensitive and NK-resistant targets. This effect was found to be mediated by DC surface-bound IL-15 [35].

IL-2 is a well-known growth factor of antigen activated T lymphocytes. IL-2 also stimulates NK cell expansion and activation. However, it also favours Treg expansion through the high affinity sub-unit receptor IL-2Rα (CD25) expressed on these cells. IL-2 variants able to prevent Treg expansion have been generated. Among them, the IL-2 "superkine" with increased affinity to the IL-2Rβ subunit expressed on NK cells and other T effector cells [36].

IL-15 may have stimulating effects similar to IL-2 on NK cells, and also enhances ADCC, without stimulation of Treg expansion [37]. An IL-15 super-agonist, with a long half-life, has been already tested in humans and numerous clinical trials are in progress [38].

There are few preclinical studies investigating cytokine therapy for NK reactivation. However, some data on IL-6 are available. In pancreatic cancer blocking IL 6 not only inhibits tumor growth but also rescue the NK cells from suppression induced by the peripancreatic adipose tissue [39].

Prevent NK Cell Inhibition

Removing the block of NK cells is an emerging, rapidly evolving area and upregulation of some checkpoint molecules (e.g. TIGIT, CD96, PD1, KIRs, NKG2a, IL1R8) represent potential targets for NK-based immunotherapy.

Anti-PD-1

PD-1 is upregulated in several solid tumors, including head and neck cancer [40] and is associated with the inhibition of NK cell activity [41]. It has been demonstrated that PD-1 expression impairs function of intratumoral NK cells. Notably, treatment with PD-1 blockade was able to reverse PD-L1-mediated inhibition of PD-1+ NK cells [42]. Inhibition of PD-1 on NK cells may be important in particular in tumors that poorly express or do not express MHC-I, thereby evading CD-8 T cell attack, but, for the same reason, are a good target for NK cells.

Anti NKG2A

HLA-E is a non-classical MHC-I molecule, frequently up-regulated in SCCHN and is associated with low survival rates. Although MHC-Ia molecules help in cancer cell recognition through the T-cell receptor, HLA-E can be recognized by the inhibitory heterodimeric CD94/NKG2A receptor [43]. This interaction inhibits NK-cell's cytotoxic functions and prevents autoimmunity, but is also exploited by cytomegalovirus to evade antiviral immunity.

Interactions of HLA-E with CD94/NKG2A significantly impairs IL2 receptor–dependent proliferation of tumor-specific T cells that contributed to reduced cytotoxicity and cytokine production, which improved following antibody-mediated blockade treatment in vitro and ex vivo [44].

Andre P et al. demonstrated the efficacy of anti-NKG2A monalzumab in combination with anti-EGFR.

In a phase II trial of monalizumab combined with cetuximab, responses were observed in 35% of patient who were immunotherapy-naive and 18% in those who received previous chemotherapy. The combination was well tolerated and 93% of adverse events (AE) were of grade 1–2 severity with only 6% of patients experiencing treatment-related grade 3–4 AE. Eight out of 26 patients (31%) achieved a confirmed response (1 complete and 7 partial), 54% had stabilization of disease (SD) [43].

The first patient cohort of the study UPSTREAM (patients not eligible for one of the biomarker-driven cohorts, after platinum progression) treated with single agent monalizumab (10 mg/kg) every 14 days, were reported at ESMO 2019. The sub-study did not meet its primary objective (progressive disease 78%) although 59% of patients had received prior treatment with anti PD1/PD-L1. We hypothesize

that blocking the inhibitory axis CD94/NKG2A/HLAE alone might be not sufficient to reverse an immunosuppressive TME.

Anti KIR2

NK cell activation is partially controlled by KIRs upon binding with their ligands. Preclinical hematological studies reported activation of NK through mismatches between KIRs on donor NK cells and recipient MHC class I molecules, with improved relapse-free survival and overall survival [45]. The efficacy and safety of the first-in-class anti-pan-KIR2D agent lirilumab was explored in several clinical trials. Lirilumab can be safely administered but the efficacy in monotherapy is disappointing. Contrary to this, combinations with anti-PD1 antibody and antiCTLA-4 (136 with nivolumab; 22 with ipilimumab) were well-tolerated, with encouraging preliminary results. In SCCHNl the Lirilumab plus nivolumab cohort showed an objective response rate (ORR) of 24%, with durable responses. Notably, increased PD-L1 expression was strongly associated with improved probability of objective response [46].

Anti TIM-3

Interestingly, resistance to anti-PD-1 monoclonal antibodies (mAbs) might depend on up-regulation of alternative immune checkpoints, including TIM-3, LAG3, TIGIT etc. Recent studies showed that T-cell immunoglobulin mucin 3 (TIM3) participates in the regulation of Tregs, and correlates with immunosuppressive microenvironment (Galectin-9, Foxp3, CD68 and CD163) [47].

The increased surface levels of TIM-3 on NK cells in cancers induce NK cell impairments [48], while TIM-3 blockade results in increased NK cell cytotoxicity both in vitro and ex vivo [49]. In SCCHN anti TIM3 reduces Treg activation and decreases CTLA4 and TIGIT.

Currently, therapeutic approaches combining the administration of anti-TIM-3 and anti-PD-1 antibodies showed that the adaptive resistance to PD-1 blockade can be overcome [50].

Several studies are ongoing in phase I both in solid and hematological malignancies as monotherapy or in combination with an anti-PD-1 mAb or anti-LAG3 mAb (NCT03489343, NCT03311412, NCT02817633, NCT03680508, NCT04139902, and NCT03744468).

Anti LAG-3

Lymphocyte activation gene 3 (LAG-3) is an inhibitory receptor on T cells, which increases the effect of Tregs and shows relationship with T cell exhaustion. LAG-3 suppresses immune responses in several tumors, including Hodgkin's lymphoma,

gastric cancer, breast cancer, and other solid tumors. T cells co-expressing both LAG-3 and PD-1 may show a greater degree of exhaustion compared with those expressing LAG-3 alone [51]. Combining anti-LAG-3 mAb and anti-PD-1 mAb synergistically enhances T cell activity, [52] and a phase I/II clinical trial with the combined treatment is ongoing (NCT01968109).

Blockade of LAG-3 pathways has shown to enhance T-cell and NK cell activity, leading to increased antitumor activity and limiting tumor burden in several preclinical studies [52].

In this context, different anti-LAG-3 mAb are currently being used in phase I and phase II clinical trials as monotherapy (NCT03489369 and NCT03250832) or in association with other immune checkpoints inhibitors (NCT04150965, NCT02658981, NCT01968109, NCT03005782, NCT04080804, NCT02676869). A number of additional LAG-3 antibodies are currently in preclinical development.

Anti-TIGIT

T-cell immuno receptor with immunoglobulin and ITIM domains (TIGIT), can suppress T-cell activation and promote T-cell exhaustion. TIGIT and CD96 are co-inhibitory receptors expressed on both T and NK cells and compete with the activating NK cell receptor DNAM-1 for binding to the poliovirus receptor (PVR;CD155) and Nectin2 (CD112) [53]. These receptors participate in a balanced system to control NK cell effector functions. The expression of TIGIT is highly variable among different cancer types and it is highly expressed on tumor-infiltrating NK cells [54].

Notably, the therapeutic effects of anti-TIGIT and anti-PD-L1 monotherapy, or anti-TIGIT and anti-PD-L1 combinations depend on the presence of NK cells [55], indicating the importance of NK cells in checkpoint-targeted immunotherapy. Currently, several ongoing clinical trials (phase I and phase II) focus on testing the feasibility of targeting the TIGIT pathway and improving therapeutic effects through combination with existing immunotherapies, including anti-PD-1 agents (NCT04150965, NCT03119428, NCT04047862, and NCT03563716), mainly in solid tumor patients.

Increase ADCC Through Engineering of Monoclonal Antibodies

Many approved mAbs are of the IgG1 isotype. Fc region in IgG1 includes two N-linked biantennary complex-type oligosaccharides. The Fc region induces ADCC through its interaction with the Fcγ receptor family. However, ADCC activity is influenced by the structure of the Fc region. In physiological conditions, mAb Fc

region links to the FcγRIIIa of the effector cells with low affinity, in competition with a specific serum IgG.

The therapeutic mAbs can be engineered to remove fucose residues from the Fc N-glycans. Afucosylated mAbs exhibit strong ADCC activity compared to fucosylated counterpart due to much higher binding affinity to FcγRIIIa. Strong ADCC activity is also maintained at low antigen density, a situation in which the fucosylated mAbs cannot induce detectable ADCC [56, 57].

There is a growing interest in afucosylated mAb and many studies are in progress. Some afucosylated mAb are already approved in clinical practice, such as obinutuzumab (anti CD20), mogamulizumab (anti CCR4) and benralizumab (anti IL5Rα) [58].

Improve NK Cells Trafficking and Homing in the Tumor

In many tumors, NK cells are not in direct contact with tumor cells, but rather, they are restrained in the stroma surrounding tumor nests. It happens even if NK cells express the chemokine receptor CXCR3 and the specific chemokines CXCL9 and CXCL10 are released. Therefore, the endogenous production of chemokines may be insufficient for NK cell recruitment to tumor nests [11].

Chemerin is a super agonist of NK trafficking. It is a chemokine that plays a pivotal role in both immune response, lipid metabolism and in the regulation of programmed cell death, including autophagy and apoptosis. The chemerin chemotactic receptor CMKLR1 is expressed on NK cells, macrophages and subsets of dendritic cells.

Chemerin is released in an inactive form (prochemerin) and is converted into active chemerin in inflamed areas. Chemerin is down regulated in many tumors, but restoring its expression may increase NK infiltration and tumor suppression. Indeed, in a breast cancer model, Pachynski RK et al. forced overexpression of chemerin by tumor cells obtaining significant recruitment of NK cells and T cells within the TME [59]. However, the clinical development of chemerin is hampered by its potential side effects [60].

Lee J et al. recently suggested a novel approach. These authors developed an antibody-based NK-cell-homing protein (NRP-body), namely an antibody able to link to a specific tumor antigen, which drives a cargo domain containing CXCL16, the NK chemoattractant. When the antibody links to the tumor antigen, CXCL16 is released in the TME reaching a very high concentration. In a pancreatic cancer model, the NRP-body increased NK-cell infiltration into tumors. Preclinical results showed promising effects [61].

CAR–NK

Compared to CAR-T cells, CAR-transduced NK cells (CAR-NK) exhibit several advantages, (Table 5.1) such as safety in clinical use, the mechanisms by which they recognize cancer cells, and their abundance in clinical samples. Human primary NK cells and the NK-92 cell line have been successfully transduced to express CARs against several tumors, with most mature results in hematological cancers. Moreover, toxicities concerns appear less serious than CAR-T, cytokine release syndrome (CRS) is not reported although NK cells release IFN-γ, IL-3 and GM-CSF, which may result in a different form of CRS (with few systemic inflammatory response and toxic death).

In the last years to counteract these potentially fatal toxicities, CAR- NK cells are modified with an inducible suicide gene able to be activated pharmacologically to turn off the waterfall [62].

NK-92 cells engineered to CAR-NK was recently approved by FDA for clinical trials, and it was already tested in patients with melanoma, sarcoma, colorectal cancer, renal cell cancer (benefit in 5/11 patients) and NSCLC (benefit in 3/4 pts). To date, the used targets of CAR-NK include different cancer antigens such as CD19, CD20, CD244, HER2, CD38, epithelial cell adhesion molecule (EPCAM), disialoganglioside (GD2), EGFR variant III) [63].

Liu et al. generated cord blood derived CAR-NK by transducing NK cells with a retroviral vector to incorporate the genes for CAR CD19, IL-15 (a cytokine crucial for NK cell persistence), and the caspase-9 suicide gene. CAR.19/IL-15/iC9-NK showed additional activity of CAR-NK compared to CAR-T as NK cells preserve the intrinsic capacity to recognize and target tumor cells. Moreover, as above reported, IL-15 drives NK cell expansion and persistence, as demonstrated by longer persistence and anti-tumor activity compared with CAR.19-transduced NK cells lacking IL-15. Finally, these cells can be easily eliminated by pharmacological activation of Caspase 9 [64].

In acute myeloid leukemia NK cells (NK-92) were transduced with a third generation CAR lentiviral construct containing both CD28 and 4-1BB costimulatory molecules, and were infused after salvage chemotherapy in three patients. CAR NK92 cells were irradiated to prevent both excessive expansion and to treat parenteral cells derived from a lymphoma patient. The study failed to demonstrate

Table 5.1 Advantages of CAR NK cells over CAR T cells

CAR-NK	CAR-T
Prepared "off-the-shelf"	Requires autologous cells
Low secretion of cytokines (no CRS)	Risk of CRS
Cheap	Expansive
Maintains their natural receptors (NKG2D, NKp30…)	Likelihood of relapse related to a loss of CAR-targeting antigen

CAR Chimeric antigen receptor, *CRS* Cytikines released syndrome

clinical efficacy but as the "first in human study" it opens the way to optimize this potentially efficacious treatment [65].

Exciting results in pancreatic cancer models have been reported also with cryopreserved NK cells. Systemic administration of NK cells induced greater in vivo tumor growth suppression when compared with gemcitabine. The potent antitumor effect of NK cells was obtained by increasing infiltration into desmoplastic tumor tissues, apoptosis and IFN-γ, and by inhibition of TGFβ [66].

A promising strategy to increase efficacy of NK is to create NK cell engagers (NKCEs): multifunctional antibodies targeting tumor antigens, NKp46 and CD16. The goal is to increase tumor-cell destruction by bringing tumor cells and NK cells together. The new generation of trifunctional NKCEs targets the two activating receptors, NKp46 and CD16, on NK cells and a tumor antigen on cancer cells. Trifunctional NKCEs were more potent in vitro than clinical therapeutic antibodies targeting the same tumor antigen [67].

Despite the usefulness of NK cells, NK-cell therapy is limited by tumor cell inhibition of NK-cell homing to tumor sites, thereby preventing a sustained antitumor immune response.

Ongoing researches on this topic will hopefully provide new tools to overcame this issue (see above in the: 'Improve NK Cells Trafficking and Homing to the Tumor' section).

Conclusions

NK cells represent one of the most important tools of the immune system. They are involved in immune surveillance, control of metastatization, and in the fight against tumor cells inside the tumor.

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Chapter 6
Biomarkers for Immune Modulatory Treatment in Head and Neck Squamous Cell Carcinoma (HNSCC)

Danny Rischin

Background

Immune checkpoint inhibitors have changed the standard of care in recurrent/metastatic mucosal head and neck squamous cell carcinoma. Initially in the 2nd-line post platinum based chemotherapy setting [1, 2], and more recently based on the results of the Keynote-048 trial in the 1st-line R/M setting [3]. Although responses can be durable, only a minority of patients respond. Hence, the need for predictive markers to ensure these therapies are provided to patients most likely to benefit, whilst sparing patients who are unlikely to benefit from these treatments.

Potential Predictive Biomarkers for Immune Checkpoint Inhibitors

1. Immune checkpoint ligand expression e.g., programmed death-ligand 1 (PD-L1)
2. Markers of a T-cell inflamed microenvironment e.g., gene expression profiles
3. Markers of tumour neoepitope burden e.g., tumour mutation burden
4. Multidimensional quantitative immunohistochemistry (IHC)/immunofluorescence (IF) e.g., PD1/PD-L1

D. Rischin (✉)
Department of Medical Oncology and Sir Peter MacCallum Department of Oncology, Peter MacCallum Cancer Centre and University of Melbourne, Melbourne, VIC, Australia
e-mail: danny.rischin@petermac.org

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PD-L1

PD-L1 is the most studied predictive biomarker for response to immune checkpoint inhibitors. However, the field has been hampered by a number of factors including the use of different antibodies, measurement of tumour versus immune cells versus both, variable scoring criteria, and variable expression and cut-offs across tumour types [4]. In Table 6.1, the antibodies employed for some of the more common immune checkpoint inhibitors in use are shown, as well as the cells scored. With regard to staining tumour cells the antibodies behave similarly, with the exception of SP142 that stains a lower percentage of cells. However, in general these antibodies are not interchangeable, and it is best to use the same antibody and ideally the same assay as was used in the relevant trial in that cancer [4]. In general, good reproducibility has been demonstrated for scoring of tumour cells, but this is not the case for measuring immune cells only [5].

In R/M HNSCC there does seem to be a correlation between PD-L1 expression and response, as well as survival, albeit not in all studies (Table 6.2). In R/M HNSCC, as in other cancers, a range of assays and scoring criteria has been used. However, in this manuscript the focus will largely be on the assays used in the key pembrolizumab

Table 6.1 PD-L1 antibodies

	Nivolumab	Pembrolizumab	Durvalumab	Atezolizumab
Primary antibody	28–8	22C3	SP263	SP142
Scoring	Tumour	Tumour – TPS Tumour + immune cells - CPS	Tumour	Tumour and immune cells

Table 6.2 Correlation between PD-L1 expression and response in R/M HNSCC

28–8	PD-L1 ≥ 1% TC	PD-L1 < 1%		
Nivolumab	**18%** (n = 96)	**12%** (n = 76)		Ferris et al. [6]
SP263	PD-L1 ≥ 25% TC	PD-L1 < 25% TC		
Durvalumab	**16%** (n = 112)	**9%** (n = 67)		Zandberg et al. [7], Siu et al. [8]
SP142	PD-L1 IC ≥ 5%	PD-L1 IC < 5%		
Atezolizumab	**24%** (n = 25)	**14%** (n = 7)		Colevas et al. [9]
22C3	PD-L1 CPS ≥ 20	PD-L1 CPS ≥ 1	PD-L1 CPS < 1	
Pembrolizumab		**22%** (n = 127)	**4%** (n = 25)	Chow et al. [10]
Pembrolizumab	**23%** (n = 133)	**19%** (n = 257)	**4%** (n = 44)	Burtness et al. [3]
	TPS ≥ 50%	TPS < 50%		
Pembrolizumab	**26%** (n = 65)	**11%** (n = 179)		Cohen et al. [2]

trials in R/M HNSCC that have led to approvals based on PD-L1 expression. In the 1st-line setting worldwide and in the 2nd-line setting in Europe use of pembrolizumab for R/M HNSCC first requires evaluation of PD-L1 expression.

The first phase 3 trial in R/M HNSCC was the trial of nivolumab versus standard of care (investigators choice—methotrexate, docetaxel or cetuximab) [1]. Patients were enrolled regardless of PD-L1 expression, and it was also not a stratification factor. Based on this trial nivolumab was approved for treatment in platinum resistant R/M HNSCC in all-comers, i.e., no restriction based on PD-L1 expression. In an exploratory analysis, tumour PD-L1 expression did not appear to be predictive of benefit [6].

In the pembrolizumab trials in HNSCC the PD-L1 22C3 pharmDx companion diagnostic assay has been used. Two scoring methods are available:

1. The tumour proportion score (TPS), which is the percentage of viable tumour cells with partial or complete membrane staining at any intensity
2. The combined positive score (CPS), which is the ratio of the number of PD-L1–expressing cells (tumour cells, lymphocytes, macrophages) to the number of all viable tumour cells × 100

The PD-L1 CPS score has been shown to have good reproducibility in a gastric cancer study [11]. In an exploratory analysis of the Keynote-012 HNSCC cohort, measurement of tumour + immune cells seemed to be more predictive of response to pembrolizumab than measurement of tumour cells only [10]. In the Keynote-040 trial pembrolizumab was compared to standard of care (investigators choice—methotrexate, docetaxel or cetuximab) in patients who had received prior platinum [2]. Eligibility required submission of a tissue sample for PD-L1 assessment and PD-L1 TPS (≥50% vs. <50%) was a stratification factor. 26% of the population had PD-L1 TPS ≥50%, and when analysed by TPS scores the benefit of pembrolizumab appeared to be predominantly in this population. In Europe, the EMA approved pembrolizumab for platinum pre-treated HNSCC in patients with PD-L1 TPS ≥50%. In an exploratory analysis when analysed by PD-L1 CPS (≥1% vs. <1%) 83% of the population had CPS ≥1, and it was predictive of benefit.

There has been a preliminary report of a posthoc analysis of efficacy outcomes based on PD-L1 scoring techniques in Keynote-040 [12]. Standard receiver operating characteristic curves were generated for TPS and CPS for patients receiving pembrolizumab versus SOC to demonstrate the relationship between pembrolizumab and SOC at each cutoff. Concordance between TPS and CPS cutoffs was 77% at a cutoff of 1, 91% at a cutoff of 20, and 95% at a cutoff of 50. At lower expression levels, CPS detects a larger fraction of pembrolizumab responders than TPS while maintaining similar survival results. At higher expression levels, CPS ≥50 can be used interchangeably with TPS ≥50%. Based on these results it was concluded that CPS is a valid scoring method for determining PD-L1 status in patients with HNSCC.

The Keynote-048 trial evaluated the role of pembrolizumab alone or in combination with platinum-5-FU chemotherapy compared to the standard of care, the Extreme regimen of platinum, 5FU and cetuximab in patients receiving 1st-line

systemic therapy for R/M HNSCC [3]. PD-L1 expression based on TPS (≥50% vs. <50%) was a stratification factor, with 22% having TPS ≥50%. Key populations for the primary, secondary and exploratory endpoints were defined by PD-L1 CPS scores: ≥20, ≥1 and the total population. 40–45% of the population had CPS ≥20 and 85% had CPS ≥1. In this trial pembrolizumab monotherapy was shown to be superior to the Extreme regimen in the CPS ≥20 and ≥1 populations but not in the total population. The combination of pembrolizumab with chemotherapy was shown to be superior to the Extreme regimen in all three populations. For both monotherapy and the combination with chemotherapy there was increasing benefit (overall survival and response) with increasing PD-L1 CPS. Based on the Keynote-048 results, pembrolizumab approvals for use in the 1st-line R/M setting have been contingent on tumour PD-L1 expression as assessed by the CPS. The FDA restricted approval of pembrolizumab monotherapy to patients whose tumours express PD-L1 ≥1, but approved the combination with platinum and 5FU for the total population. The EMA approved both monotherapy and the combination with platinum and 5FU for 1st-line treatment of metastatic or unresectable R/M HNSCC in adults whose tumours express PD-L1 with a CPS ≥1.

PD-L1 expression using CPS enriches for the population likely to benefit from use of an immune checkpoint inhibitor, and can identify a population unlikely to derive much benefit. However, it is a weak predictor of benefit as only a minority of patients with PD-L1 CPS ≥20 achieve a response and prolonged survival.

Gene Expression Profiling

Several signatures have been identified that are predictive of response to immune checkpoint inhibitors. These include the ‘Teff’ signature [13] defined by three genes (PD-L1, CXCL9, and IFNγ) and is associated with responses to atezolizumab in patients with non-small cell lung cancer and the ‘T-cell inflamed gene expression profile’ (GEP) [14] consisting of 18 interferon-gamma responsive genes and is associated with responses to pembrolizumab in melanoma and 9 different solid tumors, including HNSCC.

The T-cell inflamed GEP score is higher in patients with HNSCC who responded to pembrolizumab, with area under the receiver operating characteristic curve of 0.768 [15]. GEP was significantly correlated with PD-L1 expression in HNSCC ($r = 0.51$), which is consistent with PD-L1 expression regulation by T-cell derived IFNγ.

Tumour Mutation Burden

There is a correlation between the median mutation burden of a given tumour type and the probability of response to an immune checkpoint inhibitor [16]. HNSCC has a moderate TMB, with median number of coding somatic mutations per megabase of 5.0 [17]. TMB of HNSCC is similar to oesophago-gastric and urothelial tumours, and considerably lower than more responsive tumours such as melanoma, cutaneous SCC and mismatch repair deficient tumours.

In HNSCC patients treated with pembrolizumab, TMB is higher in responders, with area under the receiver operating characteristic curve of 0.617 [15]. There was no correlation between TMB and either GEP or PD-L1. In another study higher TMB was associated with benefit from anti-PD1/PD-L1 in HPV negative HNSCC [18].

Combination of Tumour Mutation Burden and Gene Expression Profiling

The combination of TMB and GEP had joint predictive utility in identifying HNSCC responders and non-responders to pembrolizumab in a study of 105 patients [15]. There were no responders in the patients with low TMB and low GEP, and only one responder in the group with high TMB but low GEP. The highest response rate was in the group with both high TMB and high GEP – 37%. The group with low TMB but high GEP had an intermediate response rate of 16%.

Multidimensional Quantitative IHC/IF

A recent meta-analysis compared biomarker modalities for predicting response to immune checkpoint blockade [19]. It concluded that modalities that permit assessment of more than one biomarker were promising, for instance multiplex immunohistochemistry or immunofluorescence e.g., evaluating PD1 to PD-L1 proximity. These strategies may improve the positive predictive value that remains low with single modality predictive biomarkers. These techniques take into account the spatial importance of tumour immune interactions and the contribution of protein marker co-expression. The area under the receiver operating characteristic curve for multiplex IHC/IF was 0.79 that was considerably higher than for single factors e.g., PD-L1, GEP or TMB.

Tissue Resident Memory (T_{RM}) Cells

Tissue resident memory cells are a subset of T cells that occupy tissues without recirculating. They are characterised by expression of CD103 and CD69 and are usually CD8 and CD4 positive. T_{RM}s have a role in infections and cancer immunosurveillance [20].

High levels of intratumoural CD103 positive immune cells (≥30%) in patients with HPV associated oropharyngeal cancer treated predominantly with chemoradiation is associated with an excellent outcome independent of stage [21]. This was demonstrated in a retrospective training cohort from the Peter MacCallum Cancer Centre with a hazard ratio of 0.13 (95% CI 0.02–0.94, P = 0.004) and confirmed in an independent validation cohort from the Princess Alexandra Hospital with a hazard ratio of 0.16 (95% CI 0.02–1.22, P = 0.02). The 5 year survival estimates for the patients with high intratumoural CD103 was 100% in both cohorts while in the patients with low (<30%) intratumoural CD103 it was 82% and 88% (Fig. 6.1—pooled results from the two cohorts). In both cohorts, which were unselected i.e., contained both low and high risk HPV OPSCC, the CD103 high group represented 20% of the population.

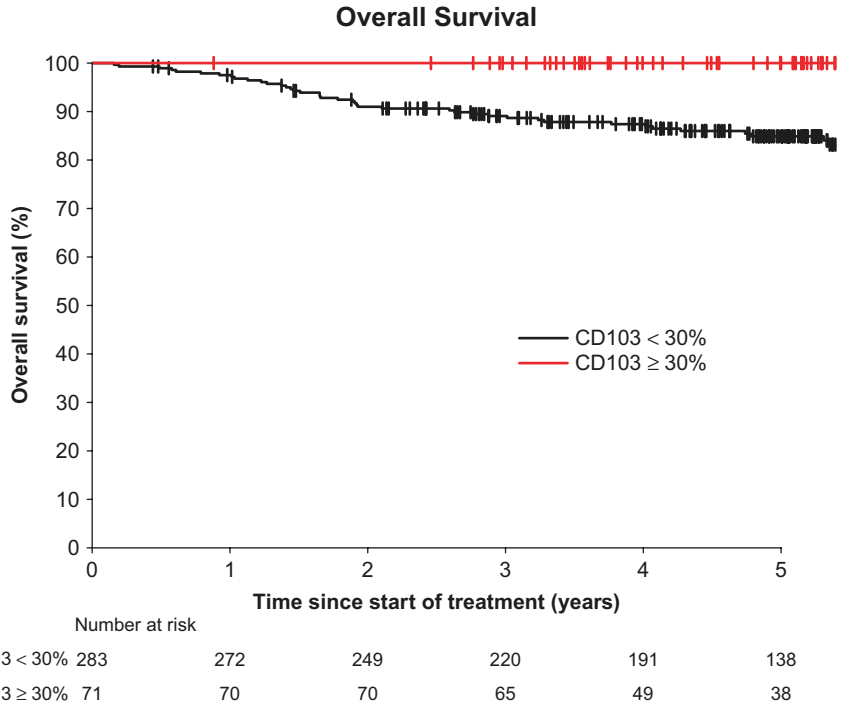

Fig. 6.1 Overall survival by intratumoural CD103 expression in locoregionally advanced HPV oropharyngeal SCC (combined Peter MacCallum and Princess Alexandra cohorts)

Tumours with CD103+ cells co-expressed CD69 and CD8 on multispectral immunofluorescence consistent with T_{RM}s. Tumours with high intratumoural CD103 also had higher expression of genes identified in a single cell gene expression analysis of T_{RM}s [22], as well as gene signatures associated with responses to pembrolizumab [14] and atezolizomab [13].

In another study there was expansion of CD103+ cells in biopsies of melanoma patients early during treatment with an anti-PD1 agent, which was greater in responding patients [23]. It has been suggested that high levels of CD103 CD8 tumour infiltrating lymphocytes in non-small cell lung cancer may be a predictive biomarker for sensitivity to immune checkpoint blockade [24]. It is reasonable to speculate that patients with CD103 + HPV-associated oropharyngeal cancer may be particularly sensitive to immune checkpoint blockade. While this population would be expected to be rare in the recurrent/metastatic setting, this locoregionally advanced population would be ideal candidates for de-escalation strategies in general, and in particular de-escalation trials that incorporate immune checkpoint blockade.

Conclusion

For the first time in HNSCC we have treatments approved based on the results of a companion diagnostic. Pembrolizumab for first-line treatment of recurrent metastatic disease requires assessment of PD-L1 expression as measured by the CPS. The PD-L1 CPS score enriches for populations more likely to respond, but the false positive predictive value remains high. Better predictive biomarkers are required, and while some show promise, clinical utility in HNSCC has not been established.

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Part II
Primary Disease

Chapter 7
Novel Approaches in Surgical Management: How to Assess Surgical Margins

Frail Biological Basis with Promising Future Perspectives

Marco Ferrari, Nausica Montalto, and Piero Nicolai

Introduction

Understanding the physical interface between tumor and host is a fascinating topic, as it dictates our current ability to appreciate the mechanisms of local growth of tumor and plan a resection with an adequate cuff of surrounding normal tissues. Despite many uncertainties regarding the definition of "adequate margins" that should be achieved by surgeons, there is strong evidence that clear resection margins are one of the main predictors of local control and overall survival in carcinomas of the upper aerodigestive tract. As a consequence, the presence of positive margins together with extranodal extension are the main factors supporting the use of chemotherapy in association with radiotherapy in the adjuvant setting [1, 2].

The present manuscript provides a basic historical, biological, and practical background on the concept of margins, which is essential to appreciate the importance of future perspectives in the field of margin control for tumors of the head and neck.

M. Ferrari · N. Montalto · P. Nicolai (✉)
Section of Otorhinolaryngology – Head and Neck Surgery, Department of Neurosciences, University of Padua, Padua, Italy
e-mail: nausica.montalto@aopd.veneto.it

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Historical Background: The Concept of "Margin"

The concept of margin in oncologic surgery is almost six centuries younger than the word "cancer", which was coined by Hippocrates in view of the appearance of blood vessels surrounding a tumor and resembling the claws of a crab [3, 4]. Thereafter, cancer was considered mostly as a "humoral disease", which was consequently deemed as non-curable through simple surgical excision. Galen should be credited for being the first to hypothesize that cancer can infiltrate surrounding tissue even beyond the sensitivity of the naked eye, an intuition driven by the observation that tumors tend to regrow in scars [5]. This assumption led to conclude that cancer should be removed together with a cuff of apparently normal tissue, which still remains the pillar of surgical oncology. Although the contribute of Galen in understanding cancer is considered as controversial [6], the observation that a tumor can early return in areas adjacent to where it was completely excised can be considered as the first insight into the concept of margins. Thus, it can be estimated that the concept of "surgical margins" was born in the second century, which means almost 600 years after Hippocrates. In the nineteenth century, Virchow and Lebert observed that a cancer is formed by "cancer cells", which have the ability to invade neighboring tissues in small groups, yet not producing macroscopic changes in the early phases [5]. This new understanding of cancer provided an essential explanation to the observation of Galen, thus corroborating that cancer can be theoretically cured through excision of adjacent tissues. Despite its ancient birth, the concept of surgical margins first settled in oncologic surgery at the end of the nineteenth century, with Halsted being one of the most distinguished oncologic surgeons to concretely apply this thought to surgical practice [7]. Although biological comprehension of cancer has seen a large number of steps forward since then, the basic concept of surgical margins has remained unchanged, namely removing enough tissue to ensure that all cancer cells are included in the surgical specimen. On the other hand, the contemporary understanding of cancer biology suggests that cancer cells dissemination occurs from even early-stage tumors (also at a systemic level), thus rising some doubts on the belief that "removing all cancer cells" is the actual mechanism through which cancer is cured [8].

Current Biological Rationale of Margins in Head and Neck Surgery

The recommendation to leave a margin of normal tissue surrounding the visible tumor stands in the awareness that tumor cells can subtly extend far beyond the macroscopic boundary of the tumor. In the head and neck, oral squamous cell carcinoma represents the most frequently analyzed cancer to assess the pattern of growth towards adjacent tissues. The histologic morphology of the interface between tumor and surrounding soft tissues has been classified in five patterns with increasing

Fig. 7.1 Patterns of local invasion of soft tissues according to Brandwein-Gensler *et al*. [9]

degree of aggressiveness (Fig. 7.1) [9]. Type 1 pattern is defined as "broad pushing front", meaning that the tumor grows expansively and does not release groups of cells beyond its surface. Type 2 is described as "finger-like" as the tumor front displays some appendices irregularly protruding towards neighboring tissues. From type 3 to type 5, non-contiguous groups of cells with heterogeneous shape and distance from the tumor front are observed. In the type 3 front, only tumor islands, which look like "fingers" that grow up to the point of detaching from the tumor, are observed. Smaller cell groups, strands, or even single cells located within 1 mm from the main tumor surface fall under the definition of type 4 front. Type 5 front of invasion, finally, displays the so-called "satellites", which consist of either a cell or a group of cells located 1 mm beyond the tumor front. The ability to subclinically infiltrate surrounding soft tissues such as fat, striated muscles, fascial structures, and loose connective areas intuitively increases with the type of invasion front. Oral cancer was also used to analyze the pattern of invasion of bone, with special reference to the mandible. Two modalities of extension towards bone have been observed: in the erosive pattern, the tumor causes bone resorption by activating osteoclasts along a broad front of invasion; in the infiltrative pattern, tumor cells grow between bony trabecula by partially maintaining the microscopic and macroscopic bony architecture [10]. Some authors surmised that the infiltrative pattern might represent a later phase of invasion of bone compared to the erosive pattern. Parallel to these mechanisms of infiltration of adjacent tissues, cancers can acquire the ability to grow along nerves and/or vessels, which all together provides tumor cells with a dense network of pathways to move distantly from the clinically appreciable mass [11–13].

Special Elements of Challenge in the Head and Neck Area

The head and neck probably represents one of the most challenging areas of the human body to achieve adequately and homogeneously wide margins.

Although a number of factors contribute to the challenge, the need to preserve several vital functions most commonly compete with the delineation of a wide margin all along the tumor surface (Fig. 7.2a). In fact, the head and neck are dense in neurovascular structures and essential effector organs such as the brain, eyes, tongue, and larynx, which constantly place the surgeon and multidisciplinary team in front of dilemmas on resectability *versus* non-resectability or preservation *versus* ablation.

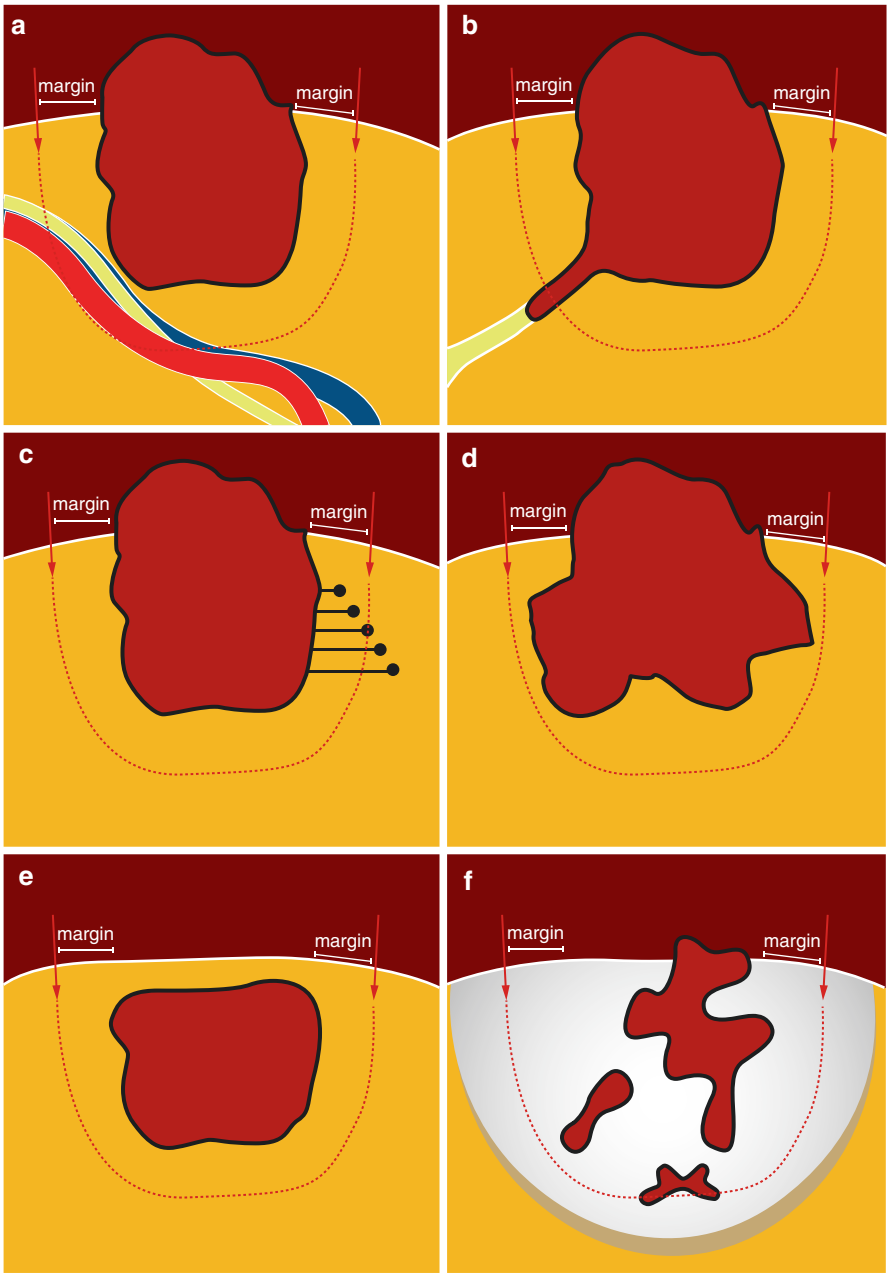

Fig. 7.2 Special elements of challenge in the management of margins in cancers of the head and neck. (**a**) Adjacency to critical neurovascular structures. (**b**) High density of nerves and vessels providing cancer with a network of escape routes. (**c**) Heterogeneous propensity towards subclinical extension into adjacent tissues. (**d**) Complex 3-dimensional shape. (**e**) Deep location of the tumor. (**f**) Multifocal tumor dispersed into previously treated tissues

The density of neural and vascular structures also provides cancers with a dense network of potential escape routes (Fig. 7.2b). This further complicates the management of tumors displaying perineural and lymphovascular spread, as the vectors of microscopic growth of the disease might be numerous, thus making the genuine extension of the tumor deeply counterintuitive compared to the macroscopic shape of the lesion.

Biological heterogeneity is another element of complexity characterizing tumors of the head and neck. Besides the well-known variety of cancer types that exquisitely affect specific areas (*i.e.*, sinonasal tract, salivary glands), several degrees of biological aggressiveness have been observed within a single histology (Fig. 7.2c). There is evidence that tumors pertaining to the same histological category can display widely different propensity to grow beyond the macroscopic boundaries of the lesion through budding, satellitosis, pagetoid growth, perineural spread, permeative bone invasion, or other mechanisms [14–21]. This fact poses an additional challenge, since a tumor, even though labelled with a reliable preoperative diagnosis, might potentially be amenable to a "close-margin" excision (*i.e.*, when microscopic local extension is limited) or could instead require a "wide-margin" resection (*i.e.*, when microscopic groups of cells deeply invade adjacent tissues) as far as is known prior to surgery.

The 3-dimensional shape of the tumor also hinders adequate and regular delineation of margins (Fig. 7.2d). While often resembling a plaque or a sphere in the early phases of growth, advanced tumors of the head and neck acquire a 3-dimensional morphology that mirrors the complexity of subsite anatomy. This translates into a substantially increased chance of misorienting the plane of dissection with respect to one or other components of the tumor [22].

The deep location of a tumor, which means that the lesion is located underneath an uninvolved epithelial plane, is not a rarity in the head and neck (Fig. 7.2e). It can result from either the origin of the tumor (*e.g.*, salivary cancers, mesenchymal tumors), its growth pattern (*i.e.*, submucosal growth in mucosal carcinomas) or tumor history (*e.g.*, deep or nodal recurrences). Cancers with no superficial components force surgeons to infer the 3-dimensional configuration of the lesion based on imaging, palpation, and knowledge of anatomy, yet with a non-negligible risk for the resection to be misled.

Finally, improvement and implementation of non-surgical strategies bring to the operating theater an increasing number of patients with a tumor recurring within an irradiated and/or medically treated area (Fig. 7.2f). Similarly, refinements in surveillance strategies allow identification of post-surgical recurrences that are often suitable for surgical re-excision. Post-treatment presentation frequently implies a cancer that is multifocally dispersed within uninvolved yet deeply altered tissues, thus remarkably increasing the chance of leaving microscopic residual disease irrespective of the attention posed towards margin delineation.

These elements being considered altogether, surgical margins have been unsurprisingly a hot topic in head and neck oncology over the last decades.

Practical Determinants of Margin

Owing to the aforesaid elements of complexity, oncologic surgeons have developed strategies to optimize margin delineation. Similar to the principles guiding elective treatment of lymph node levels, these strategies are probabilistic in nature, meaning that they are intended to maximize the probability to also include the occult portion of the disease in the resection. This, however, has the cost to unnecessarily resect uninvolved tissue in some patients, or to remove an insufficient thickness of microscopically involved tissue in others.

Three main theoretical approaches have supported the establishment of surgical rules to properly delineate margins.

The "metric approach" consists of the identification of a spatial cut-off that ensures all tumor cells are included in the resection in the majority of cases [23]. This can be objectively measured at definitive pathology. Since the distance between tumor and specimen surface shrinks during intraoperative cutting and throughout post-surgical processing, the actual margin thickness needs to be estimated. In oral cancer, for instance, since a 5 mm pathologic margin was identified as a prognostic cut-off in several studies, a shrinkage rate of the surgical specimen accounting for 21–32% and varying with tissue type and size, at least a 1-cm actual margin is precautionarily recommended [24, 25]. Main argumentations against the metric approach are that a universal cut-off can be adequate, excessive, or insufficient depending upon histology and tumor-specific biology, and that 1 cm margin is hardly ever achievable in some head and neck sites (*i.e.*, sinonasal tract, skull base).

The "barrier approach" is based on the assumption that tumor expansion is contained by some anatomical structures, which usually consist of fascial layers, muscles, or bones [23]. This approach leads surgeons to identify and follow specific anatomical planes that surround the tumor, even though it implies to delineate the dissection plane with an irregular distance from the tumor surface. The main flaws of this approach are in the poor recognizability of some of these barrier-structures at definitive pathology, alongside the scarce demonstrability that they actually serve as barriers against tumor local progression.

The "compartment approach", finally, is based on the surmise that tumor cells tend to follow specific anatomical structures or vectors dictated by tissue architecture [26]. Though sounding similar to the barrier approach, this way of conceiving tumor progression is less optimistic on the capability of some structures to prevent local cancer progression. Rather, cancer cells would expand owing to a "pressure growth" that pushes cancers towards the pathways of least resistance (*e.g.*, between muscular fibers or fascicles).

As for all competing theoretical models aimed at explaining a biological phenomenon, the reality probably lies somewhere in the middle. Most likely, cancers progress through preferential pathways (either because of least resistance or due to a biological gain of function such as perineural spread), while also stochastically infiltrating surrounding tissues with some structures (*e.g.*, bone, cartilage) probably

serving as physical barriers against tumor expansion. Moreover, distribution between these modalities of local expansion can obviously vary among malignancies.

A paradoxical fact on recommendations for margin width lies in the technique-dependent threshold defining "clear margins". A cancer of the upper aerodigestive tract would be defined as completely resected with a threshold of 5 mm of pathologically uninvolved tissue if operated on with open surgery, 2–5 mm if through transoral robotic surgery, 0.5–2 mm if via transoral laser surgery, and regardless of metric measurements provided that adjacent structures are not infiltrated in case endoscopic transnasal resection has been performed [27–36]. On the one hand, this difference is understandable as it expresses the need to define as either "adequate" or "inadequate" a resection performed with a given technique. On the other, it reflects that the definition of margin is currently far from being biology-driven [37].

"Frailty" of Cutting Through Healthy Tissue

The concept of "free margin" grounds on the belief that tissue uninvolved by cancer is healthy. However, evidence dating back to the 1990s suggested that tissues surrounding mucosal cancers bear molecular alterations typically found in malignancies [38]. These observations are in agreement with the multistep model that explains cancer development and progression. In fact, precancerous cells that gradually accumulate all the mutations necessary to become cancer also proliferate, thus giving rise to a number of cells that are preconditioned towards malignant transformation. This might also explain the propensity of cancers induced by long-term exposure to a risk factor (*i.e.*, tobacco smoking) towards recurrence, field cancerization, and synchronous/metachronous malignancies. Consequently, instead of conceiving cancer as a well-defined mass, preconditioning of the surrounding mucosa contributes to make it more comparable to an ill-defined "cloud" of genetic alterations centered around the visible disease and variably extending to the adjacent mucosa (Fig. 7.3).

Current Intraoperative Margin Evaluation

For mucosal cancers, which represent the majority of head and neck malignant tumors, delineation of margins is required on both the superficial aspect, meaning that the surgeon has to decide how far from the visible tumor the mucosa has to be cut, and during dissection of deep tissues. For superficial delineation of margins, surgeons rely on sight and palpation, with some technologies (*e.g.*, narrow band imaging) augmenting the ability to identify altered tissues mostly owing to optical changes [39, 40]. Delineation of deep margins is based on palpation, imaging

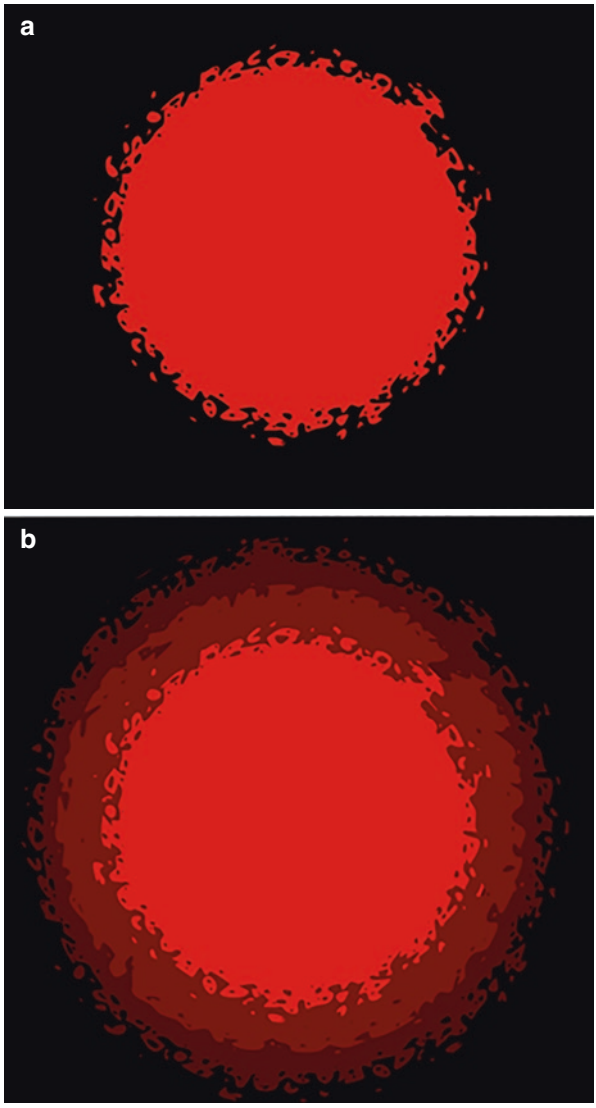

Fig. 7.3 Discrepancy between the common representation of cancer (**a**) and actual distribution of precancerous alterations in adjacent tissues (**b**)

interpretation, and the consequent 3-dimensional configuration that the surgeon creates in his/her mind. Sight is currently excluded from the ideal strategies to define the deep margin of resection, as it would imply the deep portion of the tumor to be exposed, which is a suboptimal scenario as opposed to leaving the tumor surrounded by a cuff of normal tissue.

Frozen sections allow intraoperative microscopic assessment of resection margins. Two main approaches to perform frozen sections for margin assessment are traditionally available: the defect-driven (also defined as patient-driven) technique consists of sampling the surgical bed, whereas in the specimen-driven technique

tissues to be analyzed are harvested from the surgical specimen. There is no consensus on which technique yields the best accuracy in terms of intraoperative margin evaluation. Some evidence suggests that specimen-driven frozen sections might provide a higher chance of achieving wide negative margins as compared to defect-driven approach [41]. Moreover, positive frozen sections on the surgical specimen may also represent an independent negative prognostic factor, whereas defect-driven frozen sections have not been demonstrated to carry any relevant prognostic information [42]. This could be explained by the fact that sampling on the surgical specimen leads the surgeon to address the most critical margin relative to the palpable mass, whereas analysis of the surgical bed requires inferring the initial situation of the tumor. However, some authors have reported that circumferential sampling of the surgical bed has an almost excellent negative predictive value, though with suboptimal positive predictive value [43]. Irrespective of the specific technique employed to sample tissue to be sent for frozen section analysis, a meta-analysis demonstrated that achieving negative margins by extending the resection based on a positive frozen section does not equate to an initially negative margin, nor does it significantly increase the local control rate [44]. These data should not be misinterpreted as suggesting uselessness of achieving negative margins through additional resection following a positive frozen section. In fact, in the same meta-analysis, local recurrence-free survival of patients with positive margins is reported being close-to-significantly ($p = 0.055$) worse compared to those with negative margins achieved through additional specimens on a positive frozen section [44]. As a consequence, one can conclude that obtaining negative margins upfront represents the best case scenario from a prognostic standpoint, but radicalization on a positive frozen section is still to be recommended based on the currently available data.

Future Directions: "Know Your Enemy"

Borrowing the aphorism of Sun Tzu from "*The art of war*", the first step to improve our ability to locally control cancer should consist of "knowing cancer". In particular, it is a common observation that every head and neck cancer has its own specificity in terms of local progression, which is not reliably expressed by the current systems of classifying and describing tumors.

For instance, it has been demonstrated that tongue squamous cell carcinoma has a particular propensity to subclinically invade the so-called "T-N tract", which roughly corresponds to the connective space including the sublingual area up to the level IB [45]. This confirms that tongue cancer can grow eccentrically with respect to the epicenter of clinically appreciable disease, which has not been observed in other oral cavity subsites whose cancerization shares analogous epidemiological and histopathological characteristics. This data being acquired, a modification of the surgical technique defined as "compartmental tongue surgery" has been implemented by some groups, aiming at addressing this particular characteristic of tongue cancer. Indeed, based on preliminary and retrospective data, compartmental tongue

resection seems to provide improved oncologic outcomes compared to standard wide-margin resection [26, 46]. These findings possibly confirm that focusing attention on the most probable escaping route of tumor might translate into better control of cancer.

Another example of deepening the understanding of cancer local behavior is the relationship between histologic growth pattern and topographic gross extension. For instance, it has been revealed that perineural and lymphovascular invasion substantially drive local extension of cancers of the maxillary sinus regardless of their histology [47]. In particular, tumors displaying lymphovascular invasion tend to grow with a caudal direction and give nodal metastases, while those with perineural invasion more frequently invade superior, medial, and posterior structures. Should detection of perineural and lymphovascular invasion be reliably detectable before surgery, the resection could be extended accordingly towards the most critical areas.

In view of this evidence, head and neck oncologic surgeons should be avid in knowing the local behavior of cancers with a histology-, site-, and possibly biology-level precision. Therefore, future research on local tumor extension in the head and neck should primarily assess the relationship between the cancer's specificities and escape routes, in order to guide surgeons towards the most critical areas and possibly improve outcomes.

Future Directions: Enhanced Tumor Visualization

Another strategy to improve local control is to augment the way cancer is "seen" during ablation. The most promising and accessible technology to support this refinement is represented by surgical navigation systems. Although most frequently employed to minimize intraoperative complications and optimize precision of reconstruction, cross-sectional imaging-based navigation could also provide the surgical team with a more precise image of tumor extension. This has been shown in a preclinical setting, where the employment of navigation with 3-dimensional rendering of the tumor extension significantly increased the adequacy of margin delineation in models of advanced cancers variably extending within the craniomaxillofacial skeleton [22]. Over a total of 381 simulated osteotomies, the use of surgical navigation decreased the rate of gross margin involvement from 18.1% to 0.0%. Moreover, some groups have published their experience in using navigation to improve the margin status of resections of advanced cancers of the head and neck, showing encouraging results [48–50]. Despite the limited number of patients reported in these preliminary experiences (24 overall), the employment of navigation led to obtain free margins in a high percentage of patients affected by locally advanced cancer of the head and neck.

By basing the 3-dimensional representation of the tumor on radiologic data, navigation-guided resections might also benefit from incorporating relevant information into cancer rendering. For instance, the tumor can be rendered together with an isotropic expansion to provide a visual representation of a metric margin.

Moreover, cancer rendering could also include fusion of functional and cross sectional imaging, possibly increasing the accuracy of tumor mapping [50]. In this sense, whichever future methodology is capable of better depicting the actual tumor extension could be incorporated in the representation of tissue to be resected through surgical navigation.

However, the accuracy of surgical navigation is constrained by precise and lasting registration alongside the presence of a bony framework that limits motions of soft tissue. For this reason, navigation is most likely useful in the setting of tumors strictly attached to the craniomaxillofacial skeleton, whereas cancers invading mostly soft tissues would be less accurately rendered.

Future Directions: Augmented Mapping of the Surgical Bed

The latest and most promising advent in the field of surgical margins control is application of bio-optical imaging technologies to search for tumor localizations that would otherwise be undetectable by the naked eye [51]. Employment of this technology to improve delineation of the superficial margin of resection has been already demonstrated to be beneficial. On the contrary, optical imaging to detect potential residues of the tumor into the surgical bed and accordingly guide frozen section is still an ever-changing field. The most promising optical imaging modalities which could meet this need are fluorescence-based imaging, hyperspectral imaging, and Raman spectroscopy. Fluorescence-based imaging relies on either natural (*i.e.*, autofluorescence imaging) or targeted fluorescence (*i.e.*, through biological probes attached to fluorophores) of cancer tissue. Hyperspectral imaging consists of dividing electromagnetic waves beyond the 3-band division of the human eye and even beyond the spectrum of visible light. By collecting and elaborating this optical information, it is possible to infer biological information of a tissue under analysis. Raman spectroscopy is able to depict the molecular fingerprint of a tissue by taking advantage of light scattering as a consequence of vibration of intramolecular bonds. All these imaging modalities rely on the common principle of collecting bio-optical characteristics of tissues and render them in a way that is appreciable to the surgeon's eye.

Recently, van Keulen *et al*. published a series of 20 patients who were operated on for head and neck squamous cell carcinoma by targeted fluorescence-surgery [52]. All patients were injected with panitumumab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, conjugated to the fluorophore IRDye800CW. The surgeon could therefore visualize in real time the distribution of EGFR through a handheld camera prior to incise tissues. The authors demonstrated that tumor-to-background ratio, which represents the ability to distinguish the tumor from surrounding tissues, was satisfactory irrespective of age, gender, tumor size and site, and EGFR expression. Though preliminary in nature, these data are encouraging, as they demonstrate feasibility of the workflow and suggest that targeted-fluorescence imaging is reliable. Analysis of the actual benefit of this technology in terms of intraoperative margin status evaluation will represent an essential future step.

Halicek *et al*. published a study on 293 fresh specimens obtained from resection of head and neck squamous cell carcinomas in 102 patients and analyzed with reflectance-based hyperspectral imaging [53]. The authors found that hyperspectral imaging could distinguish squamous cell carcinoma from uninvolved tissue with an area-under-curve ranging between 0.80 and 0.90 compared to histopathological microscopic evaluation. The time span required to obtain hyperspectral-based evaluation of the surgical specimen was estimated to be around 2 min. This study provided promising data on the classification performance of hyperspectral imaging calculated from a large dataset. However, application of this methodology to the surgical bed would require optimization for potential confounders such as blood and cauterized tissues.

Barroso *et al*. have demonstrated the utility of Raman spectroscopy in identifying positive margins on 26 mandibulectomy specimens, with diagnostic accuracy as high as 95% [54]. Yu *et al*. achieved a 99.3% sensitivity and 94.3% specificity in distinguishing tongue squamous cell carcinoma with respect to normal tissue by applying a deep learning method to Raman spectral data obtained from 24 fresh specimens [55].

The above-mentioned references represent just selected publications among a large and constantly increasing number of studies demonstrating and progressively refining the diagnostic performance of bio-optical imaging techniques on fresh tissues harboring cancer. The following step will probably be to apply these technologies intraoperatively and quantify the actual benefit they can confer to outcomes.

Conclusions

Adequate control of margins is an urgent need in head and neck surgical oncology. Our current understanding of local progression of cancer is still inadequate, especially considering the variety of histologies and biological behaviors characterizing the head and neck area. Consensus should be reached to obtain a solid and biology-driven definition of “adequate margins”, which could be transversally applied to a given cancer irrespective of the surgical technique employed to excise it. On the other hand, technologies such as surgical navigation and bio-optical imaging will probably be implementing our current way of ablating cancers, possibly translating into better delineated surgical specimens and improved outcomes.

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Chapter 8
The Surgical Approach to Elderly Patients with HNSCC

Andreas Dietz

Introduction

Recently, some reviews and recommendations regarding treatment of head and neck cancer in elderlies have been published by Petr Szturz, Paolo Bossi and Jan Vermorken [1, 2]. These papers point out that the age of 70 (or even 75) as a cut-off defining the elderly has been broadly accepted and adopted by the "National Institute on Aging" and the "National Institutes of Health" [3]. This cut-off point may better capture the reality in terms of biological alterations occurring with advancing age, because aging is associated with a progressive loss of functional reserve of multiple organ systems, increased prevalence of chronic diseases, enhanced susceptibility to stress, and fluctuations in social support and economic resources [4]. Regarding prevalence of head neck surgery, data from New Zealand showed number and age of patients undergoing major head and neck cancer surgery peaked in the age group of 71–80 years [5].

Chronological age does not sufficiently correlate with biological parameters and provides only limited information for personalized management. Therefore Szturz et al. points out that in clinical practice, the crucial step is to distinguish a fit-old individual, who will likely withstand a radical treatment with curative intent, from a frail-old patient, who will probably not tolerate such approach. To deliver optimal patient care at an individual level a team approach represented by a multidisciplinary tumor board is essential. These meetings should offer a collaborative review of each case with special attention to disease factors (site, stage, biology, and risk factors for locoregional or distant relapse), patient factors (age, sex, performance and nutritional status, comorbid conditions, oral health, life-style habits, and socioeconomic background), treatment options, and patient preferences [1, 2]. With

A. Dietz (✉)
Clinic of Otolaryngology, Head and Neck Surgery and Department of Head Medicine and Oral Health, University of Leipzig, Leipzig, Germany
e-mail: andreas.dietz@medizin.uni-leipzig.de

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special focus on indication for primary or secondary (salvage) surgical procedures some specific factors must be taken into consideration.

Functional Physiological Age Related Issues with Impact on Selection for Surgical Treatment

Physiology of aging is characterized by a couple of differently distinct biologic developments with relevant impact on assessment for feasibility of surgical procedures. To check all these factors would be very time consuming and unrealistic in daily routine. Nevertheless, knowledge about these factors is mandatory and can sharpen the view by checking some representative indices for selecting the right patients (Table 8.1).

There are several factors going along with worse functional outcome after surgery (and other treatments) if not taken into consideration. The upper esophageal sphincter (UES) contraction reflex and the sensitivity of the complex swallowing process mainly at the level of the larynx entrance is reduced in advanced ages (Involvement of cranial nerves like vagus, trigeminus, glossopharyngeus, accessories, hypoglossus and plexus cervicalis composite all structures flexible and mobile). Additionally, the trigger to swallow is reduced and as well as protective mechanisms like coughing or harrumphing. Therefore, the danger of silent or definitive aspiration increases and can cause fatal complications like pneumonia. Kawamura

Table 8.1 Practical factors relevant for surgical indication going along with physiological aging [6–10]

• The healing of skin wounds is significantly prolonged
• The compliance of the cardiovascular system is reduced with resulting in hypertension
• The upper esophageal sphincter (UES) contraction reflex is reduced
• The elasticity of the chest wall is reduced
• There is loss of supporting tissue of the pulmonary airways
• There is an altered thermoregulation due to both changes in muscle and fat mass and a reduced metabolism
• The sympathetic activity is decreased
• The compensatory reaction of the autonomic nervous system to stress and volume losses is reduced
• The tolerance to a reduced number of oxygen carriers (Hb value) is reduced
• There is an earlier indication for transfusion than in younger patients
• There is a decreased respiratory drive on hypercapnia and/or hypoxia
• The is a reduced volume of distribution
• There is a decreased hepatic and renal clearance
• There is a higher sensitivity of the central and peripheral nervous system to anesthetics and muscle relaxants
• On average, the need for anesthetics in patients >80 years is about 30% lower than in those aged 20

et al. could demonstrate, that the frequency elicitation of UES contraction reflex decreases significantly with age while the magnitude of change in UES pressure remains unchanged, indicating a deleterious effect of aging on the afferent arm of this reflex. This reflex is altered in some dysphagia patients [7]. This age-related difference in swallowing function, sensibility for aspiration and successful rehabilitation can play a major role in finding the right surgical procedure, like trade off in favor for total compared to partial laryngectomy in elderlies for instance. Especially in supraglottic laryngectomies the positive correlation between increasing age and the occurrence of aspiration pneumonia should be considered. Therefore, preoperative pulmonary function tests (FEV1, VC) can be helpful to consider risk of postoperative aspiration pneumonia [9]. Compared to the relevance of swallowing the quality of voice is less important and of secondary interest for the late quality of life outcome especially in elderlies.

Equally relevant as one of the most significant predictors for successful outcome after surgery in elderly patients is the preoperative lung function. The 30-day mortality rate following upper-airway and thoracic invasive surgery on average is 6% in elderly patients. Up to 50% of the causes of death in these patients are related to pulmonary complications/comorbidities [6]. Improved preoperative preparation (e.g. breathing gymnastics), the development of modern anesthetics as well as the optimized perioperative monitoring (relaxometry, pulse oximetry) have been able to contribute significantly to the fact that the perioperative risk in the old patient is not significantly increased by age itself [6]. In general, pulmonary function decreases with advanced age and can cause major problems if the surgical procedure is not fitting into the performance precondition. Interestingly, restriction of the thorax expansion capacity by delivery of a pectoralis major myocutaneous (PMC) flap (very common procedure in reconstructive head and neck surgery) and tight wound closure of the overlying skin can cause severe problems after surgery. Pulmonary atelectasis has been reported in patients undergoing these procedures, and many of these patients are heavy smokers and drinkers and have associated cardiopulmonary disorders. Flap harvest and donor site closure may lead to impairment of pulmonary function after delivery of pectoralis major myocutaneous (PMC) flap in surgical reconstruction in patients with cancer of the head and neck. Talmi et al. evaluated prospectively patients undergoing extirpation of head and neck tumors with PMC reconstruction. Patient age, smoking history (pack-years), anesthesia duration, percentage predicted pre- and postoperative FEV1, percentage-predicted pre- and postoperative FVC (forced vital capacity), and preoperative SaO_2 (oxygen saturation) were evaluated. A series of 11 patients, 5 of whom smoked, could be evaluated postoperatively. Preoperative FEV1/FVC was more than 70% and FEV1 more than 75% predicted in all patients. A decrease in FVC was observed in seven of the 11 patients, which ranged between 2% and 27% without any clinically obvious respiratory manifestations. A baseline SaO_2 of more than 96% was noted in all patients. Four of nine postoperative chest X-rays demonstrated atelectasis. The authors conclude, that alternative methods of surgical defect closure should be considered in patients with severe preexisting pulmonary disorders [10].

Assessment of Comorbidity for Surgical Treatment

In general prevalence of comorbidities in head and neck cancer patients is of importance, and that is true not only for elderly patients Since chronic abuse of tobacco and alcohol are still the main risk factors for head and neck cancer both factors also cause many other diseases. Pulmonary and cardio-vascular disorders are the main limiting factors for radical and extensive surgical procedures. In elderlies, the mixture of additional age related diseases and tobacco/alcohol related comorbidities could be a complex challenge for indicating the individual treatment in the single patient. Table 8.2 summarizes the frequent surgery-relevant diseases in older age.

Elderly patients (70+ years) have a high prevalence of comorbidity resulting in a high frequency of polypharmacy defined as a daily use of five drugs or more. Jorgensen et al. [11] compared 30,122 cancer cases with 120,485 controls (42.6% >70 years) and found mean drug use of 5.12 in elderlies with cancer 5.12 and 4.07 in controls in general (not specific for head and neck cancer).

In the experience of most head and neck surgeons recovery after extensive surgical procedures is also linked to the age. Patients recovery can be prolonged in elderlies, even when the operation went smoothly. Grammatica et al. published recently a retrospective multi institutional study in the "Older Old (>75)" and "Oldest Old (>85)" undergoing free flaps for advanced oral cancer (the majority of the reconstructions were performed by radial forearm flap and ALT (anterior lateral thigh flap); about 10% had fibula/scapula flaps). Pre-operative assessment was performed by the American Society of Anesthesiologists (ASA) and the Adult Comorbidity Evaluation 27 (ACE-27) scores. Complications after surgery were grouped as medical or surgical, and major or minor according to the Clavien-Dindo scale. The majority (67%) of patient met ASA-3 criteria (severe systemic disease), 63.5% met the ACE-27 score 2 criteria (moderate comorbidity) and 8.3% had severe comorbidity (ACE-27 score 3). 38% had a history of smoking and 47.6% of alcohol abuse. The mean operation time in minutes was 553.5 (range 230–890 min). Overall, 52 (61.9%) patients had at least one complication: ASA score, diabetes mellitus, and duration of general anesthesia (DGA) significantly impacted the complication rate at multivariate analysis. Patients with diabetes suffered from 61.1% complications in contrast to only 31.8% in non-diabetic patients. 20.2% of patients had major

Table 8.2 Frequent surgery-relevant diseases in older age [6]

• Arteriosclerosis
• Lung emphysema
• Chronic obstructive pulmonary disease
• Malnutrition
• Diabetes type II
• Osteoporosis
• Parkinson's disease
• Alzheimer's disease
• Dementia

surgical complications (11% flap necrosis). 10.7% of patients had major medical complications; Smoking and ASA-4 category showed close-to-significance p-values in multivariate analysis. The authors conclude that lengthy DGA (pivotal factor) and in-hospital stay should be carefully considered especially when dealing with advanced age patients. Therefore, surgical teams should be encouraged to reduce the duration of surgery by operating with ablative and reconstruction teams simultaneously. Pre-operative assessment and aggressive management of glycemia in patients with diabetes is mandatory [12].

Keeping an eye on the factor "duration of general anesthesia" which is prognostic for complications in elderlies, some surgical procedures should be balanced by extent of resection, degree of reconstruction and safety. For example, some borderline stages of larynx carcinomas, which could be successfully treated by partial laryngectomy in younger patients but with high risk of long time aspiration in elderlies, could be considered for total laryngectomy or primary chemoradiation. Laryngectomy can be conducted in a short time with minimal trauma by preserving very carefully the surrounding tissues and keeping the operation field very small. Indication management for elective Neck dissection in N0-situations in elderlies can be cautious. Compared to primary chemoradiation this surgical approach is quick, guarantees complete tumor removal und ends up with less late dysphagia for instance.

Age and Special Surgical Procedures

Although the finding that age in regard of surgical procedures is not an independent negative prognostic factor in head and neck carcinoma patients, there is a widespread mental conflict that suggests that you are better off at being young. Most older patients and their families are more reluctant to agree to major surgical interventions [13]. Overall, there are no prospective randomized studies explicitly addressing age and standard of care in head and neck cancer surgery.

Before the 1960s, the operative mortality rate for elderly patients undergoing elective surgery was two to six times higher than that in the general population [14]. In the early 1970s, McGuirt et al. published the first data addressing head and neck surgical outcome in elderlies [15]. The incidence of both major and minor surgical complications was comparable between the cohorts above and below 70 years of age. However, medical complications, mostly of cardiovascular and pulmonary origin, were higher by 8% in the elderly subgroup. Perioperative mortality rates, defined as death within 30 days of operation, were 7.4 and 1.4% in older and younger 70 years subjects, respectively [15]. According to the literature, age "per se" is not an independent contraindication for a surgical intervention in tumors of the head and neck area [16]. Claymen et al. stated in 1998 based on his data in a small retrospective study that although the older age group (>80) had a higher frequency of morbid preoperative conditions, there were no significant differences in perioperative or postoperative complications between the two groups. Careful preoperative staging and evaluation of associated medical illnesses, as well as skillful

perioperative and postoperative management, are essential for reducing operative morbidity and mortality [17].

In general, all head and neck procedures should be adjusted to the functional status of the individual patient as already mentioned in this chapter. Therefore, the multidisciplinary team should include specialists like phoniatricians, voice specialists and logopedics to asses preoperative swallowing competence and other functional relevant factors. In this context FEES (functional endoscopic evaluation of function) became one of the main investigations as part of the functional staging of the (not only) elderly patient.

Free flap procedures are feasible independent of age, as already mentioned in this chapter (Grammatica). Also, Tarsitano et al. presented data about safety of microsurgical free-tissue transfer and described the rates of major surgical complication being 9% in young patients and 11% in elderly patients (>75). They found no significant difference between the two groups in the rates of major and minor flap complications, morbidity or long-term functional outcome [18].

However, the mortality rate in elderly patients has declined in the past 40 years. Today, the overall surgical mortality rate is about 0.9–2.4%, even for patients with cardiac disease, largely as result of safer anesthesia techniques [19]. Both the studies reported by Taristano et al. and Grammatica et al. showed that the ASA score (not age) in multivariate analysis was the only variable associated with an increased complication rate. The perioperative mortality was addressed in a large retrospective study of 810 patients aged over 65 years, where the rate was calculated at 3.5% [20]. Smaller series later published by other investigators showed similar findings even in the oldest-old category [1, 2].

Conclusion

Head and Neck cancer surgery is feasible independent of age. The preoperative assessment of comorbidity in elderly patient in order to avoid surgical complications is of major importance. Equally relevant as one of the most significant predictors for successful outcome after surgery is the preoperative lung function and the operation time. Close coordination with anesthesia and rapid postoperative mobilization are essential for this. Decision-making and treatment based on specific assessment in an experienced multidisciplinary team is key.

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Chapter 9
Contemporary Opportunities in Nonsurgical Management of Locoregionally Advanced Head and Neck Squamous Cell Carcinoma

Shao Hui Huang, Avinash Pilar, Jishi Li, Zhiyuan Xu, and Brian O'Sullivan

Introduction

Mucosal head and neck squamous cell carcinoma (HNSCC) generally refers to carcinoma arising from the mucosa of the oro-/hypo-pharynx (excluding nasopharynx), larynx, oral cavity, and carcinoma of unknown primary origin presenting with cervical lymph node metastasis (CUP). Over the past decade, the landscape of HNSCC has changed dramatically owing to the rapid emergence of HPV-mediated

S. H. Huang
Department of Radiation Oncology, The Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada

Department of Otolaryngology-Head and Neck Surgery, The Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada
e-mail: shaohui.huang@rmp.uhn.ca

A. Pilar
Department of Radiation Oncology, The Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada
e-mail: avinash.pilar@rmp.uhn.ca

J. Li · Z. Xu
Department of Clinical Oncology, The University of Hong Kong – Shenzhen Hospital, Shenzhen, China
e-mail: lijs@hku-szh.org; xuzy@hku-szh.org

B. O'Sullivan (✉)
Department of Radiation Oncology, The Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada

Department of Otolaryngology-Head and Neck Surgery, The Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada

Department of Clinical Oncology, The University of Hong Kong – Shenzhen Hospital, Shenzhen, China
e-mail: brian.osullivan@rmp.uhn.ca

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[HPV(+)]oropharyngeal carcinoma (OPC) and a steady decrease in smoking-related/HPV-negative [HPV(−)] HNSCC, the latter almost certainly explained by the success of smoking cessation strategies. The 8th edition TNM (TNM-8) now separates HNSCC into two major categories: HPV(+) and HPV(−) HNSCC [1, 2] with different staging classifications. Examples introduced in the TNM-8 include the migration of almost 50% of HPV(+) oropharyngeal cancer (OPC) from Stage IV in the traditional 7th edition TNM to Stage I in the TNM-8, the important introduction of *depth of invasion* in oral cancer that influences migration to more advanced T-categories, and the assignment of node-positive (N+) disease with extranodal nodal extension (ENE) to higher N categories in HPV(−) disease. As a consequence, the semantics of "locoregionally advanced HNSCC" (LAHNSCC) is also evolving.

Achieving locoregional control (LRC) has traditionally been the primary focus of management of LAHNSCC due to the challenge in achieving it with conventional treatment approaches in use in the pre-HPV era. As well, recurrence in this location almost uniformly has significant implications for symptomatology, function, severe morbidity (involving the integrity of airway, neurovascular, and musculoskeletal structure), swallowing, and nutrition in addition to the hardships and risks associated with salvage management. Hence, a long-established sentiment prevailed that LAHNSCC was a "loco-regional disease" and less effort was devoted to negating the risk of distant metastasis (DM). Concurrent *cisplatin*-based chemoradiotherapy (CCRT) has represented the gold standard for organ preservation treatment in LAHNSCC since the publication of the MACH-NC meta-analysis which demonstrated significant improvement in LRC and OS with the addition of chemotherapy to radiotherapy (RT) [3, 4]. Despite this, the LRC rates remain unsatisfactory for many HPV(−) LAHNSCC. About 40% of patients experience locoregional failure (LRF) [5] and less than 50% of HPV(−) LAHNSCC patients survive more than 5 years [6]. Efforts have continued to explore other systematic approaches to enhance LRC in this population.

As is the case for HPV(−) LAHNSCC, HPV(+) OPC is also facing challenges, although of a different nature. While LRC can be achieved in >80% HPV(+) OPCs [5–8], most of these patients received intensified treatment and can expect to live for many years but are vulnerable to severe late toxicities that significantly affect quality of life in many cancer survivors. In addition, DM has emerged as one of the major challenges for this population and approaches confronting this outcome are relatively sparse. To improve the therapeutic ratio of HPV(+) LAHNSCC, the current overall research focus in this population has shifted towards two scenarios: safe de-intensification for the low relapse risk group, while innovative approaches to improve LRC and mitigate the risk of DM remain priorities in the high relapse risk group.

Non-surgical approaches for HNSCC have also evolved in parallel with accumulating knowledge about disease biology and clinical behavior, advances in technologies, availability of novel treatment approaches, and emerging evidence from clinical trials and prospective/retrospective studies. While surgery remains a mainstay in management to ensure local control, and radical RT with/without chemotherapy is similarly hallowed in the overall management philosophy, changes in

approach for different presentations are under evaluation. In this review, we summarize recent research findings in non-surgical approaches for both HPV(+) and HPV(−) LAHNSCC, including revisiting the efficacy of traditional chemotherapy agents, the role of epidermal growth factor receptor (EGFR) inhibition, the potential to refine chemotherapy regimens (including new agents and sequencing), and the combination of immunotherapy with RT.

Definition of LAHNSCC in HPV(+) HNC

LAHNSCC has historically referred to stage III/IV disease that included T3-T4 or any N-positive presentations. In the HPV(+) TNM-8 classification, the TNM-7 T1-T2_N1-N2b subset has been re-classified as stage I disease with very high LRC and low DM risk [5]. Since no stage IV group exists for non-metastatic HPV(+) OPC/CUP, stage II and III would naturally be considered as LAHNSCC. However compelling data indicates that outcome heterogeneity still exists within stage I. Stage I disease with radiologically identified extranodal extension (rENE+) has emerged as a strong prognostic factor for higher DM and mortality risk [9], and a proposal exists to classify it as N3, and therefore stage III disease [10]. By extension it seems prudent to also combine HPV(+) TNM-8 *stage I* disease with rENE+, together with *stage II/III*, as LAHNSCC group.

The nominal components of the main risk strata for HPV(−) LAHNCC have not changed in TNM-8 and continue to refer to stage III and IV disease, including all non-metastatic (M0) HPV(−) LAHNSCC excepting the T1-T2 N0 subset. However, the criteria contributing to individual T and N categories have been refined which has resulted in criterion-based stage modification. A *depth of invasion* (DOI) parameter has been added as a new T-category modifier for oral cavity SCC and migrates so called "thicker" tumours (correspondingly those with higher DOI) to a more advanced T-category. Clinical and pathological descriptors for ENE have also been introduced that assign a higher N-category. Such changes in definitions warrant re-interpretation of historical data and impact present and future clinical trial design.

Trials on HPV(+) LAHNSCC

Revisiting the Role of Cisplatin and Cetuximab in HPV(+) LAHNSCC

With the recognition of HPV(+) HNSCC as a new disease, clinical trials are addressing HPV(+) HNSCC separately from HPV(−) disease. The most established "tool" for LAHNSCC is *cisplatin* chemotherapy combined with RT. *Cetuximab*, an FDA approved EGFR inhibitor, has also been used in LAHNSCC following a randomized

trial (IMCL-9815) that showed superior LRC with *cetuximab* combined with RT compared to RT alone for LAHNSCC; however HPV status was unknown at the time of the trial [11] and the RT outcomes may not reflect the results expected from contemporary precision RT techniques which were unavailable during the period of the trial. The efficacy and toxicity of *cisplatin* and *cetuximab* with RT on HPV(+) HNSCC were recently compared in the two HPV(+) phase-III randomized trials: RTOG 1016 (NCT01302834) [7] (comprising 39% T3-T4 tumours) and De-ESCALaTE HPV (NCT01874171) [8] (comprising 34% T3-T4 tumours). Both trials showed inferior efficacy of *cetuximab* compared to *cisplatin* in HPV(+) OPC, mainly attributable to higher LRF with *cetuximab*. The differential effect on DM reduction with *cisplatin* versus *cetuximab* was significant in De-ESCALaTE HPV (2-year DM: 3% vs. 9%, p = 0.009) but marginal in RTOG 1016 (5-year DM: 8.6% vs. 11.7%, p = 0.09). Regarding outcomes according to TNM-8, the De-ESCALaTE HPV trial showed that the differential effect of *cisplatin* vs. *cetuximab* exists in both stage I/II (98.4% vs. 93.2%, p = 0.043) and stage III diseases (2-year OS: 93.3% vs. 67.1%, p = 0.030). The toxicity profile also did not favor *cetuximab*. The failure of *cetuximab* to optimize outcomes in the loco-regional treatment of HPV(+) OPC is probably not surprising when one considers that HPV(+) OPC rarely expresses EGFR [12]. An additional intriguing observation of the RTOG 1016 trial is the relatively high LRF in the *cetuximab* arm compared to other reported outcomes with RT alone in HPV(+) cohorts [13, 14]. Compromised outcomes of *cetuximab* in HPV(+) OPC was also observed in the RTOG 0522 trial (NCT00265941) [15]. It showed a trend towards higher hazard ratio (HR 1.57, p = 0.12) with the addition of *cetuximab* to *cisplatin* chemotherapy which was opposite to that found with HPV(−) OPC (HR 0.86, p = 0.31). These paradoxical observations raise an unsubstantiated possibility for *cetuximab* to be interfering with radiosensitivity in the treatment of HPV(+) OPC.

Notwithstanding any additional nuances, both aforementioned phase III trials have cemented the place of *cisplatin* as a potent radiosensitizer to enhance LRC although it is less effective in abrogating the risk of DM. *Cisplatin* combined with RT remains the gold standard for the treatment of HPV(+) LAHNSCC while *cetuximab* is not suitable for this disease. Several important questions regarding chemotherapy remain unresolved. For example, there is no robust data to indicate which subgroups of patients truly benefit from *cisplatin* chemotherapy and there remains uncertainty about the optimal dose of *cisplatin* for HPV(+) OPC patients. A retrospective study suggests that a cumulative dose of *cisplatin* >200 mg/m^2 seems necessary for TNM-8 stage III (T4 or N3) HPV(+) OPC [16]. Another prominent question is whether weekly *cisplatin* is equally effective compared to three-weekly high dose *cisplatin* (a trial is currently under development). The NRG HN-002 trial (NCT02254278) showed that weekly *cisplatin* with reduced RT dose (60 Gy in 30 fractions, 5 fractions per week) is very effective for T1-T3N0-N2b HPV(+) OPC minimal smokers (<10 pack-year smoking) with 2-year progression free survival of 90.5% [17]. However, the trial shows that, while LRC is achievable with *cisplatin* combined with a modest RT dose reduction, *cisplatin* also appears to be less potent in fully mitigating DM risk. For example, the RTOG 0234 trial, although without knowledge of HPV status, showed that *docetaxel* in combination with cetuximab seemed more effective compared to *cisplatin* with cetuximab in DM reduction (2-year DM: 13% vs. 25%) in the

postoperative setting of general LAHNSCC [18]. In essence, more effective systemic agents are needed to eradicate microscopic metastasis overall and in HPV(+) LAHNSCC due to the prominence of this end-point in the management of the disease.

Refining "Old Tools" for HPV(+) LAHNSCC: Dose, Fractionation, and Volumes

Although most HPV(+) LAHNSCC have good outcomes, RT intensification is still needed for a subset of HPV(+)LAHNSCC. In addition to *cisplatin* radiosensitization, other traditional intensification "tools" include hyper-fractionation with augmented RT doses, shortened overall treatment time (acceleration) [19], or hypoxia modification (e.g. nimorazole combined with radiotherapy). Studies have shown that an acceleration using six fractions compared to five fractions per week improved the outcome of HPV(+) OPC [20]. The NRG HN-002 trial (NCT02254278) also indicated that even in "low-risk" minimal smoking N0-N1 HPV(+) OPSCC, modest dose intensification by fractionating 60 Gy in 30 fractions over 5 weeks (6 fractions per week) rather than 6 weeks for the treatment period may still be beneficial [17]. Hypoxia modification has not shown effectiveness in HPV(+) OPC although it improves outcomes in HPV(−) LAHNSCC [21].

Another traditional "tool" under active study in HPV(+) OPC is refining the elective RT volumes. Villaflor et al. [22] conducted a phase II trial and showed that volume reduction (omitting the elective volume that ordinarily treats regions of the neck that are not overtly involved by disease) in patients with complete or partial response (amounting to at least a 50% volume reduction) after induction chemotherapy appears to be safe. Patients in the subsequent OPTIMA trial [23] also received risk-stratified dose-volume reduction and de-escalated RT volumes which were limited to the first echelon of uninvolved nodes with promising results. Long-term follow-up of the trial patients with additional patients treated following OPTIMA outlines presented in ASCO 2020 confirmed safety and excellent functional outcomes with this approach [24]. The HN10 trial (NCT03822897) of the Canadian Clinical Trials Group (CCTG), a phase II single-arm trial of Elective Volume Adjusted De-Escalation Radiotherapy (EVADER) for TNM-8 stage I-II HPV(+) OPSCC is currently recruiting and adjusts the prophylactic RT neck volumes according to the initial sites of disease presentation (e.g. the presenting subsite in the oropharynx, laterality of the primary site, and the extent of neck node disease).

Addressing Distant Metastasis Endpoint: Role of Induction Chemotherapy

Induction chemotherapy has been proven to be effective in DM reduction in nasopharyngeal carcinoma [25, 26], another viral-related pharyngeal cancer. GP (*gemcitabine-cisplatin*) induction chemotherapy appears to have similar efficacy in

DM reduction with lower grade 3–4 toxicities compared to the TPF (docetaxel-cisplatin-fluorouracil) regimen. However, the role of induction chemotherapy in HPV(+) OPC is yet to be defined. The phase III DeCIDE trial (NCT00117572) [27] compared TPF induction chemotherapy followed by cisplatin-CCRT vs. cisplatin-CCRT alone in N2-N3 HNSCC [61% were OPC, of which the majority were HPV(+)]. The induction chemotherapy cohort showed a significant reduction in DM as the first site of failure ($p = 0.043$), but this difference did not translate into an OS difference. A possible reason is that the trial was based on the TNM-7 classification and many N2 HPV(+) OPC enrolled in the trial had traditional N2b disease with T1-T2 categories which today would be considered low risk by TNM-8. In turn this could have diluted a putative benefit of induction chemotherapy. Similarly, the phase III PARADIGM trial (NCT00095875) [28] investigated the role of TPF induction chemotherapy followed by carboplatin-CCRT vs. cisplatin-CCRT alone in LAHNSCC (tumour HPV status was not tested) and also did not find a survival benefit. The trial was terminated early due to slow accrual. More recently, the single-arm phase II ECOG 1308 trial [29] and the OPTIMA trial [23] both suggested a promising role for induction chemotherapy in DM risk reduction, as well as a risk stratification tool for refining subsequent treatment including, most importantly, the potential to administer a less intense locoregional approach in appropriately responding cases following the induction regimen.

Quest for Additional Risk Stratification Parameters

Although TNM-8 stratifies HPV(+) OPC patients' prognosis better than TNM-7, it is recognized that outcome heterogeneity exists, especially in stage I disease [9]. Recently, rENE+ was observed to carry strong prognostic value, mainly impacting DM. A resulting proposal considers that all cases with rENE+ should be classified as N3b disease since it portends higher risk of DM and worse OS among all non-metastatic (M0) HPV(+) OPC [10]. The study also found that the addition of cisplatin could negate the LRF risk with rENE+ but does not appreciably negate DM risk. Therefore, strategies addressing the DM endpoint are urgently needed. One of the challenges of implementing rENE+ as a risk stratification factor is how to reliably assess rENE+. For example, "conglomerate", "matted" nodes, and "coalescent" nodes could all indicate evidence of rENE+ in addition to irregular nodal borders and adjacent structure invasion [10]. Radiologist training and standardization of taxonomy is needed. Computer-assisted intelligent machine learning may also enhance sensitivity and objectivity in recognizing rENE+ [30, 31]. Notably, the need to restrict the designation of rENE+ to only cases with obvious and unequivocal criteria is potentially important. "*Overcall*" of rENE+ by inclusion of cases where extranodal disease is either not actually present or of minimal degree could obscure the very deleterious true impact of unequivocal rENE+, especially on DM and mortality. Recent evidence suggests that the associated risk surpasses that of other accepted prognostic factors, including TNM stage and its categories, and smoking history.

Besides rENE+, researchers are also investigating other biomarkers for risk stratification of HPV(+) LAHNSCC. Dynamic biomarker such as the pre-treatment tumour growth velocity [32], response to induction chemotherapy [29], or the temporal pace of morphological [33] and functional (by FDG PET or hypoxia imaging) [34] volume reduction during the early phase of the RT course are potential candidates for risk stratification and merit investigation with response-adapted clinical trials.

HPV genotyping may also have a potential role for risk stratification. High-risk HPV includes α-7 HPV subtype (e.g. HPV-18, 39, 45) and α-9 HPV subtype (e.g. HPV-16, 31, 33, 35) [35–37]. The majority (>95%) of HPV(+) OPC is caused by HPV-16 followed by HPV-35 and HPV-31, and rarely by HPV-18 and HPV-45 [38–41]. Emerging data suggest that patients with an α-7 HPV subtype (e.g. HPV-18) OPC do not have as good prognosis as those caused by an α-9 HPV subtype (e.g. HPV16, 31, 33, 35, etc.) [35–37]. Whether a patient with α-7 HPV driven OPC should be excluded from de-intensification trials remains to be determined.

Liquid biopsy has shown a promising role in risk stratification as well. A recent report from Fakhry et al. [42] showed that oral HPV DNA viral load detected using oral rinse decreased rapidly with therapy, and persistent detection was associated with increased risk of recurrence and death. Analysis of tumour HPV DNA holds considerable promise as a biomarker for treatment response and risk of progression. Chera et al. [43] demonstrated the potential role of plasma circulating HPV DNA in disease surveillance.

Emerging Role of Immunotherapy in HPV(+) LAHNSCC

Emerging evidence suggests that the host immune system plays a significant role in the outcome of cancer patients. HPV(+) OPC is an immunogenic tumour [44, 45], rendering it a potential target tumour site for immunotherapy. A recent study revealed that a majority of HPV(+) OPC had PD-L1 overexpression, especially those with a minimal smoking history (93%), and was higher than in HPV(−) OPC (70%) although the prognostic value of the finding remains uncertain [46].

Currently, available immunotherapy strategies include passive immunotherapy (e.g. immune checkpoints inhibitors, immune co-stimulatory antibodies, tumor-infiltrating lymphocytes, and chimeric antigen receptor [CAR] T cells) and active immunotherapy (e.g. vaccines, immune adjuvant cytokines, and oncolytic viruses) [47]. Thus far, immune checkpoint inhibition is the most commonly investigated immunotherapy strategies for HNSCC. Several strategies exist to block the intrinsic inhibitory immune checkpoint pathways. For example, programmed cell death protein (PD-1)/programmed death-ligand 1 (PD-L1) pathway blockade restores the activity of anti-tumour T cells that have become dormant while cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) blockade allows for activation and proliferation of more cytotoxic T-cell clones and reduces T-cell mediated immunosuppression. PD-1 blockade has shown promising results in the recurrent/metastatic

setting [48–51], which prompted approval of *nivolumab* or *pembrolizumab*, both PD-1 inhibitors, by the Food and Drug Administration (FDA) for treatment of recurrent/metastatic HNSCC.

Theoretically, radiotherapy can be synergistic with immunotherapy to enhance its effect [52]. For example, RT may prime the immune system to release and/or expose tumour-specific antigens to elicit tumour-specific T cell responses [52, 53]. Conversely, RT could also suppress the immune system when a high RT dose is delivered to large volumes of hematologic cells [54]. The balance of priming or suppressing the immune response may depend on RT dose, fraction size, delivery time, as well as the irradiated volume. Not surprisingly, the focus has shifted to novel approaches including investigation of the role of immunotherapy combined with RT in the definitive setting (Table 9.1). KEYNOTE 412 (NCT03040999), a phase III trial (n = 780), examined the addition of pembrolizumab to CCRT compared to CCRT alone for LAHNSCC, including T4 or N3 HPV(+) OPC and p16-negative stage III/IV (except TNM-7 T1-T2N1) OPC and larynx/hypopharynx/oral cavity SCC. The trial has completed recruitment and results are awaited. The JAVELIN Head and Neck 100 trial (NCT02952586) (n = 697) [55] was designed to evaluate the addition of *avelumab* (a PD-L1 inhibitor) to CCRT for LAHNSCC including HPV(+) T4 or N2c-N3 (TNM-8 stage II/III) disease and stage III/IV HPV(−) LAHNSCC. However, an interim analysis of the trial results suggested a lack of efficacy leading to termination of accrual [56]. Nonetheless, such trials may be able to shed light on whether PD-L1 expression is a harbinger of adverse prognosis, while at the same time confer useful prediction by indicating a possible benefit of anti-PD-L1 immunotherapeutic agents.

Besides PD-1/PD-L1 inhibition (thereby blocking immune-suppressing ligands) that unleashes T-cell anti-tumour function, CTLA-4 blockage could enhance T-cell activation and is also under evaluation in HPV(+) OPC. Since PD-1/PD-L1 and CTLA-4 block different target pathways, it is hypothesized that targeting both PD-1/PD-L1 and CTLA-4 pathways may have additive or synergistic activity, although toxicity is unknown. One such trial is the CTTG HN.9 trial (NCT03410615) which was designed with the intent of comparing two arms containing RT with either concurrent-adjuvant *durvalumab* (PD-L1 inhibitor) versus *durvalumab* and *tremelimumab* (CTLA-4 inhibitor) compared to a third arm comprising standard of care *cisplatin*-CCRT. Several EORTC centers are also currently joining this trial. Notably, the *tremelimumab* arm has been terminated prematurely due to excessive adverse events.

Research in HPV(−) LAHNSCC

In contrast to the numerous trials and a variety of investigational approaches targeting HPV(+) HNSCC, the trial arena for HPV(−) HNSCC remains relatively quiet. The outcome of HPV(−) LAHNSCC with the current standard of care (high dose cisplatin-CCRT) remains unsatisfactory. Novel strategies are urgently needed for

Table 9.1 Selected phase II and III immunotherapy trials for non-metastatic LAHNSCC

Trial name (Starting year)/Sample Size	Eligibility criteria	Arms and interventions	Outcomes
Trials addressing HPV-positive oropharyngeal carcinoma			
NCIC CTG HN.9 NCT03410615 **(2018)** Phase II noncomparative study N = 180	**Intermediate risk** HPV(+) OPC (TNM-8): – T1–2 N1 (smoking ≥10 PY) – T3 N0-N1 (smoking ≥10 PY) – T1–3 N2 (any smoking history).	• **Arm 1** (comparator): RT (70 Gy/35f/7w) + CDDP days 1, 22 and 43 • **Arm 2** (experimental): RT (70 Gy/35f/7 week) + IO (durvalumab on days 7, 22) + Adj IO (durvalumab × 6 cycles) • **Arm 3** (experimental): RT (70Gy/35fr/7 week) + IO (durvalumab on days 7, 22) + Adj IO (durvalumab+ tremelimumab) → closed to accrual due to AE	**Primary endpoint** – Event-free survival **Secondary endpoints** – QoL (FACT-HN) – Acute and late toxicity – LRC, DMFS – Cost effectiveness of IO – Dysphagia scores
HCC 18-034 NCT03715946 **(2018)** Phase II N = 135	**Resectable intermediate risk** HPV(+) OPC (TNM-7): • **Tobacco< 10 PY, T0–3**: – >N2b (>5 LN +), or – N2c/N3, or – ENE+ (>1 mm) or margin+ • **Tobacco> 10 PY, T0–3**: – Any N2/N3, or – ENE+ (>1 mm) or margin+	Trans oral surgery followed by de-intensified adj RT (45–50 Gy/25f, 6f/w) + IO (nivolumab × 6 cycles)	**Primary endpoint** – Progression-free survival **Secondary endpoints** – PEG tube dependence – Acute and late toxicity – QoL (FACT-HN) – LRC, DMFS
NRG-HN005 NCT03952585 **(2019)** Phase II/III N = 711	p16(+) OPC with **low-risk** features (TNM-8): T1–2, N1 or T3, N0 with smoking <10 PY	• **Arm 1** (comparator): RT (6f/w × 6 weeks) + CDDP (days 1, 22) • **Arm 2** (experimental): Reduced dose RT (5f/w × 6 weeks) + CDDP (days 1, 22) • **Arm 3** (experimental): Reduced dose RT (6f/w × 5 weeks) + IO (nivolumab × 6 cycles)	***Phase II*** **Primary endpoint** – PFS ***Phase III* (1 or 2 experimental arms from the phase II)** **Co-primary endpoints** – Non-inferiority of PFS – Superiority of QoL (MDADI) **Secondary endpoints** – LRC, DMFS – Acute and late toxicity – QoL (EORTC QLQ-30)

(continued)

Table 9.1 (continued)

Trial name (Starting year)/Sample Size	Eligibility criteria	Arms and interventions	Outcomes
Trials addressing HPV-negative head and neck squamous cell carcinoma			
IMSTAR-HN (2018) NCT03700905 **(2018)** Phase III N = 276	**Resectable**, **p16(−)** LAHNSCC (OPC/larynx/hypopharynx/Oral) planned for surgery: – Any T3-T4, N0-N3, M0 – Any N2a-N3, T1-T4, M0	• **Arm 1** (comparator): Surgery + SOC Adj therapy (low-risk: adj RT: high-risk: adj CRT) • **Arm 2** (experimental): Neoadjuvant IO (nivolumab) + Surgery + SOC Adj + Adjuvant IO (nivolumab × 6 months in Arm 2a; nivolumab+ipilimumab × 6 months in Arm 2b)	**Primary endpoint** – DFS **Secondary endpoints** – LRC, DMFS, OS – Acute and late toxicity QoL (EORTC QLQ-30)
ADHERE EORTC 1735-HNCG NCT03673735 **(2019)** Phase III N = 650	**Resected**, non-metastatic p16(−) LAHNSCC with **high-risk** features (TNM-8): • OPC/larynx/hypopharynx/Oral: – pStage III-IVA **High-risk features include:** ENE and margin+ (<1 mm)	• **Arm 1** (comparator): Post-op CRT (66 Gy/6.5w + CDDP days 1, 22 and 43) + placebo (one dose before CRT and for 6 months after CRT) • **Arm 2** (experimental): Post-op CRT (66 Gy/6.5w + CDDP days 1, 22 and 43) + IO (durvalumab one dose before CRT and for 6 months after CRT)	**Primary endpoint** – PFS **Secondary endpoints** – LRC, DMFS, OS – Acute and late toxicity – Quality of life measures (EORTC-QLQC 30 and HN 35)
Trials addressing both HPV-positive and HPV-negative head and neck squamous cell carcinoma			
JAVELIN HEAD AND NECK 100 NCT02952586 **(2016)** Phase III N = 697 **Note:** The trial was stopped in March 2020following interim analysis	LAHNSCC planned for CRT (TNM-7): • **p16(+) OPC**: – T4, N0-N3, M0 – N3, T1-T4, M0 • **p16(−) OPC**: – Any T3-T4, N0-N3, M0 – any N2a-N3, T1-T4, M0 • **Larynx/hypopharynx/oral**: – Any T3-T4, N0-N3, M0 Any N2a-N3, T1-T4, M0	• **Arm 1** (comparator): RT (70 Gy/35f/7w) + concurrent CDDP (days 1, 22, 43) + concurrent placebo (start with RT and for 12 months) • **Arm 2** (experimental): RT (70 Gy/35f/7w) + CDDP (days 1, 22, 43) + concurrent IO (avelumab start with RT and for 12 months)	**Primary endpoint** – PFS **Secondary endpoints** – OS, LRC, DMFS – Acute and late toxicity – Response rates

KEYNOTE 412 NCT03040999 **(2017)** Phase III N = 780	LAHNSCC planned for CRT: • **p16(+) OPC**: – T4, N0-N3, M0 – N3, T1-T4, M0 • **p16(−) OPC**: – Any T3-T4, N0-N3, M0 – Any N2a-N3, T1-T4, M0 • **Larynx/hypopharynx/oral**: – Any T3-T4, N0-N3, M0 – Any N2a-N3, T1-T4, M0	• **Arm 1** (comparator): RT (70 Gy/35f/7w or 6w) + CDDP × 2–3 cycles + placebo (17 cycles, 1 before RT, 2 during and 14 after) • **Arm 2** (experimental): RT (70 Gy/35f/7w or 6w) + CDDP × 2–3 cycles + IO (pembrolizumab × 17 cycles, 1 before RT, 2 during and 14 after)	**Primary endpoint** – Event-free survival **Secondary endpoints** – OS – AE – QoL (EORTC-QLQC 30 and HN 35)
REACH GORTEC-2017-01 NCT02999087 **(2017)** Phase III N = 688	LAHNSCC planned for CRT (includes both **p16(+)** and **p16(−)** (TNM-7): Stage III-IVB Oral cavity/OPC/larynx/hypopharynx	• **Arm 1** (comparator): RT (70Gy/33 f/6.5w) + concurrent CDDP (days 1, 22 and 43) • **Arm 2** (experimental): RT (70 Gy/33f/6.5w) + cetuximab (weekly × 8 cycles) + concurrent/adj avelumab (starting with RT × 12 months) • **Arm 3** (comparator- unfit patients): RT (70 Gy/33f/6.5w) + concurrent cetuximab (weekly for 8 cycles) • **Arm 4** (experimental-unfit patients): RT (70 Gy/33f/6.5w) + cetuximab (weekly for 8 cycles) + concurrent/adj IO (avelumab starting with RT × 12 months)	**Primary endpoint** – PFS **Secondary endpoints** – OS – Adverse events – QoL (EORTC-QLQC 30 and HN 35)
KEYNOTE 689 NCT03765918 **(2018)** Phase III N = 704	**Resectable**, LAHNSCC planned for surgery (TNM-8): • **p16(+) OPC**: – Stage III (T4, N0-N2) • **p16(−) OPC/larynx/hypopharynx/oral**: – Stage III-IVA	• **Arm 1** (comparator): Surgery + SOC Adj therapy (low-risk: adj RT: high-risk: adj CRT) • **Arm 2** (experimental): Neoadjuvant IO (pembrolizumab × 2 cycles) + surgery + SOC Adj therapy + Adj IO (pembrolizumab × 15 cycles)	**Primary endpoint** – Major pathologic response – PFS **Secondary endpoints** – OS – pCR – Adverse events – QoL (EORTC-QLQC 30 and HN 35)

(continued)

Table 9.1 (continued)

Trial name (Starting year)/Sample Size	Eligibility criteria	Arms and interventions	Outcomes
NIVOPOSTOP GORTEC 2018-01 NCT03576417 **(2018)** Phase III N = 484	**Resected**, LAHNSCC with **high-risk*** features (TNM-8): • **Oral cavity/OPC/larynx/ hypopharynx**: – pStage III-IVA • **p16(+) OPC**: – pT3-T4, N1, >20 PY * **High-risk features include:** – ENE, multiple PNI, multiple pN+ (≥4), margin+ (≤1 mm)	• **Arm 1** (comparator): Adj CRT (66 Gy/6.5w + CDDP days 1, 22 and 43) • **Arm 2** (experimental): Adj CRT (66 Gy/6.5w + CDDP days 1, 22 and 43) + IO (nivolumab on days 1, 22 and 43)	**Primary endpoint** – DFS **Secondary endpoints** – OS – Acute and late toxicity – QoL (EORTC-QLQC 30 and HN 35)
NRG-HN004 (2017) NCT03258554 Phase II/III N = 523	**CDDP ineligible**, LAHNSCC planned for RT (TNM-8): • **p16(+) OPC**: – Stage III (T4, N0-N2) – Stage I-II (selected based on smoking status) • **p16(−) OPC/larynx/ hypopharynx/oral**: – Stage III-IVB	• **Arm 1** (comparator): RT (70 Gy/35f/7w) + concurrent cetuximab (×8 weekly cycles) • **Arm 2** (experimental): Standard RT (70Gy/35fr/7 week) + concurrent IO (durvalumab for 7 four-weekly cycles)	***Phase II*** **Primary endpoint** – PFS – Dose-limiting toxicity ***Phase III*** **Primary endpoint** – OS **Secondary endpoints** – LRC, DMFS – Acute and late toxicity – QoL (EORTC QLQ-30/HN-35, MDADI, EQD-5)
WO40242 NCT03452137 **(2018)** Phase III N = 400	Non-metastatic, **LAHNSCC** completed definitive local therapy [includes both **p16(+) and p16(−)**]	• **Arm 1** (comparator): Adj placebo (×16 cycles) • **Arm 2** (experimental): Adj IO (atezolizumab × 16 cycles)	**Primary endpoint** – PFS – OS **Secondary endpoints** – Adverse events – QoL (EORTC-QLQC 30)

HPV(+) Human papillomavirus-positive, *OPC* Oropharyngeal cancer, *p16(+)* p16 positive, *p16(−)* p16 negative, *LAHNSCC* Locally advanced head and neck squamous cell carcinoma, *PY* Pack-year, *adj* Adjuvant, *RT* Radiotherapy, *CDDP* Cisplatin, *IO* Immunotherapy, *CTx* Chemotherapy, *CRT* Chemoradiotherapy, *ENE+* Extranodal extension positive, *margin+* Margin positive, *SOC* Standard of care, *70 Gy/35f/7w* 70 Gy in 35 fractions delivered over 7 weeks, *f/w* Fractions per week, *AE* Adverse event, *QoL* Quality of life, LRC Locoregional control, *DMFS* Distant metastatic-free survival, *PFS* Progression-free survival, *OS* Overall survival, *pCR* Pathologic complete response

this population. Several immunotherapy trials targeting both HPV(+) (TNM-8 stage II/III) and HPV(−) LAHNSCC (TNM-8 stage III/IV) were described earlier and results are awaited. Recent genomic studies show that molecular alterations in HPV(−) LAHNSCC are common, which may provide valuable targets for immunotherapy. Another strategy is the investigation of mutated p53 [57, 58] and studies addressing novel pathways, such as Wee-1, are relevant in this regard [59, 60] as mentioned below in discussing *Window of Opportunity* trials.

Window of Opportunity Trials Exploring Targeted Agents, Including immunotherapy

One of the more active and potentially rewarding research areas for HPV(−) HNSCC is in the *Window of Opportunity* trial paradigm. *Window of opportunity* trials are studies where patients receive one or more new compounds between the time of cancer diagnosis and initiation of standard (mainly surgery) or investigational treatment [61]. It leverages the potentially idle time before treatment is initiated to investigate novel agents without significantly delaying the standard of care therapy [62]. Treatment response assessment can, therefore, be based on pre- and post- investigational treatment imaging and biopsy. *Window of opportunity* trials may, therefore, improve our understanding of pharmacodynamic parameters, and help to identify biomarkers for better patient selection. Oral cavity SCC is an ideal disease site for such trials. Several immunotherapy *Window of Opportunity* trials are ongoing (Table 9.1). The "*WISTERIA"* trial (RG_15–139, NCT03028766) [35] is evaluating the role of AZD1775 (a small molecule WEE1 inhibitor), administered before and after surgery in patients with LAHNSCC. The "*SNOW-001*" trial (NCT03575598) is another example in which the role of sitravatinib (a tyrosine kinases inhibitor) is evaluated combined with nivolumab administered before surgery in oral cavity SCC.

Hypoxia Modification and Smoking Cessation

Hypoxia has been identified as a contributor to radio-resistance and LRF in HNSCC [63, 64]. Several methods have been investigated to overcome this problem [65] but without broad success [66]. For example, investigators have attempted to reduce hypoxia by blood transfusion [67] or by the administration of erythropoietin [68, 69] with RT, but disappointingly found such efforts to be not only unhelpful but apparently deleterious. Conversely, hypoxic cell radiosensitizers (e.g. nimorazole) combined with RT enhanced its effectiveness [21, 70–72] but the effect appears to be confined within the HPV(−) LAHNSCC subgroups with hypoxic tumours [21, 72]. A similar phenomenon was also observed in the TROG 0202 trial which tested the addition of tirapazamine, a hypoxic cell cytotoxin, with CCRT [73]. However,

identifying patients with hypoxic tumours prior to RT is challenging. Various hypoxia gene signatures have been proposed although their value and availability remain to be determined [74–76] and trials addressing them have proved challenging, including tight turn-around time for the assay in different jurisdictions (especially if these are remote from the testing facility), and more recently competition with other strategies for the same patient groups (most obviously related to the recent provocative developments focusing on immunotherapy).

Perhaps, one of the most potent and available strategies to tackle tumour hypoxia is smoking cessation. Studies have shown that current smokers have the highest risk of disease recurrence and toxicity from RT compared to "never smokers" [77–80]. Evidence exists that smoking cessation could reverse blood hypoxia levels immediately to the level of "never smokers" and the LRC of such "recent quitters" appears to revert to a similar level as "never smokers" [81]. It seems imperative for radiation oncologists and health care professionals to evaluate the smoking history in HNSCC patients and promote smoking cessation strategies at the initial consultation as well as subsequently in the patient experience. The majority of current smokers appear prepared to discuss smoking cessation and accept therapy [82].

Patients Unfit for Chemotherapy

As noted, outcomes of HPV(−) LAHNSCC are unsatisfactory even with full intensity (300 mg/m^2) of high dose cisplatin CCRT. Many (>60%) are unable to receive full chemotherapy intensity [16, 83] or unable to tolerate chemotherapy at all due to poor general condition including organ (e.g. liver, kidney, cardiac) impairment, older age or frailty, and other comorbidities [84]. Moreover, elderly patients may not benefit from chemotherapy to the same degree [3]. Options are limited and novel approaches are needed in this under-investigated subset of LAHNSCC. Immunotherapy has emerged as a potential tool to improve outcome due to its different toxicity profile compared to traditional systemic treatments. Recently, the NRG HN-004 trial (NCT032558554) has been initiated to address this population, including both HPV(+) and HPV(−) cases. This randomized phase II/III trial is investigating the role of durvalumab (PD-L1 inhibitor) with RT compared to cetuximab with radiation for LAHNSCC who are unable to receive cisplatin due to contraindications.

Conclusion

The landscape of LAHNSCC has changed and requires different trial questions. The disease is now generally divided into two major types: HPV(+) and HPV(−). Risk stratification (staging) and new parameters (e.g. ENE determined clinically or radiologically and pathologically) can facilitate new trial designs by enriching trial

populations for the treatment under investigation, but also minimizing dilution of effect by exclusion of patients who are unlikely to require the intervention under study. Trials are addressing HPV(+) and HPV(−) LAHNSCC separately under the same principles of risk refinement and treatment optimization. Active research areas for non-surgical approaches include choice of RT dose/fractionation/volumes and combinations/sequences of systemic agents with radiation. Novel systemic agents, especially immunotherapy agents, are emerging but their role in the definitive treatment setting remains to be refined. *Window of Opportunity* trials may facilitate patient selection, identify potential therapeutic targets, and expedite drug development. A proportion of patients with LAHNSCC are unsuited for chemotherapy, such as the elderly and the frail, and may need different approaches but trials addressing these patients' needs are at a nascent phase. Efforts in this area will guide future treatment strategies in order to enhance oncologic and functional outcomes of our vulnerable head and neck cancer populations.

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Chapter 10
High-Dose Three-Weekly or Low-Dose Weekly Cisplatin during Radiation, What to Prefer?

Petr Szturz and Jan B. Vermorken

Introduction

Since 1990s, chemoradiotherapy has been fulfilling an important role in the management of locally (and/or regionally) advanced squamous cell carcinoma of the head and neck (LA-SCCHN). In laryngeal and hypopharyngeal cancers, the first-generation trials on organ preservation demonstrated that induction chemotherapy followed by radiotherapy could spare total laryngectomy in more than half of patients without jeopardizing overall survival [1, 2]. About a decade later, the second-generation trials compared different administration schedules of chemoradiotherapy, and the Radiation Therapy Oncology Group (RTOG) 91–11 study showed the highest yields of larynx preservation when chemotherapy and radiotherapy had been delivered concurrently [3, 4]. In the same period, the latter schedule emerged as a preferred alternative to radiotherapy alone in unresectable cases without distant metastases and after surgical removal of locoregionally advanced disease in the presence of close or positive margins or extracapsular spread [5–7]. Subsequently, the recommended standard-of-care regimen has consisted of normofractionated external beam radiotherapy (2 Gy once per day five times weekly) combined with three cycles of concurrent high-dose three-weekly cisplatin at a dose of 100 mg/m^2, both in the definitive and adjuvant settings.

P. Szturz
Department of Oncology, Lausanne University Hospital (CHUV), Lausanne, Switzerland

J. B. Vermorken (✉)
Department of Medical Oncology, Antwerp University Hospital, Edegem, Belgium and
Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium
e-mail: JanB.Vermorken@uza.be

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While the addition of concomitant systemic treatment to radiotherapy positively impacts on locoregional control and survival, albeit to a limited extent, at the same time it does increase acute and late adverse events. Approaches to deal with these shortcomings are focussing either on modifications in radiotherapy or chemotherapy or both. An important step forward has been the implementation of intensity-modulated radiotherapy (IMRT) techniques leading to a reduction of acute and in particular late treatment-related toxic effects [8, 9]. In parallel, many efforts have been undertaken to ameliorate chemotherapy, but they did not produce any further practice-changing results. Theoretically, adjustments in systemic treatment schedules and finetuning of exposition to anticancer agents can modulate acute toxicity, whereas the objective of new systemic drugs or drug combinations has been primarily to improve efficacy. In this respect, the success story of immune checkpoint inhibitors in the recurrent and/or metastatic setting sparked hopes for patients with LA-SCCHN who are currently being offered participation in several large-scale randomized trials, which are ongoing in many centres globally, as further addressed below.

In this work, we will focus on weekly low-dose cisplatin, as an alternative to the standard, high-dose regimen, given concurrently to definitive or adjuvant radiotherapy in LA-SCCHN in order to decipher whether this change in administration schedule can influence acute toxicity as hypothesized above and what effect it may have on survival parameters. We will explore the comparison between weekly and three-weekly cisplatin at different levels of evidence according to the European Society for Medical Oncology (ESMO) grading consensus system and conclude with practice-oriented recommendations (Fig. 10.1) [10].

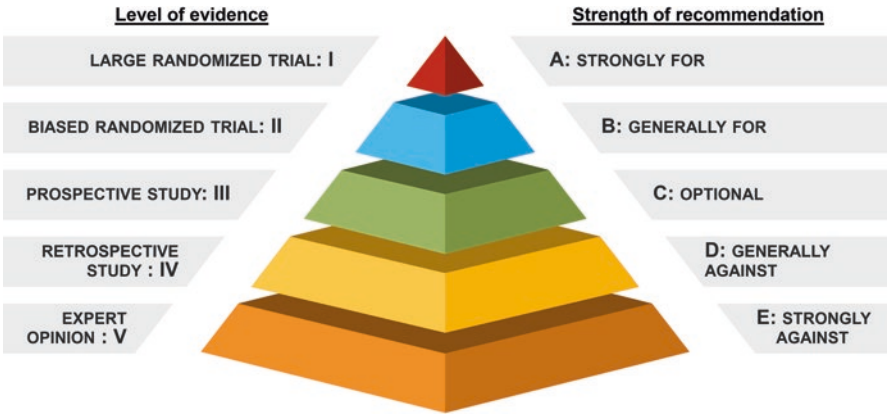

Fig. 10.1 Grading consensus system of clinical practice recommendations according to the European Society for Medical Oncology (ESMO) [10]. © Copyright PresentationGO.com (Pyramide)

High-Dose Three-Weekly Cisplatin

Its position as the current standard of care has been built on the results of four large phase III trials published between 2003 and 2004 [3, 5–7]. Additional supporting data were provided in one smaller randomized study from 2004 [11]. The total intention-to-treat population of all these five trials together, three of which were conducted in the definitive and two in the adjuvant settings, equalled 1763 patients. Comparing concurrent chemoradiotherapy with radiotherapy alone, significantly enhanced disease-free survival (or its analogous measure), locoregional control, and overall survival were observed in favour of high-dose three-weekly cisplatin, which on the other hand had neither a significant influence on the incidence of distant metastases nor on response rate. More recently, enrolling exclusively treatment-naive human papillomavirus (HPV)-positive oropharyngeal cancer cases, the role of this regimen has further been reinforced by two large phase III trials, RTOG 1016 and De-ESCALaTE, in which the comparator arm comprised bioradiation with single-agent cetuximab [12, 13]. Here again, high-dose three-weekly cisplatin unequivocally outperformed the latter arm in terms of locoregional control rate and survival. In addition, the De-ESCALaTE trial, including only low-risk oropharyngeal cancer cases (HPV-positive, smoking history of less than ten pack-years), found a significant decrease in distant metastasis after cisplatin treatment (3% versus 9%, $p = 0.0092$). Taken all seven trials together, the total intention-to-treat population reached up to almost 3000 patients (Table 10.1). Of note, contrastingly to the remaining studies, the RTOG 1016 study employed an accelerated radiotherapy technique with six fractions over five days weekly aiming at the standard total dose of 70 Gy. As a general rule, altered fractionation radiotherapy has been combined with only two cycles of concurrent high-dose cisplatin given its shorter overall duration.

The efficacy, toxicity, and compliance of three-weekly high-dose cisplatin were explored in three meta-analyses of aggregate data, separately evaluating chemoradiotherapy based on conventional and on altered fractionations in the definitive and post-operative settings [14, 15]. Among 31 prospective trials using conventionally fractionated radiotherapy, model-based estimates of 5-year overall survival were 39% and 51% in the definitive and adjuvant settings, respectively. Relative to radiotherapy alone, patients treated with the combined regimen experienced more grade III-IV acute toxicity. About 40% developed mucositis, up to one fourth difficulties with swallowing, and at least 20% bone marrow suppression. As a result, only about two thirds of them could receive all three planned cycles of high-dose cisplatin [14]. Due to a limited number of eligible trials with altered fractionation, the respective meta-analysis could be performed only in the definitive setting. The estimated 5-year overall survival increased to 57% and compliance with both cisplatin cycles was as high as 92%. Nevertheless, severe acute adverse events remained frequent: 40% mucositis and dysphagia and about one out of five patients suffered from hematotoxicity [15].

Table 10.1 Overview of phase III trials exploring conventional radiotherapy with 3 cycles of 100 mg/m^2 cisplatin[a] versus radiotherapy alone or bioradiation with cetuximab. Arrows pointing up symbolize significant improvements achieved by the addition of cisplatin, while the equals signs indicate a lack of statistically significant difference

Author, year	Setting	ITT	Benefit of CRT vs. RT alone				
			RR	DFS	LCR	DM	OS
Adelstein, 2003 [5]	Definitive	295	=	↑	nr	=	↑
Forastiere, 2003 [3, 17]	Definitive	547	=	↑	↑	↑/=	=
Fountzilas, 2004 [11]	Definitive	128	=	↑	nr	nr	↑
Cooper, 2004 [6]	Adjuvant	459	nr	↑	↑	=	=
Bernier, 2004 [7]	Adjuvant	334	nr	↑	↑	=	↑
		1763					
			Benefit of CRT vs. BRT alone				
Mehanna, 2019 [13]	Definitive	334	nr	↑	↑	↑	↑
Gillison, 2019 [12]	Definitive	849	nr	↑	↑	=	↑
		2946					

ITT intention-to-treat population of the entire study (all definitive trials had tree arms); *(C)RT* (chemo)radiotherapy; *vs.* versus; *RR* response rate; *BRT* bioradiation with cetuximab; *nr* not reported; *DFS* disease-free survival (progression-free survival in Bernier et al. and Gillison et al., disease-specific survival in Adelstein et al., time to progression in Fountzilas et al., recurrence rate in Mehanna et al.); *DM* distant metastases (benefit not confirmed in Forastiere 2013); *OS* overall survival

[a]altered fractionation radiotherapy with 2 cycles of 100 mg/m^2 cisplatin in Gillison et al.

Data on late toxicity should be regarded with caution because their reporting is scarce and usually biased due to difficulties with long-term follow-up of study participants. Although published results did not confirm that adding cisplatin leads to a significant increment in late side effects when compared with radiotherapy alone, it is plausible to assume the opposite. Not only has late toxicity been associated with long-term exposure to circulating platinum, but also the worse survival of patients randomized to the concurrent chemoradiation arm in the RTOG 91–11 trial suggests an important contribution of systemic treatment to late treatment-related morbidity [16, 17]. In this respect, it is of interest to consider the role of radiotherapy technique, especially IMRT as alluded to above. In fact, the majority of studies employed conventional two-dimensional or three-dimensional conformal planning, which has been gradually replaced by IMRT since its introduction in clinical protocols about 15 years ago. In the aforementioned meta-analyses, only six out of 38 chemoradiotherapy trials with high-dose cisplatin used IMRT but never as an exclusive method [14, 15].

Finally, it is informative to balance the results obtained from the meta-analyses of studies on high-dose cisplatin in the definitive setting that opened for accrual between 1981 and 2011 and those obtained from the two most recent phase III trials, De-ESCALaTE and RTOG 1016, which recruited patients from 2011 to 2016 (Table 10.2). Compliance to normofractionated radiotherapy was better in the De-ESCALaTE study than observed in the meta-analysis, which partly could be attributed to the use of IMRT. Surprisingly, a much lower percentage of patients in

Table 10.2 Compliance and toxicity based on per protocol calculations and expressed in percentages in patients with locally and/or regionally advanced squamous cell carcinoma of the head and neck treated with three-weekly high-dose cisplatin given concurrently to definitive radiotherapy [12–15]

	Normofractionated		Altered fractionation	
	Meta-analysis	De-ESCALaTE	Meta-analysis	RTOG 1016
Compliance				
RT: Completed without interruption	84	93	76	nr
RT: Completed as prescribed	90	100[a]	95	95[b]
CT: Received all planned cycles	71	38	92	93
CT: Received at least 200 mg/m^2	96	84	83	nr
CT: Received at least 2 cycles	92	90	92	93
Severe acute toxicity (gr 3–4)				
Anemia	8	2 (SAE)	5	3
Thrombocytopenia	4	1 (SAE)	3	1
Leukopenia	19	nr	18	12
Neutropenia	18	2 (SAE)	18	15
Febrile neutropenia	5	4 (SAE)	5	5
Mucositis and/or stomatitis	42	15 (SAE)	40	42
Xerostomia	2	0 (SAE)	4	8
Dysphagia	26	8 (SAE)	40	37
Nausea and/or vomiting	16	28/30 (SAE)	17	19/12
Weight loss	12	4 (SAE)	4	8
Anorexia	6	10 (SAE)	8	22
Laryngeal toxicity	8	1 (SAE)	3	<1
Nephrotoxicity	5	7[c]	5	3
Neurotoxicity	2	6	3	<1
Ototoxicity	3	2	2	3
Skin toxicity	11	4	13	8
Diarrhea	2	4 (SAE)	1	1
Constipation	2	10 (SAE)	1	1
Infection	5	12	6	2
Mortality during CRT or within 30 days after completion				
Grade 5 toxicity	3	nr	1	1
30-day mortality	4	nr	3	2
Late toxicity				
Overall prevalence (gr 3–4)	20	30[d]	43	20
Xerostomia (gr 1–2)	59	nr	72	85
Xerostomia (gr 3–4)	10	nr	6	2
Dysphagia (gr 3–4)	10	nr	12	4
Subcutaneous fibrosis (gr 3–4)	5	0	2	0

RT radiotherapy; *CT* chemotherapy; *gr* grade; *SAE* serious adverse events; *nr* not reported

[a]Defined as having received at least 65 Gy

[b]Defined as having received at least 66.5 Gy

[c]Renal and urinary disorders

[d]Including grade 5 toxicity

De-ESCALate received all planned cisplatin cycles, which is more difficult to explain. Patient factors (demographics), treatment factors (hydration schema, antiemetic regimen), and physician factors (motivation based on literature data [18]) all might have played a role in this. Despite the lower exposition to cisplatin, overall survival was excellent with a 2-year estimate of 97.5%. Otherwise, interpretation of toxicity in De-ESCALaTE is hampered by incomplete data reporting and substitution of grade III-IV acute toxicity for serious adverse events [13, 14]. Concerning altered fractionation, probably owing to a smaller target dose of cisplatin, compliance and severe acute side effects were comparable between RTOG 1016 and the older trials included in the meta-analysis on altered fractionation schedules using the high-dose cisplatin regimen. The lower overall prevalence of severe late toxicity in RTOG 1016 than in the meta-analysis may pertain to the use of IMRT in this study. This could have also contributed, along with superior prognosis of HPV-positive oropharyngeal cancer patients, to the unprecedented 5-year overall survival of 85% relative to the other phase III trials [12, 15].

Low-Dose Weekly Cisplatin

Low-dose weekly cisplatin regimens have gained attention primarily in an attempt to improve treatment tolerance by decreasing acute side effects. Here, we will show that the supporting evidence for these schedules is considerably weaker than what we have for the high-dose cisplatin regimen, mainly with respect to efficacy and late toxicity. This does not necessarily mean that a low-dose regimen is inferior, but more data are needed to substantiate the arguments. First, the mere definition remains elusive. It is generally accepted that weekly cisplatin should be given once per week during the course of radiotherapy but due to local variations in radiotherapy protocols, the number of administrations varies between six and seven and may be further perturbated by unpredictable factors sometimes producing space for an additional eighth cycle. Ranging usually between 20 and 50 mg/m^2, an even greater dilemma arises when choosing the individual cisplatin dose [19]. Consequently, the concept of cumulative dose has been introduced as a possible solution to this conundrum. Retrospective evidence suggests that the overall survival benefit observed in several clinical trials was driven by patients who received a cumulative dose of at least 200 mg/m^2 and this particularly if they had HPV-positive cancer of the oropharynx [13, 18, 20, 21]. Although this hypothesis has never been confirmed prospectively, its adaptation in clinical practice can be useful in situations when the risk of serious toxic complications escalates near the end of treatment [22]. In fact, it remains unknown whether pushing the total dose beyond 200 mg/m^2 adds an additional advantage in overall survival or whether this benefit, reported recently in the literature, merely reflects a process of selecting patients with better health status who in general have a better prognosis and are able to tolerate quantitatively more chemotherapy [23]. In this respect it is intriguing to note that in De-ESCALaTE, patients who received all three cycles of 100 mg/m^2 cisplatin did not experience

more toxicity events than the total population, and grade III-V late adverse events even seemed to be less common [13].

Analogously to high-dose cisplatin, the low-dose regimen underwent prospective evaluation versus radiotherapy alone in four trials randomly assigning a total of 834 patients [24–28]. When looking at Table 10.3 summarizing these studies, the first thing to notice is a seemingly positive correlation between the cumulative dose of cisplatin and the efficacy of weekly regimens. Exploring seven weekly doses of 20 mg/m^2, the largest and oldest study was published 24 years after the last patient had been accrued. The delay was probably due to the disappointing results which nonetheless contribute to our better understanding of cisplatin pharmacodynamics. In the chemoradiotherapy arm, overall survival was numerically lower and both acute and late toxicity significantly higher than in the comparator arm [24]. A benefit in overall survival was seen only in the two studies in which the majority of patients received a cumulative dose of at least 200 mg/m^2 cisplatin. They were reported by Sharma et al., who had chosen seven weekly doses of 40 mg/m^2, and Bachaud et al. with seven to nine doses of 50 mg/m^2 [26–28]. Of note, the last study, by Ghosh-Laskar et al. (seven to eight doses of 30 mg/m^2), had to be prematurely closed because of poor accrual. Despite the initial plan to administer at least 210 mg/m^2, the median cumulative dose of cisplatin was only 180 mg/m^2, and this fell short of translating the significant yields in disease-free survival and locoregional control into a meaningful gain in overall survival [25]. Taken the four studies together, the addition of weekly cisplatin increased the frequency and severity of acute adverse events with less pronounced impact on late toxicity, the latter of which reached statistical significance only in the first study reported by Quon et al. and was not reported in the study by Sharma et al. [24, 26].

Table 10.3 Overview of randomized trials exploring conventional radiotherapy with weekly low-dose cisplatin versus radiotherapy alone

			Benefit of CRT vs. RT alone					Total cisplatin [mg/m^2]	
Author, year	Setting	ITT	RR	DFS	LCR	DM	OS	Planned	Received
Quon, 2011 [24]	Definitive	371	=	=	nr	nr	=	140	nr
Ghosh-Laskar, 2016 [25]	Definitive	199	nr	↑	↑	=	=	210–240	180[a]
Sharma, 2010 [26]	Definitive	176	↑	=	=	=	↑	280	92%[b]
Bachaud, 1991 [27]	Adjuvant	88	nr	↑	↑	=	↑	350–450	59%[c]
		834							

Arrows pointing up symbolize significant improvements achieved by the addition of cisplatin, while the equals signs indicate a lack of statistically significant difference

ITT intention-to-treat population of the entire study (tree arms in Ghosh-Laskar et al.); *(C)RT* (chemo)radiotherapy; *RR* response rate; *nr* not reported; *DFS* disease-free survival (failure-free survival in Quon et al., progression-free survival in Sharma et al.); *DM* distant metastases; *OS*, overall survival

[a]Given median cumulative dose

[b]Of patients received planned cumulative dose

[c]Of patients received all planned cycles (at least 7, corresponding to a cumulative dose of 350 mg/m^2)

To further report on the outcomes of Iow-dose cisplatin, we will refer to the previously mentioned meta-analyses. They were conceptualized to compare the standard, high-dose three-weekly cisplatin with a weekly regimen complying with the vaguely defined dose and frequency criteria. Altogether, 38 trials were included in the high-dose arms and 21 in the low-dose arms of the three meta-analyses performed separately in the definitive conventionally fractionated, adjuvant conventionally fractionated, and definitive altered fractionation chemoradiotherapy settings. By involving uncontrolled studies and selected arms of otherwise ineligible randomized trials, one of their major limitations was that the final populations they compared were not intended to be compared and differed thus qualitatively but also quantitatively. Nevertheless, they fill the gap because there are hardly any unbiased randomized trials comparing these two schedules [19]. The results pertaining to weekly cisplatin will be presented in the following paragraph in relation to what has already been stated about the three-weekly schedule.

High-Dose Three-Weekly Versus Low-Dose Weekly

Aiming at a comprehensive approach to the topic, we will provide a step-wise evaluation and a concise overview of available evidence divided into 5 levels according to the model adopted by ESMO (Fig. 10.1) [10].

Level V Evidence

The lowest level of evidence is based on expert opinions, cross-sectional studies, case reports, and case series. Consequently, the assumptions underpinning low-dose weekly cisplatin in comparison with the high-dose regimen include a better short- and long-term tolerance without jeopardizing outcome, improved compliance, timely dose adjustments, enhanced radiosensitization, reduced risk of radioresistance, and lower costs due to outpatient administration [14]. Of them, those exploring efficacy, toxicity, and compliance were explored at higher levels of evidence and will be further discussed below. Radiobiological properties per se and logistical aspects have clinical relevance primarily if they influence patient outcomes, and they have not been studied separately in prospective cohorts. Cost-effectiveness issue have recently been gaining increasing attention, but data are mostly available for new medicines. Based on a small retrospective study of 62 patients, the incremental cost-effectiveness ratio (ICER) for the addition of three-weekly cisplatin to radiotherapy was calculated at $3303-per-quality-adjusted life year (QALY) [29]. An analysis of healthcare expenditures in the cisplatin arm of the De-ESCALaTE trial revealed total costs of £13,517 per patient at 24 months post-treatment [30]. Unfortunately, similar analyses are neither available for a weekly

regimen versus radiotherapy alone nor for a comparison between the two cisplatin schedules under question.

Level IV Evidence

Retrospective cohort and case-control studies provide a higher level of evidence but are still difficult to pool. As summarized in one of our publications, the results of such studies comparing weekly versus three-weekly cisplatin are conflicting and do not allow us to make firm conclusions, albeit that an overall impression of their outcomes tends to endorse the high-dose regimen [14].

Level III Evidence

Moving on to non-randomized prospective trials we present here the key results of the set of three meta-analyses mentioned above [14, 15]. In the adjuvant setting of conventionally fractionated chemoradiotherapy, data from nine trials could be retrieved, six on high-dose and three on low-dose cisplatin. While no differences in late toxicity ($p = 0.5938$) and compliance ($p = 0.5747$) were observed, severe acute toxicity favoured the weekly schedule with significantly less dysphagia ($p = 0.0026$) and weight loss ($p < 0.0001$). However, the latter findings should be interpreted with caution as they are based on only one trial using weekly cisplatin. In the definitive setting of conventionally fractionated chemoradiotherapy, 39 studies were included in the analysis, 25 in the high-dose and 14 in the low-dose cohort. There were clearly less severe acute toxicities with the weekly regimen as reflected by significantly less myelotoxicity (leukopenia: $p = 0.0083$; neutropenia: $p = 0.0024$), nausea and/or vomiting ($p < 0.0001$), and severe nephrotoxicity ($p = 0.0099$). In line with that, also the compliance was better with the weekly cisplatin regimen. No data on late toxicity were available in the low-dose cohort, precluding thus further calculations. The efficacy outcomes provided interesting insights into the role of the cumulative cisplatin dose. Although no difference in overall survival was noted in either of these meta-analyses, only about two thirds of patients in the high-dose arm could receive all three cisplatin cycles, i.e. a cumulative dose of 300 mg/m^2. But what if the target dose was not 300 but 200 mg/m^2. In that case, would it still be possible to ensure sufficient efficacy but with notably lower acute toxicity?

As alluded to above, 200 mg/m^2 might indeed provide an adequate exposition to the drug. Moreover, this is exactly the target cumulative dose used in the third meta-analysis run in the definitive setting of altered fractionation chemoradiotherapy that involved 11 studies, seven with high-dose and four with low-dose cisplatin. Here, two cycles of the former regimen generated significantly less severe acute toxicity (mucositis and/or stomatitis: $p = 0.0202$, constipation: $p = 0.0066$) and short-term mortality (toxic deaths: $p = 0.0168$, 30-day mortality: $p = 0.0154$), but also less

severe late adverse events (subcutaneous fibrosis: $p < 0.0001$) than observed with the low-dose cisplatin regimen. In line with an improved compliance ($p = 0.0353$), the vast majority of patients (95%) receiving the high-dose regimen could receive both planned cycles. Finally, patients on high-dose cisplatin during altered fractionation radiotherapy lived longer than those receiving the weekly regimen during altered fractionation radiotherapy ($p = 0.0353$). Albeit purely hypothetical, these results contribute to the ever-growing body of knowledge that supports a minimal cumulative dose of 200 mg/m^2.

Level of Evidence II

Only two prospective trials randomly assigning LA-SCCHN patients to receive either the three-weekly high-dose or a weekly low-dose regimen have been published so far (Table 10.4) [31, 32]. Owing to an increased risk of bias arising from insufficient power and cumulative dose issues, we have assigned them to level of evidence II. The first study randomized 55 patients who were treated with the same mean radiotherapy and cisplatin doses (208.5 mg/m^2 three-weekly versus 200.4 mg/m^2 weekly) but a cumulative dose of at least 200 mg/m^2 could be delivered to significantly more patients in the high-dose arm (88.5% versus 62.5%, $p = 0.047$). In spite of that, the low dose regimen proved to be more toxic ($p = 0.02$), particularly with regard to severe mucositis (38.5% versus 75.0%, $p = 0.012$). No differences in overall survival were noted at median follow-up of 12 months [31]. The accrual in the second randomized study reached up to 300 patients but concerns were raised about the different cumulative doses with 180–210 mg/m^2 being the target exposure in the weekly cisplatin arm and 300 mg/m^2 in the three-weekly arm. In the end, it was not that surprising to see the three-weekly regimen generating better locoregional control at 2 years (58.5% versus 73.1%, $p = 0.014$) but at the cost of an

Table 10.4 Phase III trials comparing radiotherapy given concurrently either to three-weekly high-dose or to weekly low-dose cisplatin

Author, Year	Therapy intent	Study arms	Inclusion period	Intention-to-treat population		Concurrent cisplatin [mg/m^2]	
				...of both study arms	...of the cisplatin arm	planned schedule	planned cumulative dose
Tsan, 2012 [31]	Adjuvant	Weekly	2008–2010	55	nr	7 × 40	280
		Three-weekly			nr	3 × 100	300
Noronha, 2018 [32]	Adjuvant (93%) and definitive	Weekly	2013–2017	300	150	6–7 × 30	180–210
		Three-weekly			150	3 × 100	300

nr not reported

increment in severe acute toxicity (71.6% versus 84.6%, p = 0.006), namely vomiting, infection, hearing loss, hyponatremia, and myelotoxicity. Compliance and late toxicity were comparable [32].

Level of Evidence I

Current evidence has not attained this level, and the presented meta-analyses do not qualify because they were not primarily based on randomized trials exploring the respective comparison. The situation may be changing soon when the results of the phase II/III non-inferiority trial of the Japan Clinical Oncology Group (JCOG1008) are shared. Aiming to enrol 260 participants in the post-operative setting, the study has been ongoing since October 2016 with the primary objective of overall survival. The target cumulative doses were set to 300 mg/m^2 and 280 mg/m^2 for three-weekly and weekly cisplatin, respectively [33].

Immunotherapy Trials

After having defined new standards of care in the recurrent and/or metastatic setting, immune checkpoint inhibitors entered clinical trial design in LA-SCCHN challenging cisplatin-based chemoradiotherapy. In this respect, the majority of randomized trials are exploring immunotherapy on top of cisplatin by either intensifying definitive or adjuvant treatment (dubbed for our purposes design concept "A") or by giving it right after the standard definitive or adjuvant chemoradiotherapy (design concept "B"). Alternatively, immunotherapy can replace cisplatin creating thus pure immunoradiotherapy regimens. In the former type of trials, high-dose three-weekly cisplatin represents the preferred administration mode.

The design model "A" has been adopted for example by the following larger (> 100 participants) trials: JAVELINE Head & Neck (NCT02952586), KEYNOTE-412 (NCT03040999), KEYCHAIN (NCT03383094), ADRISK (NCT03480672), and NIVOPOSTOP (NCT03576417). Here, sometimes preceded by a short lead-in phase with one dose of immunotherapy in eligible patients, chemoradiotherapy, as already mentioned, is typically based on the high-dose three-weekly cisplatin schedule (weekly regimen allowed in ADRISK) and combined with a programmed death-1 (PD-1) inhibitor (pembrolizumab or nivolumab) or an anti-PD-1 ligand (PD-L1) agent (avelumab or atezolizumab) in the experimental arm. Afterwards, patients are started on a maintenance phase with or without the immune checkpoint inhibitor for six to 12 months. The design model "B" stands for a classic two-arm concept where patients are assigned either to 1 year of immunotherapy or the same period of placebo or observation after having completed curative treatment as seen in IMvoke010 (NCT03452137) and EA3161 (NCT03811015). Other notable mentions comprise IMSTAR-HN (NCT03700905) and KEYNOTE-689 (NCT03765918)

which are combining both design models. Finally, cisplatin-based chemoradiotherapy has been challenged by non-chemotherapy approaches in the NRG-HN005 (NCT03952585) and REACH (NCT02999087) trials.

Concluding Remarks and Outlooks for the Future

There are many drawbacks of high-dose cisplatin which is not by far the ideal solution to administer concurrently to curative radiotherapy. Particularly, toxicity has become an issue for many patients, sometimes with life-long consequences. And this is where a weekly regimen steps in with probably the greatest benefit in better short-term tolerance, such as less nausea, vomiting, transaminase elevations, ototoxicity, serum creatinine increase, and myelotoxicity. However, this might be offset by worse survival outcomes and no benefit in late toxicity. Three-weekly high-dose cisplatin should therefore remain the reference adjunct to radiotherapy with continuous efforts to find more efficacious and/or less toxic modalities. Unfortunately, weekly cisplatin has not convincingly met these requirements yet. Nevertheless, some clinical situation may indeed prioritize this approach.

Elderly people have often numerous comorbidities, impaired autonomy, decreased organ reserves, and a limited life expectancy. Geriatric assessment tools have been developed to help distinguish older patients who are fit and can be considered good candidates for standard treatment from those who are frail and should be directed towards palliative measures, but also from those who are in-between. Although the latter group, sometimes referred to as vulnerable, is typically excluded from registration trials, these patients may still be deemed suitable for curative therapy. Notwithstanding the lack of rigorous scientific data, weekly cisplatin is one of the regimens that can be offered to them providing a compromise solution with less acute side effects and still a possible benefit in overall survival [34]. A single dose of 40 mg/m^2 can be pursued since the usual six to seven applications ensure an effective ($\geq$ 200 mg/m^2) and at the same time safe ($\leq$ 300 mg/m^2) cumulative dose. In addition, lowering the peak concentration of cisplatin, either by prolonging the infusion time or reducing cisplatin dose, can be recommended also in the presence of other relative contraindications as explained elsewhere [22].

More recently, the attention of healthcare professionals and researchers has been largely shifted towards immunotherapy which holds promise of being not only a more efficacious but mainly less toxic modality, offering thus new opportunities for frail patients as well [35]. In LA-SCCHN, several large trials are already ongoing and believed by many to become practice-changing, albeit only in high-income countries. In any case, the first results will not be available before 2021, and until then high-dose three-weekly cisplatin will retain its central position. But even later and in resource-limited regions, this schedule will not completely disappear from treatment protocols, and a choice between weekly and three-weekly cisplatin will maintain its significance for practicing physicians.

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Chapter 11
Where and when to Use Induction Chemotherapy in Head and Neck Squamous Cell Cancer

Jan B. Vermorken

Introduction

Worldwide cancer incidence and mortality are rapidly growing, and this is also true for head and neck squamous cell cancer (HNSCC). The 2018 estimates point at more than 750.000 new cases and more than 380.000 deaths [1]. The reasons are complex but reflect both aging and growth of the population, as well as changes in the prevalence and distribution of the main risk factors for cancer, several of which are associated with socioeconomic development [2, 3]. Sustained exposure to tobacco, tobacco-like products, and alcohol increase the risk of developing HNSCC [4]. Although HNSCC can arise within the oral cavity, oropharynx, hypopharynx, larynx, and nasopharynx, there has been a shift in primary site distribution, with a steady increase of oropharyngeal squamous cell carcinoma (OPSCC) and a decline in cancers of the larynx and hypopharynx, in particular in the Western world [5]. This change has been observed in parallel with a decrease in cigarette smoking and the identification of exposure to high-risk oncogenic human papillomavirus (HPV) as a risk factor for the development of OPSCC [6, 7]. This possible role for HPV in head and neck cancer was first reported in the 1990s, while the proof for a causal association between HPV and OPSCC was delivered in 2000 [8, 9]. A systematic review and meta-analysis showed that the overall HPV prevalence in OPSCC is increasing significantly over time: from 40.5% (95% CI,35.1–46.1) before 2000, to 64.3% (95% CI, 56.7–71.3) between 2000 and 2004, and 72.2% (95% CI, 52.9–85.7) between 2005 and 2009 ($p < .001$) [10]. Prevalence increased significantly initially in North America and subsequently in Europe, and the significant gap between them that existed before 2000 (50.7% vs 35.3%, respectively, $p = .008$) has now disappeared (69.7% vs 73.1%, respectively, $p = .8$).

J. B. Vermorken (✉)
Department of Medical Oncology, Antwerp University Hospital, Edegem, Belgium and Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp, Belgium
e-mail: JanB.Vermorken@uza.be

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Many earlier studies have observed that patients with HPV-positive OPSCC had a distinct epidemiology when compared to patients with HPV-unrelated OPSCC, i.e. they were statistically younger, were more likely male, had fewer comorbidities, and reported less tobacco exposure but higher numbers of (oral) sex partners [11–13]. The prognosis for these younger patients with HPV-positive OPSCC was substantially better than that for patients with HPV-negative tobacco-related cancers treated similarly [5]. However, more recently, several studies portend that the population of elderly patients with HPV-positive OPSCC is expanding [14–16]. In fact, the age at OPSCC diagnosis is increasing for both HPV-positive and HPV-negative patients, and a rising proportion of older patients have HPV-positive tumors. In an analysis of the National Cancer Database (with 119,611 OPSCC patients) Rettig et al. [14] showed that although patients of ≥70 years of age with HPV-positive OPSCC had improved survival compared to those with HPV-negative OPSCC (adjusted hazard ration [aHR] = 0.65, 95%CI = 0.55–0.76), the survival benefit of HPV-positive tumor status was significantly attenuated compared to younger HPV-positive patients (50–59 years: aHR = 0.45, 95%CI = 0.39–0.51; $p_{interaction} < 0.001$). The outcome of these older patients with HPV-positive OPSCC was in fact essentially similar to survival for the young HPV-negative patients [14]. These data will have implications for the therapeutic approach that clinicians need to consider for these elderly patients, taking into account the higher comorbidity score, the distinct disease characteristics, the higher rates of treatment-related toxicities, and the increased risk of non-cancer-related deaths [14].

Milestones in Systemic Therapies for Locoregionally Advanced HNSCC

Before 1980, the initial treatment of patients with locoregionally advanced stage III or IV (M0) was surgery and/or radiation therapy (RT), a choice that depended on the site of the disease, the resectability of the cancer, the performance status of the patients, and his/her comorbidities. However, with these "traditional" therapies outcome was quite poor, in particular in those with stage IV or unresectable disease. The milestones in systemic therapies are summarized in Fig. 11.1**.**

Single agent chemotherapy, in particular methotrexate was used for palliation in patients with recurrent or metastatic disease already in the 1960s. Systemic therapy was introduced as part of combined modality therapy for LA-HNSCC in the mid 1970s, initially as single agent chemotherapy with methotrexate or cisplatin, usually with palliative intent to patients with stage IV disease, M1 cancers or recurrent disease beyond salvage local treatment [17]. The utilization of cisplatin as a single agent produced a range of responses from 14% to 41% [18]. The higher response rates were seen in previously untreated patients. Subsequently, experience was obtained with combination chemotherapy, initially with cisplatin/bleomycin combinations, to which then methotrexate or vinca-alkaloids were added and ultimately

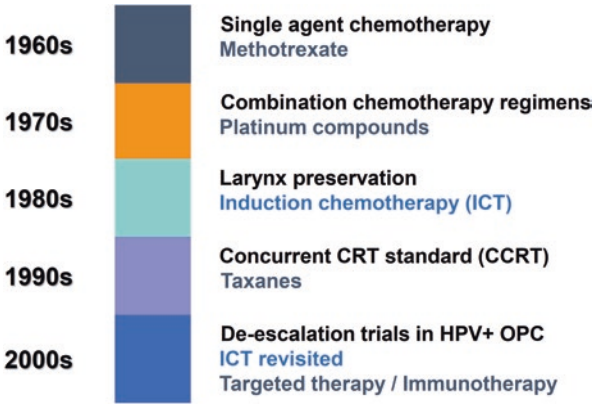

Fig. 11.1 Milestones in systemic therapy (± TRT) in head and neck squamous cell cancer

Table 11.1 Induction chemotherapy in locoregionally advanced HNSCC*

Type of induction Chemotherapy	No. of patients	CR No. (%)	PR No. (%)	OR No. (%)
Single MTX, BLM or P	188	4 (2)	81 (43)	85 [45]
Combo PB	467	34 (7)	193 (41)	227 (48)
Combo PBM	323	51 (16)	187 (58)	238 (74)
Combo PB-Vinca	474	96 (20)	231 (49)	327 (69)
Combo PF	461	162 (35)	236 (51)	398 (86)
Combo P-other	445	89 (20)	236 (53)	325 (73)

MTX metrotrexate, *BLM* bleomycin, *P* cisplatin, *PB* cisplatin/bleomycin, *PBM* PB + MTX, *Vinca* vinca alkaloid, *PF* cisplatin/infusional 5-FU, *CR* complete response, *PR* partial response, *OR* overall response. *modified from Choski et al. [19]

the cisplatin/infusional 5-fluorouracil (5-FU) regimen [19; Table 11.1). At Wayne State University in 1977, they initiated a pilot study for advanced previously untreated patients with head and neck cancer utilizing cisplatin, vincristine, and bleomycin. An overall response rate of 80% was achieved, with a 29% complete response (CR) rate [18]. With the known pulmonary toxicity of bleomycin and the in vitro synergism of 5-FU and cisplatin, they started a second pilot study with cisplatin (100 mg/m^2 IV, day 1) and 5-FU (1000 mg/m^2/day by continuous IV infusion over 96 hours), the so-called PF regimen. The response rate with that regimen was 88% overall, with a 19% CR rate [20]. Increasing the infusion time of 5-FU to 120 hrs and the number of courses from 2 to 3, increased the overall response rate to 93% and the CR rate to 54% [21]. The feasibility of the latter scheme was established and the efficacy confirmed in a multi-institutional study within the Radiation Therapy Oncology Group (RTOG). An overall response rate of 86% was obtained, with a 38% CR rate [22]. An attempt to further improve the regimen by using higher dosages of cisplatin (40 → 30 mg/m^2/day x5 for 3 cycles), given in hypertonic saline, failed to show any further improvement over the 120 hrs PF regimen [23]. Although non-randomized trials were very promising with respect to response rate and sometimes also suggesting an improvement of survival, the impact on survival could only be assessed in randomized trials. Five randomized trials executed

between 1979 and 1987 using methotrexate as a single agent for induction before local treatment were, apart from one study, all negative with respect to survival benefit [24]. In the single positive study the methotrexate had been administered intra-arterially. Looking in more detail at that study, a difference in survival was present only in patients with oral cavity cancer. Further analysis of the oral cavity cases showed that the 5-year survival difference had significance only in stage II patients. The very high response rates, and in particular the very high CR rates stimulated investigators to do randomized trials with the hope to improve survival. However, the disappointment came rather fast when early randomized trials were all negative with respect to survival benefit, apart from one in patients with oral cavity cancer, in which again chemotherapy was administered by the intra-arterial route [24]. However, apart from a high response rate in untreated patients with locoregionally advanced HNSCC, it became clear that those patients that responded well to chemotherapy subsequently also responded more favorably to radiotherapy (RT) [25]. This observation formed the rationale for the first-generation larynx preservation trials (see below).

In the 1990s, with the disappointing results with respect to survival gain in many randomized trials utilizing induction chemotherapy (ICT), the concept of concurrent chemotherapy with radiation therapy was revisited with the introduction of cisplatin given concurrently with radiation as the primary treatment for patients with inoperable and/or unresectable head and neck cancers [26]. The large individual patient-based meta-analysis, reported in 2000, demonstrated that cisplatin given concurrently with radiation (100 mg/m^2 on days 1, 22, and 43 of the RT) achieved substantially more survival benefit versus RT alone when cisplatin was given sequentially (before or after the radiation) [27, 28; Table 11.2]. That is also true for the comparison versus the at that moment considered to be the best type of ICT, i.e. the PF regimen. Since that time enthusiasm to use ICT diminished strongly and colleagues on both sides of the Atlantic started to accept concurrent cisplatin-based chemoradiotherapy (CCRT) as the preferred treatment for both patients with resectable disease and those with inoperable or unresectable disease. For the first category of patients, i.e. those with resectable disease, it was used as an adjuvant CCRT in case there were unfavorable features in the pathology specimen (positive margins and/or extracapsular extension), in the second category it was used as a definitive nonsurgical treatment (definitive CCRT). Determinative in this change of attitude

Table 11.2 Summary of the meta-analysis of the MACH-NC collaborative Group [27, 28]

Trial category	No. of trials	No. of patients	Absolute benefit at 5 years	Risk reduction	P value
All trials	65	10,850	4%	10%	<0.0001
Adjuvant	8	1854	1%	2%	0.74
Induction	31	5269	2%	5%	0.10
Induction with PF	15	2487	5%	12%	0.01
Concomitant	26	3727	8%	19%	<0.0001

PF cisplatin +5-fluorouracil combination

were four large randomized controlled trials which irrefutably showed benefit of this combined modality approach [29–32].

The first two decades in 2000 are fascinating in that new treatment approaches, initially targeted therapies, but later also immunotherapies came forward [33–40]. Both targeted therapies (in particular cetuximab) and immunotherapies (especially immune checkpoint inhibitors [CPIs]) have been practice changing. Not only were they extensively studied in the recurrent/metastatic (R/M) disease setting [34, 38–40], they also found their way in patients with LA-HNSCC [35, 36], although for CPIs that has not been fully developed yet. There arose a renewed interest in ICT since the introduction of the taxanes, which proved to be active compounds for this disease [41, 42]. Two randomized controlled trials (RCTs), one in the US and one in Europe, showed that adding docetaxel to the PF combination made this regimen more efficacious, better tolerable for the patients, did not lead to a negative effect on quality of life (QoL), and was cost-effective [43–46]. This so-called TPF regimen is now considered standard for those situations in which ICT is indicated.

Comparison of the Practice Changing TPF Protocols (TAX 323/EORTC 24971 and TAX 324)

The results of the European TPF regimen (protocol TAX 323/EORTC 24971) and the American TPF regimen (TAX 324) were published back to back in the New England Journal of Medicine in 2007 [43, 44]. In both phase III trials, LA-HNSCC patients were randomized to receive three (TAX 324) or four (TAX 323/EORTC 24971) cycles of TPF or PF as induction before local treatments. Details on the respective regimens can be found in Table 11.3. The studies were executed in different patient populations. TAX 323/EORTC 24971 included only patients with previously untreated, unresectable LA-HNSCC, while in TAX 324 there was a mixture of patients involved, i.e. those with either unresectable disease or disease of low surgical curability, as well as patients with LA-HNSCC who were candidates for organ preservation. Both studies also differed in the local treatment part of the protocol following the induction phase. In TAX 323/EORTC 24971, patients who did not have progressive disease underwent conventionally fractionated RT within 4 to 7 weeks after the completion of chemotherapy (total dose, 66 to 70 Gy) or accelerated or hyperfractionated regimens (total maximum dose 70 Gy for the accelerated

Table 11.3 TPF regimens in accordance with TAX 323/E ORTC 24971 and TAX 324

Study	TPF regimen
TAX 323/EORTC 24971 [44] – four cycles of TPF	Docetaxel (75 mg/m^2) as a 1-hour infusion on day 1 Cisplatin (75 mg/m^2) as a 1-hour infusion on day 1 5-FU (750 mg/m^2/day) by continuous IV infusion, day 1–5
TAX 324 [43] – three cycles TPF	Docetaxel (75 mg/m^2) as a 1-hour infusion on day 1- Cisplatin (100 mg/m^2) over a period of 0.5–3 hours 5-FU (1000 mg/m^2/day) by continuous IV infusion, day 1–4

regimen and 74 Gy for the hyperfractionated regimen), decided before the start of the protocol for each institution. Neck dissections could be performed, if indicated, before or after the RT. In TAX 324, all patients were assigned to receive CCRT beginning 3 to 8 weeks after the start of the third cycle of ICT (day 22 to day 56 of cycle 3). Weekly carboplatin at an area under the curve of 1.5 was given as an intravenous infusion during a 1-hour period for a maximum of seven weekly doses during the course of RT. The definitive curative radiation dose administered to the primary tumor was between 70 and 74 Gy, administered as fractions of 2 Gy per day 5 days per week. The dose administered to uninvolved lymph nodes was at least 50 Gy. Involved lymph nodes were to receive 60 to 74 Gy, depending on whether an elective neck dissection was indicated after completion of treatment. Surgery was performed 6 to 12 weeks after completion of chemoradiotherapy in patients who had an initial nodal stage of N2 and a partial response to ICT or N3 disease, or residual disease after chemoradiotherapy. Surgery was also allowed for patients who did not complete chemoradiotherapy and had resectable residual disease at the primary site or in the neck.

Both trials concluded that the overall response rate with TPF was significantly (TAX 323/EORTC 24971) or numerically (TAX 324) higher than with PF. Both TPF regimens also clearly demonstrated survival benefit over PF ICT (Fig. 11.2). About three-quarters of the patients completed both TPF and RT per protocol and 24% to 29% had treatment delays during ICT. As mentioned above, the TAX 323/EORTC 24971 regimen was associated with a more favorable safety profile than the previously standard PF regimen, likely owing to the lower overall doses of the cisplatin (75 mg/m^2 instead of 100 mg/m^2 on day 1) and 5-FU (750 mg/m^2/day x5 instead of 1000 mg/m^2/day x5). This resulted in a lower frequency of grade 3/4 stomatitis, nausea/vomiting, dysphagia, and thrombocytopenia [44]. Patients in the

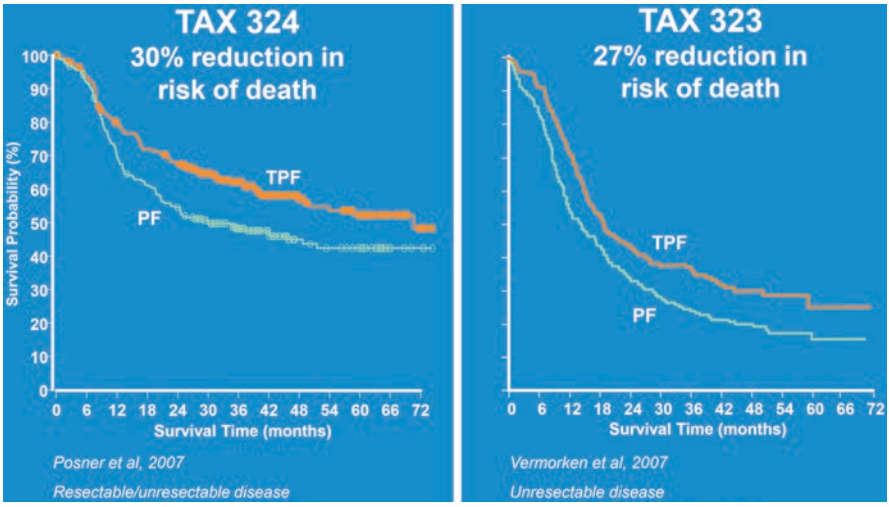

Fig. 11.2 Landmark trials of TPF versus PF in locoregionally advanced HNSCC

TPF arm had fewer treatment delays then did those in the PF group despite differences in peak neutropenia during ICT in the TPF group [43, 44]. The superiority of TPF over PF has been confirmed in a meta-analysis of pooled data from five phase III studies, including the two mentioned above [47]. This analysis concluded that the TPF regimen, compared to the PF regimen, led to benefits in progression-free survival (PFS), overall survival (OS), locoregional failure rate (LFR) and distant failure rate (DFR). Nevertheless, despite the fact that this meta-analysis confirmed that TPF was a better ICT than PF, some critical remarks were made with respect to the pooling methodology used on the five rather heterogeneous studies, the missing treatment failure data in the participating two Spanish trials [48, 49] and the EORTC trial [44], and the different follow-up treatments that were applied for the ICT responders and the ICT non-responders [50]. However, what this meta-analysis did not do, was changing the standard of care in patients with advanced HNSCC, i.e. concurrent chemoradiotherapy.

The main question that remained for most clinicians was not whether TPF was superior to PF, there was a unanimous feeling about that, but it was whether the sequential use of ICT and local therapy was superior to the concurrent use of chemotherapy and radiation. Although two previous phase III studies demonstrated benefit for ICT → RT versus RT alone, in particular in patients with inoperable/unresectable disease [51, 52], the role of ICT in connection to CCRT in patients with inoperable/unresectable disease remained controversial, due to difficulties in trial design, execution or insufficient patient accrual [53–57]. However, what most of these studies had in common was the fact that the toxicity with the combined approach was increased. Febrile neutropenia could be found as high as 11% [53] and toxic deaths have been reported even up to 6% [57]. Moreover, the use of ICT could compromise the completion of subsequent chemoradiation, which can have a deleterious effect, not only on local control, but also on survival [58]. Therefore, less toxic schemes have been investigated, such as a modified TPF regimen [59], a weekly carboplatin (AUC2) and paclitaxel (135 mg/m^2) regimen for six consecutive weeks [60] and the TPEx regimen (docetaxel and cisplatin both 75 mg/m^2 every three weeks for three cycles plus weekly cetuximab 400/250 mg/m^2) [61] are all of interest. A randomized controlled trial comparing TPF to modified TPF in fit patients is currently ongoing [62].

When to Use Induction Chemotherapy in Head and Neck Squamous Cell Cancer

For Larynx Preservation

There is an established role for ICT in larynx preservation programs for patients who otherwise would be candidates for total laryngectomy. When Wayne State University published its positive experience with the PF regimen in previously

untreated patients with head and neck cancers [21] and thereafter showed that responders nearly all (97%) were controlled by subsequent radiation, and the others did much less [25], an new concept of treatment was born, i.e. using ICT as a selection procedure. This concept was first tested in randomized trials with in the control arm patients that received the standard of care at that time, i.e. total laryngectomy with postoperative RT, and in the experimental arm patients that were treated with PF ICT followed in responders by RT and salvage surgery if required. These first generation trials are summarized in Table 11.4. The conclusions of these two trials were that the concept of larynx preservation, with the use of ICT as a selection procedure, was safe, kept the larynx in place in about two thirds of the patients and had no negative impact on survival [63–66]. The next generation of larynx preservation trials did not look only to how many larynxes could be kept in place, but took more notice of the function of the larynx. In that context a new definition of larynx preservation came forward "laryngoesophageal dysfunction-free survival" that included death, local failure, salvage laryngectomy, tracheotomy, or feeding tube at 2 years or later [67].

With the milestone of concurrent chemoradiotherapy in the second half of the 1990s (see above) next trials compared ICT followed by RT with CCRT or with alternating CT and RT [68–71]. The results of these studies are summarized in Table 11.5.

Table 11.4 Induction chemotherapy trials for larynx preservation: first generation

Study Group	Tumor size and stage	Treatment arms	No. of pts	Survival (at 5 & 10 years)	LP
VA	Larynx	TL + RND + RT	332	45% & 30%	
1991 [63]	T1-T4, N2-3	PF × 3 → RT[a]		42% & 25%	64%
EORTC	Hypopharynx	TL + RND + RT	202	33% & 14%	
1996, 2012 [64, 65]	T2-T4, N0-3[b]	PF × 3 → RT[a]		38% & 13%	62%

VA Veterans Affairs Laryngeal Cancer Study Group, *LP* larynx preservation, *TL* total laryngectomy, *RND* radical neck dissection, *RT* radiotherapy, *PF* cisplatin 100 mg/m^2 d1 + 5-FU 1000 mg/m^2, d1-5

[a]The non-responders received surgery + RT

[b]N2c was excluded

Table 11.5 Induction chemotherapy trials for larynx preservation: second generation

Study Group	Tumor size and stage	Treatment arms	No.of pts	Survival (at 5 & 10 years)	LP (10 years)
RTOG 91-11	Glottic and supraglottic	PF[1] × 3 → RT	173	58% & 39%	68%[a]
2003, 2013 [70, 71]	N0-1, N2, N3	CCRT (cisplatin)	172	55% & 28%	82%[a]
	T2, T3[+], T3[-], T4	RT	173	54% & 32%	64%[a]
EORTC 24954	Larynx and hypophar.	PF[1] × 2-4 → RT	224	49% & 34%	56%[b]
2009, 2016 [68, 69]	T2-T4, N0-N2	PF[2] alternate with RT	226	52% & 32%	56%[b]

LP larynx preservation, *PF1* cisplatin 100 mg/m^2, d1 + 5-FU 1000 mg/m^2, d1–5, *CCRT* concurrent chemoradiotherapy, *RT* radiotherapy, *PF2* cisplatin 20 mg/m^2/d, d1–5 + 5-FU 200 mg/m^2/d1, d1–5, *T3+* with fixed cord involvement, *T3−* without cord fixation

[a]*LP* larynx in place, function (voice quality, swallowing function, QoL questionnaire) evaluated

[b]*LP* larynx in place, no tumor, no tracheotomy, no feeding tube

The alternating arm in the EORTC trial had a lower dose of 5-FU (total 1000 mg/m^2 instead of 5000 mg/m^2 per cycle) and a lower total dose of radiation (60Gy instead of 70 Gy). This resulted in less grade 3 or 4 mucositis (32% in the sequential arm vs 21% in the alternating arm) and late severe edema and/or fibrosis was observed in 16% of the patients in the sequential arm versus 11% in the alternating arm. No significant differences in outcome between the two arms of the study were observed. Combined with the toxicity data the results favored slightly the alternating arm. However, due to the organizational difficulties in delivering this alternating regimen in daily practice, this regimen is rarely used [66, 68, 69]. RTOG 91–11 is a crucial trial, in that it is the only trial that compares sequential treatment (PF → RT) with cisplatin-based CCRT and a RT alone arm [70, 71]. There have been several analyses reported, all showing a higher larynx preservation rate with the CCRT arm compared with the ICT arm or the RT alone arm. At the long-term follow-up analysis, both chemotherapy regimens significantly improved laryngectomy-free survival (LFS; primary endpoint) compared with RT alone. Overall survival did not differ significantly, although there was a possibility of worse outcome with CCRT relative to ICT (HR, 1.25; 95% CI, 0.98 to 1.61; P = .08). No difference in late effects was detected, but for deaths not related to the study cancer, there was a significant disadvantage for the CCRT group compared to the ICT group (52.8% vs 69.8%, respectively, p = 0.03).

With the revival of ICT in the first decade of the twenty-first century, it was to expect that the comparison of TPF versus PF would also be studied in the larynx preservation setting. This was executed by the GORTEC (Groupe Oncologie Radiotherapie Tete Et Cou) in a phase III protocol [72]. Protocol 2000–1 was conducted in 220 patients with locoregionally advanced laryngeal and hypopharyngeal cancer, who were eligible for total laryngectomy. The European TPF schedule was compared with the standard PF regimen and three cycles at a 3-week interval were planned. The primary endpoint of the study was larynx preservation and larynx preservation was defined as a larynx in place without tumor, tracheostomy or feeding tube. Ultimately, 213 patients were treated with a median follow-up of 105 months [72, 73]. The larynx preservation rate was significantly higher with TPF than with PF (at 10 years 70.3% versus 46.5%, P = .01 in the TPF vs PF arms, respectively). The 10-year laryngeal dysfunction-free survival was 63.7% with TPF and 37.2% with PF, which was again significantly different [73]. There was no significant difference in 5-year or 10-years OS, or disease-free survival (DFS). Statistically fewer grade 3–4 late toxicities occurred with the TPF regimen compared with the PF arm (9.3% vs 17.1%, P = .038). Support for this observation comes from a subgroup analysis of the TAX 324 study, that included only patients with advanced laryngeal and hypopharyngeal cancer. Among those that had operable disease (TPF, n = 67; PF, n = 56), LFS was significantly greater with TPF (HR: 0.59; 95% CI: 0.37–0.95; P = 0.030). Three-year LFS with TPF was 52% versus 32% for PF [74].

For larynx preservation ICT with TPF is one of the two approaches that can be considered as a standard approach for patients with advanced laryngeal or hypopharyngeal cancer, who are not eligible for partial laryngectomy. The

other approach is cisplatin-based CCRT. Overall, T4 disease and tumors extending to the post-cricoid area are not eligible for larynx preservation. It is unclear for the moment which option is best. The two approaches are presently being compared in the ongoing SALTORL trial (GORTEC 2014–03).

For Treatment Intensification

As mentioned earlier, the main question that remained for most clinicians was whether the use of TPF before the cisplatin-based CCRT would lead to survival benefit. The background for that can be found in the individual patient-based meta-analysis (MACH-NC) by Pignon et al., initially published in 2000, but updated in 2009 [75]. In that analysis, a 6.5% 5-year absolute survival benefit was demonstrated for the concurrent chemotherapy/RT approach [75]. No overall survival benefit was observed with the ICT schedules, although a marginal improvement was noticed in trials that made use of the PF combination. Patterns of failure differed between the two approaches. ICT significantly improved the rate of distant metastases (HR, 0.73; 95% confidence interval [CI] 0.61 to 0.88; p = .001), but did not influence locoregional failure. However, CCRT markedly improved locoregional control (HR, 0.74; 95% 0.70 to 0.79; p < .001) with a significant but less impressive improvement in distant control (HR, 0.88; 95% CI, 0.77 to 1.00; p = .04). It seemed therefore reasonable to assume that combining both approaches could have a complementary effect on outcome. The five randomized controlled trials that compared ICT → CCRT versus CCRT alone are summarized in Table 11.6 [53–57]. Four of the five trials showed no impact of ICT on survival. The Italian study (with two types of concomitant regimens, cisplatin/5-FU + RT or cetuximab + RT) did show a survival benefit, but subgroup analysis did not show benefit for patients who received potentiation with cisplatin and fluorouracil. Two trials had accrual problems and stopped early before reaching the required number of patients, and two studies had difficulties in trial design or trial performance. Therefore, the role of ICT given before CCRT on the basis of these five trials still remains controversial.

Two meta-analyses on the usefulness of ICT before CCRT in patients with LA-HNSCC concluded that, although ICT reduced the occurrence of distant failures, this did not translate into a significant survival benefit [76, 77]. However, the most recent systematic review and Bayesian network meta-analysis, comprising 57 trials and 15,723 patients indicated that IC with TPF was significantly superior against CCRT with cisplatin (HR 0.73 95% credible interval [CrI] 0.58–0.92) [78]. Therefore, it seems that over time, more data are pointing at a real value of the TPF regimen when used in addition to cisplatin-based CCRT. **However, as indicated above, individual randomized studies so far have not given an clear answer as to whether ICT is useful for treatment intensification in daily practice. Therefore, further positioning of ICT with CCRT as standard treatment for LA-SCCHN will come from more RCTs directly comparing ICT→ CCRT with CCRT in the appropriate patient population.**

Table 11.6 Randomized trials of induction chemotherapy followed by concurrent chemoradiotherapy versus concurrent chemoradiotherapy alone in patients with locoregionally advanced HNSCC

Investigators/Trial	Population	Regimens	Survival↑	Tox↑
Hitt et al/TTCC [53]	439 pts, stage III/IV	TPF (or PF)x3→CCRT(P)	No	Yes
	Prim. endpoint: PFS	CCRT (cisplatin)		
Haddad et al/ PARADIGM [54]	145 pts, N2 and N3	TPFx3→CCRT (C or Doce)	No	Yes
	Prim. endpoint: OS	CCRT (cisplatin)		
Cohen et al/DeCIDE [55]	285 pts, N2 and N3	TPFx2→CCRT (THF)	No	Yes
	Prim. endpoint: OS	CCRT (THF)		
Ghi et al./GSTTC [56]	421 pts, stage III/IV	CCRT(PF) w/wo prior TPF	Yes[a]	Yes[b]
	Prim. endpoint: OS	BRT(cet.) w/wo prior TPF		
Geoffrois et al./GORTC 2007-02 [57]	370 pts, N2b/c, N3	TPFx3→BRT (cetuximab)	No	Yes
	Prim. endpoint: 2-yPFS	CCRT (carbo/5-FU)		

T docetaxel, *P* cisplatin, *F* 5-fluorouracil, *CCRT* concurrent chemoradiotherapy, *C* carboplatin, *Doce* docetaxel, *Cet* cetuximab, *THFX* docetaxel, fluorouracil and hydroxyurea, *BRT* bioradiation with cetuximab

[a]PFS and OS were significantly better in the ICT arms, but subgroup analysis did not show any benefit for patients who received radiation with cisplatin and 5-FU after TPF

[b]More severe neutropenia in the ICT arms, other toxicities were not significantly different

Results from the DeCIDE trial and the GORTEC 2007–02, showing fewer distant metastases in the ICT arm of the studies, suggest that there still may be patients at very high risk for developing distant metastases who could benefit from ICT. Some improvement in the N-staging in the most recent American joint Committee in Cancer staging system has been made. Features such as low neck nodes and matted nodes (a proxy for extranodal extension) are of interest in that respect. In a retrospective analysis of 321 patients treated with three cycles docetaxel/cisplatin ICT followed by CCRT (weekly cisplatin), Kim et al. reported that lower neck node involvement (level IV, Vb, and supraclavicular regions) ($p = 0.008$) and poor response to ICT ($p < 0.001$) were associated with a significantly inferior distant metastasis-free survival [79].

In contrast to the patterns of failure seen in p16-negative disease, distant failure constitute a considerable portion of treatment failures in patients with p16-positive disease [80]. The Toronto group, in their analysis, pointed at patients with T4 and N3 disease being at high risk for distant failure. In a retrospective study, comprising patients with p16-positive OPSCC with low-neck (level IV and/or Vb) and/or N3 lymphadenopathy, being at high risk of distant failure, 44 receiving ICT (docetaxel/platinum w/wo 5-FU) followed by CCRT (43 receiving platinum, 1 cetuximab) were compared with 44 patients receiving CCRT alone (38 receiving platinum, 6 cetuximab) [81]. The median age of the patients in the CCRT group was somewhat

higher (61 vs 56 years, p = 0.02). Disease control and survival outcomes were reported after adjusting for age, T-stage, N-stage and smoking status. A significant difference in distant metastases (adjusted HR 0.32, p = 0.02) and PFS (adjusted HR 0.46, p = 0.03) was observed, while OS showed a trend (adjusted HR 0.48, p = 0.09), all in favor of ICT at 3 years [81]. Finally, also protein expression biomarkers of aggressive disease could be of use in identifying patients who could benefit from ICT [82]. Examples are elevated expression of cyclin D1 and GDF15 expression as predictive markers for benefit of TPF, and acetylated tubulin as a marker for sensitivity to taxane chemotherapy [83–85]. There are also indications that excision repair cross-complementing 1 (ERCC1) expression may be of importance [86, 87]. Bišof et al. [86] reported, based on a meta-analysis of 1288 HNSCC patients who had been treated with platinum-based chemotherapy, that ERCC1 might be a predictive and prognostic factor for individualized therapies for HNSCC patients. In a study of 64 patients with oro- and hypoharyngeal cancers, who received PF induction chemotherapy before definitive local treatment, Hasegawa et al. concluded that ERCC1 was predictive for response to PF and could select those who were candidates for organ preservation [87]. The study included four clinical variables (age, sex, T-class and N-class) and 22 biomarkers which were tested on pretreatment biopsies. In multivariate analysis, next to T-class, ERCC1 expression came forward as the only independent predictive marker for response. The investigators considered that both a DNA repair pathway and an apoptosis pathway are pivotal to the mechanism underlying response to chemotherapy and suggested that further studies on ERCC1 polymorphisms and mutations and assessing apoptotic response associated with p53 activation in HNSCC were needed to clarify genetic associations with response to chemotherapy in HNSCC patients [87].

For Borderline Resectable or Unresectable Oral Cavity Cancer

Oral cavity cancer is one of the most common malignancies worldwide with geographic variation in incidence and mortality [88]. Higher incidence rates are observed in developing countries compared to developed countries. Bangladesh, Pakistan and India have the highest incidence rates of oral cavity cancer where it is the most common cancer in males and the second in females after breast cancer. As result of delay in presentation, most patients in these countries are diagnosed with advanced disease [89]. Surgery is usually the preferred upfront treatment in patients with oral cavity cancer. However, surgical resection cannot be achieved in many cases with advanced disease without major impact on patient's quality of life. The optimal care of these patients is challenging when surgical treatment is not possible. This is nicely summarized in the recent publication by Alzahrani et al. [89].

The role of induction chemotherapy in patients with resectable oral cavity cancer has been tested in two RTCs and both trials showed a negative outcome [90–92]. Licitra et al. [90] reported on 195 patients with resectable oral cavity cancer (stage

T2-T4 (>3 cm), N0-N2, M0), who were randomized to receive three cycles of PF before surgery versus surgery alone. High-risk patients (positive resection margins, extracapsular nodal spread, nodal disease [N2 or N3], vascular invasion, or perineural invasion) underwent adjuvant RT. There were three toxic deaths in the chemotherapy arm, but ICT did not lead to an improvement in OS (at 5 years 55% in both arms), locoregional relapse or distant failure. An update of this study with a median follow-up of 11.5 years showed similar results with regard to clinical outcomes [91]. Interestingly, in the late follow-up of the patients in this trial, the control group showed a higher incidence of fibrosis (40% vs 22% in chemotherapy arm) and more grade 2 dysphagia (14% versus 5% in the chemotherapy arm), which the authors ascribed to less extensive surgery carried out in the chemotherapy group (31% versus 52% in control group) and less patients receiving postoperative RT (33% versus 46% in control group). Zhong et al. [92] randomized 256 patients with stage III or IVA oral squamous cell cancer to receive 2 cycles of TPF followed by surgery and adjuvant RT or surgery and adjuvant RT alone, again showing no difference in survival. A recent meta-analysis of individual patient data of these earlier mentioned two studies confirmed the lack of clinical benefit from ICT [93]. Contrary to that, for cN2 patients, an OS benefit was found in favor of ICT ($p = 0.04$)**. Taken together, it can be concluded that there is no evidence for routine use of ICT in resectable oral cavity cancer.**

The main goal of using induction chemotherapy before surgery is to convert borderline resectable disease or clearly unresectable disease to technically resectable disease. Although there are no randomized trials to prove this concept, there are studies, most of them coming from India (not surprising with 64% of patients have clinical stage IV disease versus 2.2% in the US), that lead to the same conclusion, i.e. about 30% will become resectable, and patients in whom this is possible will do better than those in whom this not possible [94–98]. Similar results have been reported by our colleagues in Taiwan [99]. Extension of the tumor to the base of skull, prevertebral muscles and encasement or invasion of the carotid artery are absolute contraindication to surgery. In addition, Patil et al. [96] adopted criteria specifically for oral cavity cancer. These include: (1) buccal mucosa primary with diffuse margins and peritumoral edema, going up to or above the level of zygomatic arch and without any satellite nodules, (2) tongue primary (anterior two-thirds) with the tumor extending up to or below the level of the hyoid bone, (3) extension of tumor of anterior two-thirds of the oral tongue to the vallecula, (4) extension of tumor into the high infratemporal fossa, as defined by extension of tumor above an axial plane passing at the level of the sigmoid notch, and (5) extensive skin infiltration impacting the achievement of negative margin. The Indian studies mentioned above are summarized in Table 11.7. Febrile neutropenia in some of these studies was reported to be a major problem. **Nevertheless, according to in particular our Indian colleagues, who see these far advanced stages of disease much more frequently than we do in the higher income countries, ICT may be considered in patients with unresectable or borderline resectable oral cavity cancers, as it may increase the chance of resectability and subsequently might improve outcomes.**

Table 11.7 Induction chemotherapy in unresectable/borderline resectable locally advanced OSCC*

Investigators	No.of pts	Disease stage (T)	Treatments	Outcomes
Rudresha et al. [94]	116	IV (T4b)	TPF or TP (2–3x) → S	Resect. 19%; mOS 19.7 mo; mOS with NST 7.1 mo
Joshi et al. [95]	110	IV (T4b)	TPF or TP (2–3) → S	Resect. 30.9%; mOS 18.0 mo; mOS with NST 6.5 mo
Patil et al. [96]	721	IV (T4a/ T4b)	TP or TPF (2x) → S	Resect. 43%; mOS 19.6 mo; mOS with NST 8.16 mo; 24 mo LRCT rate 32% vs 15%
Rudresha et al. [97]	80	IV (T4a)	TP (2–3) → S	Resect. 23.8%; mOS 16.9 mo; mOS with NST 8.8 mo

OSCC oral squamous cell carcinoma, *TPF* docetaxel/cisplatin/5-FU, *TP* taxane/platinum, *mOS* median overall survival, *NST* nonsurgical treatment, *LRCT* locoregional control, *Resect*. resectable, *Patil's criteria

As a Selection Tool for RT Dose de-Escalation in HPV-Positive OPSCC

Treatment of patients with HPV-positive OPSCC is rapidly evolving and challenging the standard of care of definitive RT with concurrent cisplatin [100]. Several de-escalation approaches are under study, among which are radiation alone instead of radiation combined with cisplatin, radiation combined with cetuximab instead of radiation combined with cisplatin, transoral surgery followed or not by postoperative RT and ICT followed by decreased radiation dose and/or volumes for good responders. In the latter setting, ICT is used as a tool to stratify patients by treatment response. De-escalation approaches are getting major attention in patients with locoregionally advanced OPSCC, because these patients have overall a better prognosis and if treated curatively with current standard treatment (CCRT), are confronted with possible long-term toxicity issues, such as feeding tube dependency ≥2 years post RT, pharyngeal dysfunction (dysphagia), laryngeal dysfunction, mucositis, or other toxicities (e.g. infection, fistula, weight loss etc). Three US trials have reported on ICT approaches, i.e. ECOG 1308 (NCT 01084083), the Quarterback trial (NCT 01706939) and the OPTIMA HPV trial (NCT 02258659).

ECOG 1308 was a single arm phase II study in which patients with HPV-associated OPSCC (the majority having T1-3N0-N2b disease and a history of ≤10 pack-years of smoking) were treated with three cycles paclitaxel, cisplatin and cetuximab, followed by cetuximab concurrently with intensity-modulated radiation therapy (IMRT). The purpose of the study was to evaluate whether a clinical CR to ICT could select patients for reduced radiation dose as a means of sparing late sequelae [101]. Patients with CR at the primary received a reduced RT dose (54 Gy instead of 69.3 Gy). Involved lymph nodes received 69.3 Gy unless they also were judged to have completely responded. The primary end point was 2-year PFS. Of the 90 patients enrolled, 80 were evaluable and 77 received three cycles of ICT. Fifty-six patients (70%) had a CR to the ÌCT at the primary site and 51 patients continued

to cetuximab with IMRT 54 Gy. At a median follow-up of 35.4 months, the 2-year PFS and OS rates were 80% and 94% among those 51 patients. These figures were most promising (96% and 96%, respectively) for the more favorable group of patients (i.e. having <T4, < N2c and ≤ 10 pack-years of smoking). At 12 months, significantly fewer patients treated with the reduced RT dose had difficulty swallowing solids (40% v 89%; P = .011) or had impaired nutrition (10% v 44%; P = .025). The authors concluded that a reduced-dose IMRT with concurrent cetuximab was worthy of further study in favorable-risk patients with HPV-associated OPSCC.

The Quarterback trial, a phase III trial in patients with locally advanced p16-positive OPSCC and ≤ 20 pack years smoking, made use of three cycles of the American TPF regimen and clinical responders who were HPV-positive by type-specific PCR were randomized 1:2 to standard-dosed (sd) IMRT (70 Gy) or reduced-dosed (rd) IMRT (54 Gy), both combined with weekly carboplatin at AUC 1.5. The endpoints of the study were 3-year PFS and OS. The planned number of patients was 365 with 240 in the experimental arm. The original statistical plan was revised because of poor accrual. The trial terminated after 20 evaluable patients were randomized and treated (8 with sdCCRT and 12 rdCCRT). Sixteen (80%) were HPV16-positive and 4 (20%) had other high-risk (HR) variants. Fourteen (70%) had high risk features: T4, N2c, or N3. Median follow up was 56 months (range 42–70). Three-year PFS/OS for sdCCRT and rdCCRT were 87.5% vs 83.3% (log-rank test, p = 0.85), respectively. All three failures were locoregional within 4 months of completion of CCRT, 2 were in HR variants. As mentioned by the authors, the small sample size limits the interpretation of the outcome, but the study supports the potential clinical benefit of radiation dose reduction after ICT as a treatment strategy [102].

In the OPTIMA HPV trial, patients were classified as low-risk (LR) (≤T3, ≤N2B, ≤10 pack year history) or high-risk (HR) (T4, ≥N2c, >10 pack year history). Patients received ICT of three cycles of dose dense carboplatin and nab-paclitaxel. LR patients with 50% response received 50 Gray (Gy) RT (RT50) while LR patients with 30%–50% response or HR patients with 50% response received 45 Gy CCRT (CCRT45). Patients with lesser response received standard-of-care 75 Gy CCRT (CCRT75). The primary end point was 2-year progression-free survival compared with a historical control of 85%. Secondary end points included overall survival and toxicity. Sixty-two patients (28 LR/34 HR) were enrolled [103]. Of the LR patients, 71% received RT50 while 21% received CRT45. Of the HR patients, 71% received CRT45. With a median follow-up of 29 months, 2-year PFS and OS were 95% and 100% for LR patients and 94% and 97% for HR patients, respectively. The overall 2-year PFS was 94.5% and within the 11% non-inferiority margin for the historical control. Grade ≥ 3 mucositis occurred in 30%, 63%, and 91% of the RT50, CCRT45, and CCRT75 groups, respectively (P = 0.004). Rates of any PEG-tube use were 0%, 31%, and 82% for RT50, CCRT45, and CCRT75 groups, respectively (P < 0.0001) [103]. This decreased over time, being at 12 months 0%, 4% and 9%, respectively. Updated information was presented at ASCO 2020, now including 107 patients that were treated according to the same lines and now with a median follow-up of

36 months [104]. Overall, 94% of patients were alive at last follow-up (98% LR; 89% HR). Three patients developed a recurrence (2 HR and 1 LR); 2 local and 1 at distance. This OPTIMA approach demonstrated excellent oncologic and functional outcomes with long-term follow-up.

Despite these promising results, clinicians should refrain from de-escalation approaches outside clinical trials for this moment, because the safety of these approaches are still unclear. This has been reinforced by unexpected negative outcomes of two RCTs, in which cetuximab plus RT was compared with the standard-of-care cisplatin-based CCRT in p16-positive OPSCC [105, 106]**.**

Oligometastatic Disease

Another area of potential interest for the applicability of induction chemotherapy is oligometastatic disease. It is estimated that 5–47% (mean 15%) of patients will have distant metastases during the course of the disease [107]. The Surveillance Epidemiology and End Results (SEER) database revealed that 19% of patients with oral cavity or pharynx cancer presented with distant metastases at diagnosis [108]. The most common site of metastases from HNSCC is the lung accounting for up to 70% to 85%, followed by metastases to the bone (up to 20%) and liver (up to 10%). Other organs such as the brain, mediastinum, skin and bone marrow occur even more rarely [109]. There are different definitions of oligometastases for different cancers, but a consensus definition is five or fewer sites of metastatic disease [109]. Patients with oligometastatic HNSCC can be divided in two groups; (1) those who present with metastatic disease at initial diagnosis, i.e. synchronous distant metastases, and (2) those who have developed the metastatic lesions during their surveillance after their definitve treatment, the so-called metachronous distant metastases, with or without locoregional disease relapse.

Considering all patients with metastatic HNSCC as one group that should be treated with systemic therapy for palliation might not be correct. The contemporary standard of care systemic therapies result in a median survival of 10.1 to 13.6 months and it is unclear yet whether the treatment with immune checkpoint inhibitors will lead to cure [40, 110]. However, metastatic disease in HNSCC covers a wide range of disease presentations, depending not only on the site from which these metastases are originating, but also on the tumor biology and kinetics, whereby metastatic disease may vary from widely disseminated disease to oligometastatic disease.

Oligometastatic disease is a moving concept not only defined by its phenotypic metastatic burden but also by the ability to perform metastatic-directed treatments [107]. Advances in minimally invasive surgery and whole body stereotactic hypofractionated radiation therapy have opened an avenue to treat metastases in a safe, well-tolerated and relatively cost-effective manner. In a retrospective series from Germany, the authors noted a significant survival benefit for HNSCC patients who

received a specific therapy regarding distant metastases irrespective of localization as compared to a matched control cohort [111]. An analysis of patients with metastatic HNSCC in the National Cancer Database (NCDB) revealed that the patients who received high-intensity local treatment (defined as radiation doses ≥60 Gy or oncologic resection of the primary tumor) and systemic therapy had a 13% improvement in 2-year overall survival(OS) compared to patients receiving systemic therapy alone [112]. It is beyond the scope of this chapter on induction chemotherapy to discuss extensively the treatment of oligometastatic disease and the participation of local therapies therein. Suffice to say that currently, due to the lack of randomized but also sufficiently powered prospective trials, no firm recommendations can be given on how to optimally treat oligometastatic disease. However, ablative techniques have already penetrated into routine clinical practices in high-volume centers [113].

The role of induction chemotherapy in this context is primarily concerning synchronous metastatic disease at first diagnosis. Singular cases can be found in the literature were upfront chemotherapy is given with curative intent. Therefore strategies combining induction chemotherapy and upfront metastasis-directed treatments prior to locoregional therapy for the primary tumor can be anticipated [82].

Where to Use Induction Chemotherapy

Toxicity is an issue of ICT, in particular when there is not much experience with the contemporary ICT regimens. With the European TPF regimen, as given in TAX 323/EORTC 24971 [44], i.e. with prophylactic antibiotics [ciprofloxacin from day 5–15] in each cycle and dexamethasone given before the start of each cycle to prevent docetaxel-related hypersensitivity reactions, skin toxicity and fluid retention, common (≥5%) grade 3–4 adverse events included: neutropenia (76.9%), leukopenia (41.6%), alopecia (11.6%), anemia (9.2%), infection (6.9%), febrile neutropenia (5.2%) and thrombocytopenia (5.2%). 6.2% of patients discontinued treatment due to adverse events and there were 2.3% toxic deaths. With the American TPF, as given in TAX 324 [43], premedication, prophylactic antibiotics and dexamethasone were given in the manner as in TAX 323/EORTC 24971, common (≥5%) grade 3–4 adverse events included: neutropenia (83%), stomatitis/mucositis (21%), nausea (14%), dysphagia (13%), anemia/febrile neutropenia/neutropenia infection/anorexia (each 12%), vomiting (8%), diarrhea (7%), infection (6%), and lethargy [5]. 6% of patients discontinued treatment due to adverse events related to treatment and there <1% deaths due to toxic effect of study medication.

Crucially in the safe use of TPF regimens is that it is being administered by experienced oncologists, familiar with the necessary protocols and supportive care requirements to ensure patient safety and maximize adherence throughout the treatment [114]. Adequate fluid management, especially on days 1–2 during TPF

administration is crucial in preventing renal toxicity, hypovolemia, and severe fatigue. Discussing the patient in multidisciplinary team (MDT) meetings is strongly advised, considering also additional matters such as patient's psychological and nutritional status, potential for palliative care, addiction services, and speech therapy. The importance of MDT meetings have been extensively discussed during THNO-5 [115]. MDT meetings have emerged as a practical necessity for optimal coordination among health professionals and clear communication with patients, and increasingly more attention is paid to psychological aspects, quality of life, patient's rights and empowerment, and survivorship. Moreover, it has become more and more clear that treatment in higher volume centers, and experience of the center in trial participation correlate with outcomes [116, 117].

Conclusions

For more than 10 years the PF regimen has been replaced by the TPF regimen as the standard ICT regimen [43, 44]. ICT has an established role for organ preservation in advanced laryngeal and hypopharyngeal cancer and the TPF regimen has been validated in that setting. There remains uncertainty about the benefit of the sequential approach of ICT followed by CCRT, despite the fact that ICT significantly reduces the occurrence of distant metastases. It seems therefore appropriate to further study ICT in patients who have the highest risk to develop distant metastases, in particular patients with low neck nodes and matted nodes. Moreover, further studies in patients with HPV-associated OPSCC at risk for distant failure (T4 or N3 disease) could be considered for that also. Retrospective data from India suggest that ICT may play an important role in converting borderline resectable disease or clearly unresectable disease to technically resectable disease. Therefore, larger randomized trials in patients with borderline resectable cancer of the oral cavity are needed to establish the benefit of induction chemotherapy in this setting. Data are available that suggest that ICT can be used as a tool to select HPV-associated OPSCC patients for dose and volume de-escalation of RT, and retaining excellent oncologic and functional outcomes. These approaches still need to be confirmed in adequately sized clinical trials. Outside clinical trials, the utility of ICT is restricted to uniquely pragmatic clinical scenarios, such as unavoidable delay in radiation or in the situation that RT is not tolerated or feasible. This can happen when there is severe pain from advanced disease or there is impending airway compromise or neurologic dysfunction that necessitates rapid initiation of treatment [82]. Future areas of research are the role of ICT in strategies whereby ICT is combined with upfront metastases-directed treatments, the usefulness of targeted agents or immune checkpoint inhibitors in the induction setting; studies in that direction have started. Finally, the application of radiographic, proteomic and genomic biomarkers will get attention to further define prognostic groups and guide treatment selection with greater precision.

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Chapter 12
Prognostic Role of p16/HPV in Non-oropharyngeal Head and Neck Squamous Cell Cancer (HNSCC)

Stavros Gkolfinopoulos, Panagiota Economopoulou, and Amanda Psyrri

Introduction

HPV infection has been established as an etiologic and prognostic factor for a subset of oropharyngeal squamous cell carcinoma (OPC) with distinct clinical and pathologic characteristics [1]. Also established is that the expression of p16/INK4A gene correlates with HPV infection in the oropharynx and, consequently, the product of this gene, detected with immunohistochemistry (IHC), can be used as a surrogate biomarker for HPV-related OPC [2]. This information is vital for properly staging HPV(+) OPC, for determining the prognosis of the disease, and for implementing de-intensification strategies in the context of clinical trials. On the contrary, conflicting evidence exists regarding the role of p16/HPV as a biomarker in non-OPC head and neck cancer, more specifically for oral, for laryngeal and for hypopharyngeal primaries. Most of the relevant data are inconsistent and derive from retrospective and heterogeneous series of patients. Herein, a brief review of the existing information regarding the utility of p16 as a surrogate marker for HPV in non-OPC HNSCC is presented, and the potential prognostic role of p16/HPV in non-OPC primaries is analyzed.

S. Gkolfinopoulos · P. Economopoulou
Oncology Section, 2nd Department of Internal Medicine-Propaedeutic, University General Hospital "Attikon", Chaidari, Greece

A. Psyrri (✉)
Oncology Section, 2nd Department of Internal Medicine-Propaedeutic, University General Hospital "Attikon", Chaidari, Greece

Medical School, National and Kapodistrian University of Athens, Athens, Greece

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HPV Life Cycle

HPV is a small, non-enveloped, double-stranded DNA virus that encodes a total of 8–9 proteins in approximately 8000 base pairs and has the ability to infect cutaneous or mucosal tissues. The viral genome consists of three distinct regions that have specific functional properties. The early (E) region encodes proteins regulating viral transcription (E2), viral DNA replication (E1, E2), cell proliferation (E5, E6, E7), and viral particle release (E4). E6 and E7 viral oncogenes also encode proteins associated with malignant lesions that are capable of immortalizing primary human keratinocytes. The continuous expression of E6 and E7 is crucial in maintaining the cancer phenotype in infected cells, as repression of their expression reverses the malignant phenotype of HPV(+) cancer cells. The late (L) region encodes for two structural viral capsid proteins (L1 and L2). Finally, the long control (LCR) or non-coding region (NCR) regulates viral gene expression and replication [3].

HPV has a complex gene expression that requires a synchronization of transcription, mRNA stability, splicing, and polyadenylation with keratinocyte differentiation and distinct phases of the viral life cycle. The life cycle of HPV is directly related to the cellular differentiation program of the host cell. In the initial phase of the HPV life cycle, keratinocytes of the basal cell layer are infected by the virus that has passed through the above epidermal barrier through erosions and microwounds. For efficient establishment of infection from the high-risk subtypes it is vital that they infect actively dividing basal, or stem epithelial cells. Initial infection is followed by a phase of viral genome amplification; subsequently, the viral genome is maintained as an extrachromosomal circular element, known as episome, at a low copy number. Alternatively, it can be integrated into the host-cell genome. This integration usually occurs downstream of the early genes E6 and E7, often in the E1 or E2 region. HPV uses the host cell replication machinery to initiate viral DNA replication. In the case of high risk HPVs, proteins E6 and E7 promote cell-cycle progression and viral DNA replication in differentiated keratinocytes as they move towards the surface epithelium. As a result, HPV DNA replicates in a high copy number in differentiated cells located near the epithelial surface. Both E7 and E6 have a vital role in manipulating the cellular replication mechanisms in order to create the optimal environment for viral genome replication. Viral protein E6 is required for episomal genome maintenance, while E7 forces the infected cell to re-enter S-phase. The integration of HPV DNA into the host genome disrupts the expression of the main viral transcription/replication factor E2, which acts as transcriptional repressor of E6 and E7 viral oncogenes. Furthermore, the E6 protein causes degradation of the p53 tumor suppressor protein via a ubiquitin-mediated process, while the HPV-E7 protein binds cullin 2 ubiquitin ligase complex and ubiquitinates the retinoblastoma (Rb) tumor suppressor protein. Consequently, the p53 and pRb tumor suppressor pathways are dormant but active in cancer cells due to the continuous expression of E6 and E7 oncogenes. Degradation of Rb induces expression of p16INK4A, which is the hallmark of HPV(+) OPC. pRb downregulates p16 protein at the transcriptional level and low pRb levels, inversely, lead to p16

upregulation. This is the reason why HPV-associated cancers contain high p16 protein levels. The final stage of the life cycle of HPVinvolves the exit from the cell and the expression of late viral proteins L1 and L2 to enable packing of the viral genome [4].

Risk Factors for HPV(+) Non-OPC

HPV(+) OPC has distinct risk factors that are different from the common risk factors of HPV(−) OPC, e.g. excessive exposure to tobacco and alcohol. It is correlated with younger age, male gender, multiple sexual partners and higher socio-economic status, to name a few [5]. It is, however, unclear, whether these same demographic variables apply for HPV(+) non-OPC as well.

To our knowledge, there is only one study that has tried to answer that query. In a multicenter, case-control study of SCCs called the Papillomavirus Role in Oral Cancer Viral Etiology study (PROVE), Windon et al. have discovered that HPV(−) non-OPC patients were more likely to be ever smokers than HPV(+) OPC (n = 185, OR 3.28, 95%CI 1.10–10.2). Also, compared with their HPV(+) OPC counterparts, HPV(+) non-OPC were less likely to have had over 3 oral sexual partners (OR 0.29, 95%CI 0.06–0.9), more likely to have multimorbidity (OR 3.30, 95%CI 1.04–10.5), and less likely to have antibodies to HPV16 E6 (90% vs 28%, OR 0.05, 95%CI 0.02–0.2). Although this was a small study, it provided potential evidence that HPV is not an adequate factor to promote carcinogenesis in non-OPC sites, in contrast to OPC, but rather a second hit of chemical-induced carcinogenesis is required for cancer progression in these cases, as it also happens in HPV(−) HNSCC [6].

p16 as a Surrogate Biomarker for a Transcriptionally Active HPV Infection in Non-OPC

The gold standard for determining that HPV is actively contributing to the oncogenic process in OPC is the detection of the E6/E7 viral oncogene expression through quantitative reverse transcriptase–PCR (qRT-PCR) [3, 4]. Detection of HPV mRNA, however, in formalin-fixed paraffin-embedded (FFPE) tissue has variable sensitivity depending on the quality of the clinical sample. Moreover, many HPV(+) patients identified in the next-generation sequencing study by Parfenov et al. had low levels of E6/E7 expression and could be misclassified by E6/E7mRNA detection [7]. A method that is commonly used is HPV DNA detection by either in situ hybridization (ISH) or polymerase chain reaction (PCR) [2]. DNA ISH can differentiate between integrated and episomal forms of HPV in tumors but lacks sensitivity. HPV DNA PCR is a sensitive method for determination of HPV status but it lacks specificity. HPV DNA presence in tumors per se cannot prove causality, as

HPV is ubiquitously present in humans. Also, as has already been mentioned, the detection of p16 protein expression by IHC staining is used as a surrogate marker of oncogenic HPV infection. A negative autoregulatory loop between p16 and pRb has been described and degradation of pRb by HPVE7 oncoprotein leads to p16 upregulation in HPV(+) cancers [8]. p16 IHC followed by PCR for HPV DNA detection in p16(+) cases has been proposed as a reliable algorithm for determination of HPV status in paraffin-embedded OPC specimens. p16 protein expression, however, is not a reliable surrogate biomarker for HPV infection outside the oropharynx.

Using these methods, it has been found that in OPC, HPV positivity ranges between 57% and 72%, with the variation attributed to differences in assay selection and study populations [9–11]. In addition, a high concordance rate of approximately 90% is noted between HPV ISH and p16 IHC in OPC, and this is partly a result of the high rates of active and persistent HPV infection in this site [12].

In non-OPC sites, however, the rates of active HPV infection are substantially lower, as only 1.3% to 7% of non-OPCs, including cancers of the oral cavity, hypopharynx, and larynx, are HPV positive [13–15]. Similarly, the concordance rates between these two methods of HPV detection also seem to be lower. For example, in oral cavity squamous cell cancer, sensitivity of p16 IHC compared with high-risk HPV E6/E7 mRNA expression is 79%, specificity is 93%, positive predictive value (PPV) is 41%, and negative predictive value is 99% [14].

Harris et al. examined biomolecular profiles in a cohort of 25 young adults with squamous cell cancers (SCCs) of the oral tongue diagnosed between 1989 and 2007. Median age at diagnosis was 30 years (range: 20–39 years). Patients with non-squamous histology, prior history of malignancy and distant metastatic disease at presentation were excluded. Further demonstrating the discrepancy between p16 expression and HPV DNA positivity in non-OPC, p16 overexpression was observed in 11 of 25 patients, whereas HPV-16 DNA positivity was observed in none of the tumor samples by ISH and 2 of the tumor samples by PCR. Interestingly, neither of these HPV DNA(+) patients were found to be p16(+) as well. In this study, p16INK4a positivity was correlated with improved relapse-free survival (HR = 0.23, p = 0.01) and overall survival (OS) (HR = 0.28, P = 0.05). In this trial p16 positivity was correlated with favorable prognosis, while p16INK4a overexpression was not a reliable predictor of HPV positivity. The authors concluded that a mechanism alternative to HPV infection that is leading to p16 positivity may exist in this particular subset of tumors, and that p16INK4a status is the truly important prognosticmarker in HNSCC, independent of HPV infection. However, the small cohort size and the selected patient populationare serious limitations for generalization of these results [16].

Furthermore, in a study by Chung et al. p16 expression and high-risk HPV status in non-OPCs from RTOG 0129, 0234, and 0522 studies were determined by IHC for p16 and HPV DNA ISH for high-risk HPV DNA. A total of 683 eligible patients with non-OPSCC tumors, including primary sites in the oral cavity, hypopharynx, and larynx, were identified among the 1921 patients enrolled onto the above-mentioned trials. Tumors from 356 (52.1%) of 683 patients with non-OPSCC were tested for p16 expression, which could be determined in 90.4% (322 of 356) of the

tumors. HPV status could be determined in 95.5% (297 of 311). Overall, 19.3% (62 of 322) of non-OPC were p16 positive and 9.4% (28 of 297) were HPV ISH positive. p16 expression was positive in 14.1% (12 of 85), 24.2% (23 of 95), and 19.0% (27 of 142) and HPV ISH was positive in 6.5% (six of 93), 14.6% (15 of 103), and 6.9% (seven of 101) of non-OPCsfrom RTOG 0129, 0234, and 0522 studies, respectively. Cancer of the oral cavity had the highest rate of p16 positivity (21 [26.3%] of 80), followed by the larynx (31 [17.1%] of 181) and hypopharynx (10 [16.4%] of 61). Also, cancer of the oral cavity had the highest rate of HPV ISH positivity (13[14.6%] of 89), followed by the larynx (12 [7.9%] of 151) and hypopharynx (three [5.3%] of 57). HR for p16 expression were 0.63 (95% CI, 0.42 to 0.95; P = 0.03) and 0.56 (95% CI, 0.35 to 0.89, P = 0.01) for progression-free (PFS) and OS, respectively. Poor concordance was observed between p16 and HPV ISH among the subsites of the oral cavity, hypopharynx, and larynx, where oral cavity tumors had the worst concordance. Although this trial also showed that patients with p16-negative non-OPC have worse outcomes than patients with p16-positive non-OPC, HPV status was not found to be prognostic, so once again it was demonstrated that p16 was not a good surrogate biomarker for HPV positivity [17].

Finally, in a retrospective study of 409 cases of oral cavity SCC treated at 4 North American Hospitals, fifteen high-risk HPV types were detected in tumors by consensus PCR followed by type-specific HR-HPV E6/7 oncogene expression by quantitative reverse-transcriptase PCR. P16 expression was evaluated by IHC. Twenty-four (5.9%) were high-risk HPV E6/7 expression positive; 3.7% (95%CI 1.8–5.5) for HPV16 and 2.2% (95%CI 0.8–3.6) for other high-risk HPV types. HPV(+) tumors originated from throughout the oral cavity (floor of mouth [n = 9], anterior tongue [6], alveolar process [4], hard palate [3], gingiva [1] and lip [1]) and were significantly correlated with male gender, small tumor stage, poor tumor differentiation, and basaloid histopathology. In this trial, p16 IHC had very good-to-excellent sensitivity (79.2%, 95%CI 57.9–92.9), specificity (93.0%, 95%CI 90.0–95.3), and negative-predictive value (98.6%,95%CI 96.8–99.6), but poor positive-predictive value (41.3%, 95%CI 27.0–56.8) for HR-HPV E6/7 expression in oral cavity SCC [14].

Conclusively, the data at our disposal suggest that p16 is a poor surrogate marker for transcriptionally active HPV infection in non-OPC sites.

p16/HPV as a Prognostic Factor in Non-OPC

In addition to the above-mentioned studies, several other trials have attempted to elucidate the prognostic role of p16 and/or HPV status in non-OPC disease sites. Young et al. evaluated a cohort of 324 laryngeal SCC patients for the expression of p16 by IHC and for high-risk HPV E6 and E7 mRNA transcripts by RNA ISH. The median age of patients at diagnosis was 66 years (range 36–88 years). Males comprised 94% of the patients, with 95% being current or former smokers. p16 expression and HPV status were correlated with clinicopathological features and outcomes.

In this trial, 6.5% of the patients were p16(+) and only 7 cases were HPV RNA(+), all of which were also p16 IHC positive. There was no difference in OS between p16-positive and p16-negative patients with 2-year survival of 79% in each group (HR = 0.83, 95% CI 0.36–1.89, P = 0.65). Also, no statistically significant difference in OS was found between patients with HPV RNA ISH-positive tumors compared with ISH-negative tumors with 2-year survival of 86% and 71%, respectively (HR = 0.76, 95% CI 0.23–2.5, P = 0.65). The most significant strength of this study is the large cohort consisting of a single site of head and neck cancer only, namely the larynx, while its major limitation is its retrospective nature. The researchers concluded that p16 overexpression in laryngeal cancer is infrequent as are the proportion of cases with high-risk HPV transcripts, and there are no statistically significant correlations with survival outcomes [18].

Furthermore, in a retrospective, multi-institution study by Fakhry et al. 239 patients with OPC and 621 patients with non-OPC of the oral cavity, larynx, and nasopharynx, diagnosed from 1995 to 2012, were centrally tested for p16 and HPV by HPV16 DNA and high-risk HPV E6/E7mRNA ISH. The prevalence of HPV(+) tumors among cancers of the oropharynx, oral cavity, larynx, and nasopharynx was 56%, 2%, 5%, and 10%, respectively. The tumor HPV status and p16 were not of prognostic significance in HNSCCs of the oral cavity (n = 253; P = 0.22), larynx (n = 243; P = 0.72), or nasopharynx (n = 125; P = 0.23). Also, the study did not find any correlation of p16 with OS for non-OPC (P = 0.26) [19].

Also, D′ Souza et al. analyzed data from 1362 HNSCC cases diagnosed between 2002–2011 and registered in epidemiologic studies in Brazil (GENCAPO study, n = 388), U.S. (CHANCE study, n = 472), and Europe (ARCAGE study, n = 502). Tumors were centrally tested for p16 and HPV16 DNA by PCR. In total, 517 OPC and 845 non-OPC cases (397 laryngeal, 382 oral cavity, and 66 hypopharyngeal SCC) were identified. Although HPV-related OPC had similar survival benefits across these three regions, among non-OPC, neither p16 (aHR = 0.83, 95%CI = 0.60–1.14), HPV16 DNA (aHR = 1.20, 95%CI = 0.89–1.63), or p16(+)/HPV16(+) (aHR = 0.59, 95%CI = 0.32–1.08) were statistically significant predictors of mortality. The researchers concluded that the prognostic utility of HPV among non-OPC patients is limited and, although cases with dual p16 and HPV positivity appeared to have better outcome, tumor HPV/p16 testing should not be routinely done in non-OPC [20].

In addition, Lassen et al. analyzed retrospectively p16 expression via IHC in a cohort of 1294 patients enrolled in previously conducted DAHANCA-trials between 1992 and 2012. The study included patients with stage III–IV pharynx and larynx cancer treated with primary CRT. Thirty-eight percent (490/1294) of the tumors were p16-positive with a significantly higher frequency in OPC (425/815) than in non-OPC (65/479) ($p < 0.0001$). As expected, in OPC p16-positivity correlated with significantly improved locoregional control (LRC), event-free survival (EFS) and OS. However, in non-OPC no prognostic impact of p16-status was found for either endpoint: LRC (HR = 1.13 [0.75–1.70]), EFS (HR = 1.06 [0.76–1.47]), and OS

(HR = 0.82 [0.59–1.16]). This trial further suggests that, in non-OPC sites, p16 positivity is rare and does not carry any prognostic significance [21].

On the contrary, results from a retrospective analysis of 19,993 non-OPC patients registered in the National Cancer Data Base (NCDB), of whom 5070 were positive for HPV via PCR, revealed that OS was significantly higher for patients with HPV(+) versus HPV(−) non-OPC, and that the robust survival advantage of HPV was maintained in all subsites. Improved outcomes were more pronounced in patients with locally advanced compared to early stage disease. The main limitation of this trial is that, since routine HPV testing in non-OPC is not standard of care, selection bias must exist in the data set. Therefore, factors driving the decision to test for HPV status may be contributing to the improved outcomes of the HPV(+) non-OPC cohort [22].

Moreover, high-risk HPV positivity was associated with OS in certain non-OPC primaries in a large analysis of 24,470 patients diagnosed with HNSCC between 2010 and 2013 who had been registered in the NCDB. Of these patients, 9907 patients had been diagnosed with non-OPC SCCs: 1085 with SCC of the hypopharynx, 4804 with SCC of the larynx, and 4018 with SCC of the oral cavity. The rate of high-risk HPV positivity for those patients varied by primary tumor site: 17.7% of patients with SCCs of the hypopharynx were high-risk HPV(+), as were 11% and 10.6%, respectively, of those with SCCs of the larynx and oral cavity. HPV status was found to be prognostic in multiple unadjusted and propensity-adjusted non-OPC populations. HPV positivity was associated with superior OS in patients with hypopharyngeal SCC with a HR of 0.61 ($P < 0.001$), in patients with AJCC stage III to IVB laryngeal SCC (HR = 0.79; $P = 0.019$), and in patients with AJCC stage III to IVB SCC of the oral cavity (HR = 0.78; $P = 0.03$). However, as the researchers themselves have pointed out, there are certain serious limitations in this trial. First of all, the results of this trial derive from retrospective, administratively collected data. Then, important information such as patterns of response/failure to treatment, salvage therapies, cause of death and smoking status are not captured by NCDB. Finally, the method of testing is not prespecifiedby the NCDB, so HPV testing wasperformed as part of clinical care and was, therefore, heterogeneous. The results of this study, therefore, should be interpreted with caution [23].

Conclusions

The studies evaluating the prognostic impact of HPV infection in non-oropharyngeal head and neck cancers have shown conflicting results (Table 12.1). Variations in sample sizes, geography, the method of HPV detection and other factors may have contributed to this fact. It seems that p16 is a poor surrogate biomarker for oncogenic HPV infection for non-OPC disease sites. The majority of studies so far suggest that the prognostic impact of HPV positivity is reserved for the oropharynx, so routine HPV testing is not recommended for other sites.

Table 12.1 Clinical Trials investigating the prognostic role of p16/HPV in non-OPC

Trial	Trial Design	Evaluation of HPV positivity	Disease Site	N	HPV (+) cases	Survival Outcomes
Young et al (ref 18)	Retrospective, single cohort study	Expression of p16 by IHC and for high-risk HPV E6 and E7 mRNA transcripts by RNA ISH	Larynx	324	6.5%: p16(+) 7 pts.: HPV RNA (+)	**HPV (+) vs HPV (−): 2-year survival: 86% VS 71%** (HR = 0.76, 95% CI 0.23–2.5, P = 0.65)
Fakhry et al (ref 19)	Retrospective, multi-institution study	HPV16 DNA and high-risk HPV E6/E7 mRNA ISH	Larynx, oral cavity, nasopharynx	**OPC:** 239 **Non-OPC:** 621	Oropharynx:56% Oral cavity: 2% Larynx: 5% Nasopharynx:10%	**Tumor HPV status-p16 not prognostic** Oral cavity: (n = 253; P = 0.22) Larynx: (n = 243; P = 0.72) Nasopharynx: (n = 125; P = 0.23)
D'Souza et al (ref 20)	Retrospective data analysis from the studies GENCAPO, CHANCE, ARCAGE	Central analysis for p16 and HPV16 DNA by PCR	Larynx, oral cavity, hypopharynx	**OPC:** 517 **Non-OPC:** 845 (397 laryngeal, 382 oral cavity, 66 hypopharyngeal SCC)	**p16-neg /HPV16-pos: 61/125** **p16-pos /HPV16-neg: 52/122** **p16-pos /HPV16-pos: 12/39**	**No survival differences noted:** **p16:** (aHR = 0.83, 95%CI = 0.60–1.14) **HPV16 DNA:** (aHR = 1.20, 95%CI = 0.89–1.63) **p16(+)/HPV16(+):** (aHR = 0.59, 95%CI = 0.32–1.08)

Trial	Trial Design	Evaluation of HPV positivity	Disease Site	N	HPV (+) cases	Survival Outcomes
Lassen et al (ref 21)	Retrospective analysis of pts. enrolled in a DAHANCA cohort	p16 expression via IHC	Pharynx, larynx	1294	**OPC:** 425/815 **Non-OPC:** 65/479	**No prognostic impact of p16-status:** **LRC:** (HR = 1.13 [0.75–1.70]) **EFS:** (HR = 1.06 [0.76–1.47]) **OS:** (HR = 0.82 [0.59–1.16])
Ko et al (ref 22)	Retrospective analysis of non-OPC patients registered in the NCDB	HPV DNA via PCR	Oral cavity, larynx, hypopharynx	19,993	**HPV(+)/HPV(−): 5070/14,923**	**OS associated with HPV status:** **Early stage:** (HR = 0.68; 95% CI 0.51–0.92) **Late stage:** (HR = 0.46; 95% CI 0.39–0.53)
Tian et al (ref 23)	Retrospective analysis of non-OPC patients registered in the NCDB	HPV DNA via PCR	Oral cavity, hypopharynx, larynx	9907	**HPV (+) cases:** Oral cavity: 10.6% Hypopharynx: 17.7% Larynx: 11%	**OS associated with HPV status:** **Hypopharynx:** HR: 0.61 (P < 0.001) **Larynx (stage III to IVB:** HR = 0.79 (P = 0.019) **Oral cavity (stage III to IVB:** HR = 0.78 (P = 0.03)

IHC immunohistochemistry; *ISH* in situ hybridization; *HR* Hazard Ratio; *aHR* adjusted Hazard Ratio; *CI* Confidence Interval; *OS* overall survival; *OPC* oropharyngeal cancer; *NCDB* National Cancer Data Base

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Chapter 13
Is there a Role for Neoadjuvant Targeted Therapy and Immunotherapy?

Simon Beyaert and Jean-Pascal Machiels

Abbreviations

^{18}FDG-PET	18- fluorodeoxyglucose-positron emission tomography
EGFR	Epidermal growth factor receptor
G-CSF	Granulocyte-colony stimulating factor
PD(−L)	Programmed death (-Ligand)
SAE	Severe adverse events
SCCHN	Squamous cell carcinoma of the head and neck
SUV	Standardized uptake value
TPF	Taxanes, platinum-based chemotherapy and 5-fluorouracil

Introduction

The role of induction or neoadjuvant therapy to treat locally advanced squamous cell carcinoma of the head and neck (SCCHN) is controversial [1, 2]. Standard treatment remains concomitant chemoradiation with high-dose (100 mg/m^2) cisplatin when a non-surgical approach is preferred [1, 2]. The only recognized indication for induction chemotherapy is larynx preservation, and the oncological outcome is similar to that of concomitant chemoradiation in this particular setting [3]. Taxane/platinum/5-Fluorouracil (TPF) combinations have proven to be superior to

S. Beyaert
Institut de Recherche Expérimentale et Clinique (Pôle MIRO), Université Catholique de Louvain (UCLouvain), Brussels, Belgium
e-mail: simon.beyaert@uclouvain.be

J.-P. Machiels (✉)
Institut de Recherche Expérimentale et Clinique (Pôle MIRO), Université Catholique de Louvain (UCLouvain), Brussels, Belgium

Service d'Oncologie Médicale, Institut Roi Albert II, Cliniques universitaires Saint-Luc, Brussels, Belgium
e-mail: jean-pascal.machiels@uclouvain.be

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platinum/5-fluorouracil schedules, and TPF is therefore now the accepted standard for induction [4, 5].

In this chapter, we review if there is a role forneoadjuvant targeted therapy or immunotherapy in the treatment of SCCHN. We discuss two different approaches: neoadjuvant or induction studies and window of opportunity trials.

Induction Therapy with Targeted Therapy and Immune Checkpoint Inhibitors

In a neoadjuvant or induction approach, the definitive standard treatment (i.e. surgery or (chemo)radiation) is delayed to allow enough time for the neoadjuvant agent(s) to produce a therapeutic response and improve overall treatment efficacy. In this setting, the use of drugs targeting the epidermal growth factor receptor (EGFR) has been largely investigated in combination with chemotherapy.

Three trials have studied the safety and feasibility of combining cetuximab with TPF [6–8]. Specenier et al. investigated four cycles of TPF plus cetuximab (TPF-E) (cisplatin and docetaxel 75 mg/m^2 on day 1 followed by 5-fluoruracil (5-FU) 750 mg/m^2/day as a continuous infusion on days 1–5 plus cetuximab at a loading dose of 400 mg/m^2 followed by a weekly dose of 250 mg/m^2), with prophylactic antibiotics but no growth factors [6]. Induction TPF-E was discontinued in 13% of patients due to toxicity, and three out of 46 patients developed a bowel perforation. Only 65% of the patients in this study started chemoradiation. Mesia et al., using the same TPF regimen but with prophylactic granulocyte-colony stimulating factor (G-CSF) and antibiotics, observed febrile neutropenia, grade III/IV diarrhea and toxic death in 24%, 20% and 6% of patients, respectively [7]. It was therefore deemed that TPF-E leads to unacceptable toxicities. In contrast, Haddad et al. found that it was feasible to give three cycles of TPF-E with cisplatin 100 mg/m^2 day 1, docetaxel 75 mg/m^2 day 1 and 5-FU 850 mg/m^2/day as a continuous infusion on days 1–4 plus cetuximab for a total of six weeks given on days 1 and 8 of each cycle of TPF [8]. Similarly, a phase I trial combined lapatinib with TPF, but this combination also resulted in prohibitive toxicities [9].

Therefore, several single arm phase II trials evaluated the combination of cetuximab with a platinum compound and a taxane but without 5-FU [10–14]. In most of the trials, these combinations were found to be feasible, and observed objective response rates of between 70% and 97% were promising (Table 13.1).

A small number of randomized trials have compared cetuximab/platinum/taxane-based induction chemotherapy versus TP(F) [15–17]. No clinically significant differences were observed between the cetuximab-based regimens and the controls (Table 13.2). Therefore, the role of induction therapy with a targeted agent to treat SCCHN remains purely investigational.

Table 13.1 Single arm phase II trials investigating cetuximab with a platinum compound and a taxane

Regimens	N	ORR	3-year PFS rate	3-year OS
Cisplatin + Docetaxel + Cetuximab [10]	39	86%	70%	74%
Cisplatin + Docetaxel + Cetuximab [11]	54	72.2%	58.2%	90.7%
Carboplatin (AUC2) + Paclitaxel (135 mg/m2/ weeks) + Cetuximab [12]	47	96%	87%	91%
Carboplatin (AUC2) + Paclitaxel (90mg/m2/weeks) + Cetuximab [13]	30	97%	NA	NA
Carboplatin (AUC2) + Paclitaxel (90 mg/m2/ weeks) + Cetuximab [14]	63	70%	55%	78%

NA not-available; *AUC* area under the curve: *ORR* objective response rate; *PFS* progression-free survival; *OS* overall survival; *y* year

Table 13.2 Randomized phase II trials investigating cetuximab with a platinum compound and a taxane

Regimens	N	ORR	3-year PFS rate	3-year OS
Cisplatin + Docetaxel	44	82%	56%	74%
Versus				
Cisplatin + Docetaxel + Cetuximab [15]	48	81%	70%	88%
Regimens	N	ORR	400-day PFS rate	400-day OS rate
Cisplatin + Docetaxel +5-fluorouracil	50	77%	67%	86%
Versus				
Cisplatin + Docetaxel + Cetuximab [16]	50	86%	70%	79%
Regimens	N	ORR	2-year LFS rate	2-year OS rate
Cisplatin + Docetaxel + (5-fluorouracil)	180	82%	46%	68%
Versus				
Cisplatin + Docetaxel + (5-Fluorouracil) + Cetuximab [17]		81%	47%	69%

NA not-available; *AUC* area under the curve: *ORR* objective response rate; *PFS* progression-free survival; *LFS* laryngectomy-free survival: *OS* overall survival; *y*:year

Based on the promising efficacy of some window trials, immune checkpoint inhibitors are also under evaluation. A phase III trial is currently investigating the standard of care versus two cycles of neoadjuvant pembrolizumab (200 mg every 3 weeks) followed by curative-intent surgery and postoperative pembrolizumab-based (chemo)radiation [18]. One of the primary endpoints is pathological response after neoadjuvant therapy (<10% of tumor cells within the resected primary tumor and lymph nodes). The study design is depicted in Fig. 13.1.

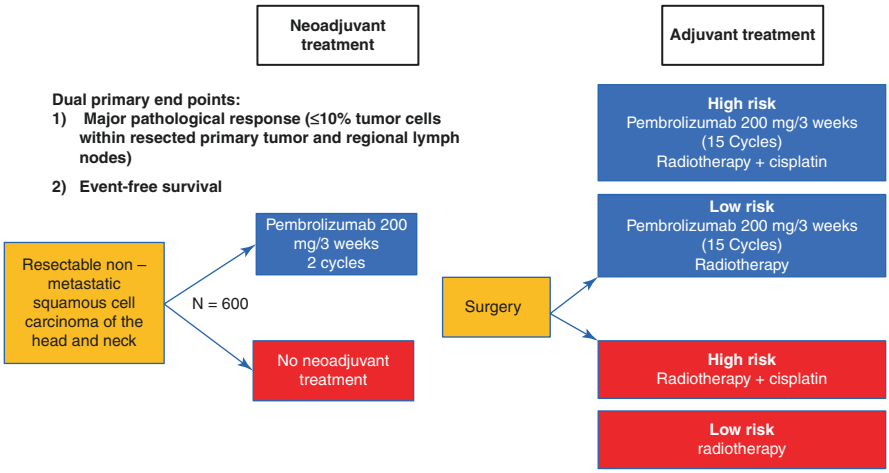

Fig. 13.1 KEYNOTE-689: Phase III study of adjuvant and neoadjuvant pembrolizumab combined with standard of care in patients with resectable, locally advanced head and neck squamous cell carcinoma

Window of Opportunity Trials with Targeted Therapy and Immune Checkpoint Inhibitors

Window of opportunity studies are trials in which patients receive one new compound in the period between their cancer diagnosis and the start of standard therapy. The primary objective of this approach is not treatment efficacy but translational research. Standard treatment is usually surgery. Tumor biopsies and anatomic and functional imaging are performed before and after investigational treatment for translational research (Fig. 13.2). The main advantage of this study design is the ability to investigate new molecules in patients who have not yet been treated by multiple anti-cancer therapies. Traditionally, drugs are often tested in patients with locoregional or metastatic recurrence whose tumors are predominantly resistant and there is a risk that the activity of these agents may be underestimated. Furthermore, the understanding of the biological and molecular effects of these tested drugs may be limited in palliative patients because it could be perceived unethical to perform additional biopsies for research purposes only. In head and neck cancer window studies, pretreatment biopsies during diagnostic endoscopy and post-treatment biopsies on the day of surgery can be performed, taking advantage of general anesthesia. The evaluation of new compounds using this approach prior to classical curative treatment provides information about molecular and clinical activity as well as predictive biomarkers [19, 20].

Window of opportunity studies aim to maximize the information gain whilst minimizing the risk to patients who are awaiting potentially curative treatment. Standard treatment should therefore not be delayed due to the investigational drugs' study procedures or side effects. Some studies have shown that curative treatment in head and neck cancer should be carried out within 20 to 28 days after diagnosis [21, 22],

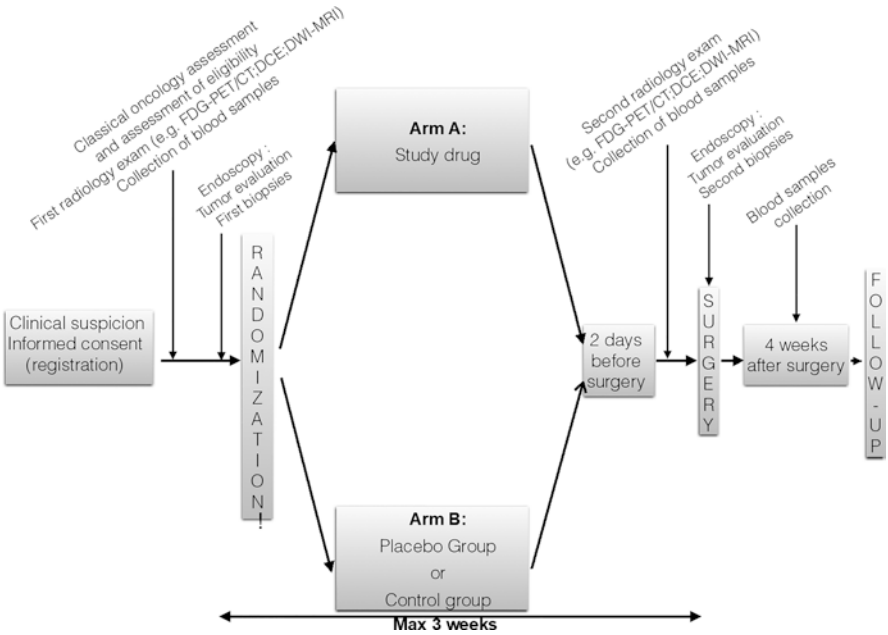

Fig. 13.2 Example of a window of opportunity study design. *FDG-PET* fluorodeoxyglucose-positron emission tomography; *DCE* dynamic contrast enhanced; *DWI-MRI diffusion*-weighted magnetic resonance imaging

making trial organization one of the main challenges for this type of study. To achieve this goal, we recommend that patients with SCCHN be included in window studies at the time of clinical diagnosis, that the time points for biopsies and imaging are prospectively pre-defined, and that the schedule, dose, and duration of the preoperative treatment are standardized and the same for all patients. Finally, to validate translational research, patients should also be randomized against a control/placebo group. If macroscopic tumor reduction is observed with the investigational compound, surgery should be performed as initially planned to ensure that the extracted surgical specimen has clear margins without microscopic tumor invasion.

Several PD1/PD-L1 monoclonal antibodies and anti-EGFR agents have been investigated in this setting (Tables 13.3 and 13.4). Interestingly, and aside from molecular activity, clinical efficacy has sometimes been detected even if the treatment period was short (<4 weeks). We will discuss some examples to highlight the advantages and drawbacks of this research approach.

Schmitz et al. [21, 23, 24] investigated cetuximab versus controls in the two weeks before curative surgery in treatment-naïve patients with SCCHN. The primary endpoint of safety was reached with cetuximab prior to surgery. Cetuximab also induced a high rate of response based on 18-fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET) evaluation and a decrease in tumor cellularity, which significantly correlated with ^{18}FDG-PET response. Four patients out of 20 also had a

Table 13.3 Window of opportunity studies with targeted therapies in SCCHN (non-exhaustive list)

Trial	N	Clinical compound	Control arm	Primary end point	Severe toxicities (i.e. grade 4)	Trial duration	Delay in surgery
Thomas [28] (2007)	35	Erlotinib	None	Tumor size and immunohistochemistry	0%	18–30 days	No
Del Campo [29] 2011)	107	Lapatinib	Placebo group (n = 36)	Apoptotic index	0%	2–6 weeks	NA
Schmitz [21] (2014)	33	Cetuximab	Control group (n = 5)	Safety and 18FDG-PET response	3% (first part) 0% (second part)	2 weeks**	No
Gross [30] (2014)	49	1. Erlotinib 2. Erlotinib +Sulindac	Placebo group (n = 12)	Ki67 modulation	0%	2 weeks	No information
Brana [31] (2014)	14	Dacomitinib	Placebo group (randomisation 2:1)	Evaluation of a genes expression signature	No information	7–11 days	No information
Bauman [32] (2014)	58	1. Erlotinib 2. Desatinib 3. Erlotinib +Desatinib	Placebo group (randomisation 1:1:1:1)	Percent change in RECIST-measurable index lesions	0%	7–21 days	No information
Machiels [25] (2018)	30	Afatinib	Control group (n = 5)	Metabolic 18FDG-PET response	0%	2 weeks	Yes (for 3 patients)
Nair [33] (2019)	64	1. Erlotinib 2. Celocoxib 3. Erlotinib +Celocoxib	Control group (n = 16)	Tumor response (clinical and MRI)	0%	21 days	No information

* Window study before curative (chemo)radiotherapy

** in the second part of the trial

NA not available; *18FDG-PET* 18-fluorodeoxyglucose-positron emission tomography; *MRI* magnetic resonance imaging

Table 13.4 Window of opportunity studies with immunotherapy and other therapies in SCCHN (non-exhaustive list)

Trial	N	Clinical compound	Control arm	Primary end point	Severe toxicities (i.e grade 4)	Trial duration	Delay in surgery
Ferris [26] (2017)	29	Nivolumab	None	Safety	0%	29 (± 7) days	No
Horton [34] (2019)	9	Nivolumab	None	Overall Response rate	0%	2 weeks	No
Uppaluri [35] (study still ongoing: NCT02296684)	21*	1. Pembrolizumab during preoperative period 2. Pembrolizumab during preoperative period + one year of Pembrolizumab during postoperative period	None	1-year Locoregionnal Recurrence	0%	2–3 weeks for the preoperative part of the trial	No
Curry [36] (2017)	50	Metformine	None	Immunohistochemistry for metabolic markers	0%	9 -24 days	No information
Berinstein [37] (2018)	27	IRX-2	None	Modulation of Lymphocyte Infiltration	0%	21 days	No
Miles B and Sikora A (study still ongoing: NCT02002182)	±30	ADXS11–001	Control group	Change in HPV E6/E7-specific CD8+ cytotoxic lymphocyte (CTL) responses in the peripheral blood and safety	No information	33 days	No information

* According to the abstract of ASCO 2017

macroscopic reduction in the size of their tumor. Gene expression analyses showed that in some patients cetuximab increased the expression of genes involved in epithelial to mesenchymal transition and activation of cancer-associated fibroblasts.

Afatinib, an irreversible pan-ErbB inhibitor, has also been investigated in a multicenter randomized window study of 25 treated patients versus five controls [25]. The primary endpoint was ^{18}FDG-PET response. Seventy percent of the patients showed a partial metabolic response and 22% of patients had a partial response according to RECIST v1.1. A high cluster 3-hypoxia score and wild *TP53* status were predictive of treatment activity. The investigational compound was considered safe even though three patients experienced surgical delay. Among them, two delays (3 and 24 days, respectively) were related to drug toxicity. We therefore believe that it is preferable to use drugs that have already proven to be safe in phase I studies in order to maximize patient safety and to protect the initiation of standard treatment. To the best of our knowledge, very few window studies in head and neck oncology have had to deal with grade ≥ 4 or unexpected side effects.

In 2017, Ferris et al. [26] conducted a window study with nivolumab, a monoclonal antibody targeting PD-1, in 29 SCCHN patients. Patients received two doses prior to surgery that was planned on day 29 ± 7. The primary endpoint was safety. The publication is still pending, but according to the ESMO 2017 abstract, grade 3–4 treatment-related adverse events occurred in four patients without delaying surgery. Tumor shrinkage, assessed by computer tomography (CT)-scan just before surgery, was observed in 48% of evaluable patients. Three patients experienced tumor reduction ≥40% (largest reduction = 75%). However, 11 patients also showed an increase in tumor size (the largest by 100%). At this stage, it is not possible to differentiate between true tumor progression or pseudo-progression.

More recently, vaccine-based therapies have begun to be investigated using window study designs. The main challenge for vaccines using this trial design is the limited period of time that short-term vaccination has available to show effective immunological effects. In this context, we recommend the use of minimally invasive samples (e.g. blood tests) to investigate the therapeutic effect of these vaccines after standard curative treatment, for example four weeks after surgery, as shown in Fig. 13.2. A meta-analysis of 239 phase I therapeutic cancer vaccine trials, conducted by Rahma et al. [27], concluded that the risk of severe adverse events (SAEs) when testing therapeutic cancer vaccines is extremely low and that AEs did not correlate with dose levels. Several window studies investigating the use of short-term therapeutic vaccination in head and neck cancers are currently in progress. First results are pending.

Conclusion

Targeted and immune therapies as induction or neoadjuvant therapy are not standard of care and should be reserved for clinical trials. In this context, a phase III trial is investigating neoadjuvant and adjuvant pembrolizumab in patients selected for a primary surgical treatment. Window of opportunity trials are important translational research tools that require careful design and an experienced team.

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Chapter 14
Is there a Role for Adjuvant Targeted and Immunotherapies in Patients with Locoregionally-Advanced Head and Neck Cancer?

Kevin J. Harrington

Introduction

Although surgery and radiotherapy are the main curative treatment modalities in patients with locoregionally-advanced squamous cell cancers of the head and neck (LA-SCCHN), their use as single modality therapies or combined as dual modality (surgery and adjuvant post-operative radiotherapy) treatment is associated with unacceptably poor outcomes for many patients. Consequently, the development of curative therapies for LA-SCCHN has seen an inexorable shift towards combinatorial approaches that include systemic treatments delivered alongside surgery and/or radiotherapy.

As is frequently the case in oncology, clinicians have used the lessons learned in treating patients in the context of relapsed and/or metastatic head and neck cancer to provide useful indicators towards therapeutic approaches that can be employed effectively in the locoregionally-advanced setting. Building on data demonstrating the benefit of systemic platin-based chemotherapy [1, 2] with or without epidermal growth factor receptor (EGFR) inhibition [3, 4] in patients with relapsed and/or metastatic head and neck cancer, clinicians have established a robust body of evidence to support the use of systemic agents in the context of treating LA-SCCHN. Thus, multimodality regimens in which radiotherapy is delivered with the addition of concomitant platin-based chemotherapy [5, 6] or a monoclonal antibody that targets EGFR [7, 8] have become standards-of-care in younger patients of good performance status. However, similar approaches involving the use of either chemotherapy or EGFR inhibition in the context of adjuvant therapy delivered after definitive or post-operative (chemo)radiotherapy have not, as yet, resulted in practice-changing outcomes.

K. J. Harrington (✉)
Head of Division of Radiotherapy and Imaging, The Institute of Cancer Research, London, UK
e-mail: Kevin.Harrington@icr.ac.uk

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In the case of immune checkpoint inhibitors (ICPI), there have also been attempts to extend the principle of applying that which is effective in the relapsed and/or metastatic setting to earlier time points within the patient pathway. ICPIs have been shown to be more effective than standard-of-care chemotherapy in both first- [9] and second-line [10–13] treatment of relapsed and/or metastatic head and neck cancer and have become new global gold-standard therapies. Importantly, in each of the practice-changing trials of ICPIs in head and neck cancer, patients have received prolonged dosing with immunotherapy and many have achieved durable and possibly permanent remissions. In many ways, the use of prolonged dosing regimens with ICPIs, even in patients who have achieved radiological complete remissions, can be seen as mimicking a course of adjuvant therapy. It is, therefore, no surprise that a number of clinical trials are currently addressing the question of using ICPIs in the adjuvant setting after the delivery of curative-intent treatment regimens, including studies involving adjuvant therapy after definitive chemoradiotherapy or surgery followed by post-operative chemoradiotherapy.

Therefore, in this chapter, we will discuss the potential role of adjuvant therapies in patients with LA-SCCHN. Data on the use of cytotoxic chemotherapy agents will be reviewed first in order to provide a context for more recent attempts to develop effective adjuvant approaches based on EGFR-targeted therapies and ICPIs.

Adjuvant Post-Operative Chemoradiotherapy Improves Outcomes in LA-SCCHN

Post-operative radiotherapy (PORT) is considered when the risk of recurrence of disease above the clavicles exceeds 20% (either at the mucosal site or in surgically-treated and -untreated nodal levels). Absolute indications for PORT include close (less than 5 mm) or involved (positive) margins at the primary tumour resection site, two or more involved cervical lymph nodes, extracapsular spread and invasion of the soft tissues of the neck. The presence of lymphovascular space invasion and perineurial invasion are relative indications for PORT that are considered in association with other factors [14].

The role of adjuvant post-operative chemoradiotherapy (POCRT), as opposed to PORT, was clarified in two seminal studies published in 2004 [15, 16] and summarised in Fig. 14.1. It is important to emphasise that, in both of these trials, the delivery of chemotherapy was restricted solely to the period of time during which the patient received adjuvant post-operative radiotherapy. In other words, there was no use of chemotherapy in a fashion that might be considered as true adjuvant therapy. The inclusion criteria for the RTOG 9501 [15] and EORTC 22931 [16] studies were slightly different, but the overall findings were remarkably similar. In the RTOG study, loco-regional control and disease-free survival were increased by the addition of concomitant cisplatin to PORT. There was a 10% improvement in 2-year loco-regional control (82% vs 72%) in favour of POCRT. In the EORTC study,

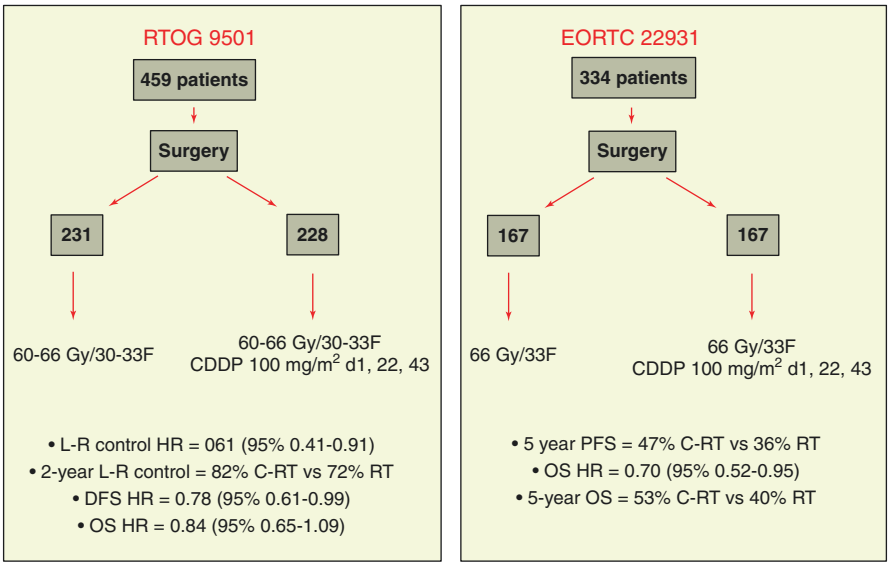


Fig. 14.1 Trial designs and main findings of the RTOG 9501 and EORTC 22931 trials of post-operative radiotherapy versus post-operative chemoradiotherapy

5-year progression-free and overall survival rates were increased by the addition of concomitant cisplatin to PORT.

The authors of those two studies subsequently amalgamated the datasets to derive consensus indications for POCRT [17]. They found that extracapsular spread in lymph node disease and the presence of microscopically involved resection margins were the only risk factors for which the impact of POCRT was significant in both trials. Therefore, these two factors have been widely adopted as absolute indications for POCRT across the globe. The combined analysis also suggested a trend in favour of POCRT in patients with stage III/IV disease, those with perineurial invasion, lymphovascular space emboli or level IV and V lymph nodes in the case of oral cavity or oropharyngeal cancers. These factors have not been accepted widely as absolute indications for POCRT.

Adjuvant Chemotherapy Is Not Effective in LA-SCCHN

The Meta-Analysis of Chemotherapy on Head and Neck Cancer (MACH-NC) collaborative group was originally published in 2000 and represented a landmark study that fundamentally changed the standard-of-care treatment paradigms for patients with stage III/IV disease [5]. That meta-analysis addressed three specific issues: (i) the effect on survival outcomes of adding chemotherapy to locoregional treatment; (ii) the effect of different chemotherapy schedules; and (iii) the role of induction/

neoadjuvant chemotherapy in laryngeal preservation. Data relating to the first two questions have provided clear guidance on the absence of a defined role for adjuvant cytotoxic chemotherapy in LA-SCCHN. Thus, although the meta-analysis, which included 63 trials involving 10,741 patients, demonstrated that survival following definitive local therapy was significantly improved with the addition of chemotherapy, neither induction nor adjuvant chemotherapy was seen to have had a significant effect on survival outcomes.

In view of the heterogeneity of the studies included in the original MACH-NC meta-analysis, a further study was conducted in which the group updated their results by including individual patient data from randomised trials that were conducted between 1994 and 2000 [6]. Data from a total of 87 randomised trials were available for the analyses. Because some trial arms were used more than once in the analysis, the total number of comparisons in the updated meta-analysis was 108 comprising data on 17,493 patients. The majority of new trial data were specifically related to the use of concomitant chemotherapy and resulted in the meta-analysis being able to draw much clearer conclusions about the value of this treatment approach. As with the previous analysis, there was a significant effect of the timing of chemotherapy with concomitant chemotherapy clearly offering the greatest benefit (hazard ratio 0.81 (95% CI 0.78–0.86). Once again, neither induction (hazard ratio 0.96 (95% CI 0.90–1.02) nor adjuvant (hazard ratio 1.06 (95% CI 0.95–1.18) chemotherapy was found to be better than locoregional therapy alone. As a consequence, there is broad agreement across the globe that adjuvant chemotherapy has no established role when delivered after definitive surgery, radiotherapy, or radical/post-operative chemoradiotherapy.

Epidermal Growth Factor Receptor-Targeted Therapies in LA-SCCHN

SCCHN very frequently show upregulated EGFR signalling. EGFR is a member of the c-erbB/HER family of transmembrane type I receptor tyrosine kinases, which comprises a total of four members (EGFR/c-erbB-1/HER1, c-erbB-2/neu/HER-2, c-erbB-3/HER-3, c-erbB-4/HER-4 [18, 19]). HER-family receptor proteins share a common structure, consisting of a glycosylated extracellular ligand-binding domain, a hydrophobic trans-membrane component and an intracellular domain with tyrosine kinase activity. When the specific (cognate) ligand binds to its ligand-binding domain on the extracellular component of a HER-family member, it causes receptor dimerization and activation of the kinase domain. This, in turn, mediates phosphorylation of target proteins, which triggers a cascade of intracellular secondary messengers that alter patterns of gene expression. In this way, binding of a protein on the cell surface can influence the cell's behaviour. It is important to note that there is no ligand for the c-erbB2/HER2 receptor and that the c-erbB3/HER3 receptor has no kinase activity. Nonetheless, these receptors are able to participate in signalling

by dimerizing with appropriate partners. For example, EGFR:c-erbB3 (HER1/HER3) and c-erbB-2:c-erbB-3 (HER2/HER3) heterodimers can exploit ligand binding to the HER3 component and kinase-mediated signalling from the EGFR or HER2 component of the partnership.

Normally, activation of HER-family receptors by their cognate ligands (growth factors) is very tightly controlled—through regulation of the expression of the receptor, the availability of the ligand and the ability of the cell to de-phosphorylate activated receptors. In this way, proliferation of normal tissues is carefully regulated to avoid unnecessary or unscheduled cell growth. In contrast, in SCCHN there is very frequently a state of independence from normal regulatory mechanisms that is driven through a number of different processes. These include: (i) manufacture and release of growth factors that stimulate HER-family receptors on the malignant cell (autocrine signalling) and on neighbouring malignant (and normal) cells (paracrine signalling); (ii) altering the number, structure or function of the surface growth factor receptors expressed on tumour cells; and (iii) by altering the signalling pathways downstream of the receptor. In contrast to other tumour types, in which EGFR gene amplification or mutation is common (e.g. lung adenocarcinoma), overexpression of the receptor, without gene amplification, is the dominant process whereby EGFR affects the pathobiology of SCCHN. The roles of HER2, HER3 and HER4 in SCCHN remain unclear. However, it is known that HER2/HER3 heterodimers are potent inducers of the PI3-kinase anti-apoptotic pathway [20] and this may be relevant to particular subsets of SCCHN, including human papillomavirus (HPV)-related disease[37].

Irrespective of the fact that SCCHN rarely shows evidence of EGFR mutation, the evidence of single-agent responses to HER-family-targeted therapies strongly supports the notion that these tumours can be reliant on signalling through these pathways in order to maintain the malignant phenotype. This reliance on activation of an oncogenic driver has been called “oncogene addiction” [21] and is seen as a potential point of therapeutic attack against a range of tumour types. Therefore, HER-family receptors represent attractive therapeutic targets in SCCHN and two main classes of drugs, monoclonal antibodies (MAB) and small molecule tyrosine kinase inhibitors (smTKI), have been developed. MAB are large molecules directed against the extracellular domain of the receptor, while smTKI inhibit the intracellular kinase domain of the receptor.

Anti-EGFR Monoclonal Antibodies Are Not Used as Adjuvant Therapies for LA-SCCHN

Anti-EGFR-targeted monoclonal antibodies (cetuximab, zalutumumab, panitumumab, nimotuzumab) have been extensively tested in patients with relapsed and/or metastatic head and neck cancers. Cetuximab has been shown to improve the outcome of first-line palliative chemotherapy [3]. In the EXTREME study, 442

eligible patients with untreated recurrent and/or metastatic SCCHN received cisplatin or carboplatin plus 5-fluorouracil every 3 weeks for a maximum of 6 cycles. Cetuximab (400 mg/m^2 loading dose, then 250 mg/m^2 per week) was administered to 222 randomly selected patients. Patients in the cetuximab arm who showed stable disease or treatment response were planned to continue with maintenance cetuximab until disease progression or unacceptable toxicity. This study showed that the cetuximab/platinum/5-fluorouracil combination prolonged median overall survival from 7.4 months to 10.1 months (P = 0.04). There were also increases in the median progression-free survival time (3.3 to 5.6 months; P < 0.001) and the response rate (20% to 36%; P < 0.001) [3]. Consequently, the EXTREME regimen was adopted as a gold-standard treatment for relapsed/metastatic head and neck cancer. In contrast, neither panitumumab [22] nor zalutumumab [23] has been registered for the treatment of relapsed and/or metastatic head and neck cancer following negative phase III trials, although the data from those trials was strongly suggestive of activity of those agents. The use of nimotuzumab is largely restricted to India and there are very limited data relating to its use in recurrent and/or metastatic SCCHN [24].

Cetuximab has also been approved as part of a curative regimen for LA-SCCHN. In a phase III study of 424 subjects with locally or regionally advanced SCCHN, locoregional control (median 24.4 vs. 14.9 months; hazard ratio: 0.68; P = 0.005) and overall survival (median 49.0 vs. 29.3 months; hazard ratio: 0.74; P = 0.03) were significantly prolonged in patients receiving radiotherapy and cetuximab compared to those treated with radiotherapy alone [7, 8]. However, in this study, there was no continued, adjuvant use of cetuximab beyond the completion of radiotherapy. In addition, both zalutumumab and panitumumab have been tested in combination with radiation/chemoradiation, again without any attempt to use them in an adjuvant phase beyond the completion of definitive treatment. Neither of these agents improved outcomes when compared to the standard therapy arms and they have not been approved in the context of LA-SCCHN [25–27]. Nimotuzumab is widely used with chemoradiotherapy in India, following the publication of a positive randomised phase III trial. However, once again, there was no use of nimotuzumab following completion of definitive loco-regional therapy [28] and, therefore, there is no evidential basis on which to deliver this therapy in an adjuvant setting.

Small Tyrosine Kinase Inhibitors Are Not Effective Adjuvant Therapies in LA-SCCHN

A number of agents have been developed to target HER-family members across a variety of different tumour types. In the context of head and neck cancer, gefitinib and erlotinib (EGFR/HER1 inhibitors) [29–31], lapatinib (HER1/HER2 inhibitor) [32, 33] and afatinib (pan-HER inhibitor) [34] have been most extensively investigated. Studies have included assessments of agents in the palliative setting for relapsed and/or metastatic disease. Despite the fact that these agents demonstrate

single-agent activity in phase I/II trials, randomised studies have failed to demonstrate clinically meaningful survival advantage relative to standard-of-care treatment and none of them is in routine clinical use for patients with relapsed and/or metastatic disease. Specifically, a randomised phase III study was conducted to compare survival in 486 patients with recurrent or metastatic SCCHN treated with gefitinib 250 or 500 mg/day or standard-of-care single-agent weekly methotrexate [35]. Neither of the gefitinib doses improved overall survival compared with methotrexate (hazard ratios 1.22 (95% CI 0.95–1.57 and 1.12 (95% CI, 0.87–1.43), respectively. The median overall survivals were 5.6, 6.0, and 6.7 months for gefitinib 250 mg/day, gefitinib 500 mg/day, and intravenous methotrexate, respectively. Afatinib has been assessed in the phase III LUX head and neck-1 study in patients receiving second-line therapy for relapsed/metastatic SCCHN [36]. A total of 583 patients were treated with afatinib (322 patients) or methotrexate ((161 patients). Afatinib significantly increased median progression-free survival (2.6 versus 1.7 months, $p = 0.03$) but did not improve median overall survival (6.8 versus 6.0 months) relative to methotrexate. In an integrated analysis of quality of life, afatinib showed a delay in deterioration of global health status, pain and swallowing problems (all $p \leq 0.03$) but such data were of insufficient weight to lead to regulatory approval of this therapy.

In the context of adjuvant maintenance therapy using HER-family-targeted therapies, there have been significant attempts to develop lapatinib and afatinib. A randomised phase III study of lapatinib administered concomitantly with chemoradiotherapy and as maintenance monotherapy in patients with high-risk surgically-treated SCCHN has been reported [37, 38]. Patients with resected stage II-IVA SCCHN, with a surgical margin ≤5 mm and/or extracapsular extension in metastatic cervical nodal disease were randomized to chemoradiotherapy (66 Gy total dose and 100 mg/m^2 cisplatin administered on days 1, 22 and 43) plus placebo or lapatinib (1500 mg/day) prior to and during chemoradiotherapy, followed by 12 months of maintenance monotherapy (either placebo or lapatinib). Six hundred and eighty-eight patients were enrolled; 346 received lapatinib and 342 received placebo. With a median follow-up of 35.3 months, the study was terminated early due to the apparent plateauing of disease-free survival events. Median disease-free survivals were 53.6 months and "not reached" for lapatinib and placebo, respectively; hazard ratio 1.10 (95% CI 0.85–1.43). No significant differences in disease-free survival by HPV status or overall survival were observed between the two treatment arms. Similar numbers of patients in both treatment arms experienced adverse events, with more patients in the lapatinib arm experiencing serious events (48% vs 40%). This study demonstrated that adding lapatinib to chemoradiotherapy and its use as long-term adjuvant therapy was safe, but did not offer any efficacy benefits compared with placebo in patients with surgically-treated high-risk SCCHN.

In the LUX head and neck-2 study, 617 patients were randomised to treatment (411 to afatinib and 206 to placebo) in a true adjuvant context [39, 40]. Eligible patients had histologically or cytologically confirmed LA HNSCC (Stage III, IVa or IVb SCC of the oral cavity, oropharynx or hypopharynx, or Stage IVa or IVb SCC of the larynx). Since HPV status was not determined for eligibility, unfavourable

risk was defined as non-oropharynx primary site or oropharynx cancer in heavy smokers (>10 pack years). Patients were required to have unresected disease prior to chemoradiotherapy. Concomitant definitive chemoradiotherapy had to have been completed no longer than 24 weeks prior to randomisation, comprising radiotherapy with curative intent to a minimum dose of 66 Gy in 33 fractions, and cisplatin or carboplatin. No evidence of disease was required on clinical and radiographic examinations (defined as no residual tumour after chemoradiotherapy (with or without R0 resection at the primary site or neck dissection). A pre-planned futility analysis, showed the study was unlikely to demonstrate a significant advantage with afatinib and the trial was halted early on the recommendation of the independent data-monitoring committee. Patients were discontinued from treatment and follow-up for disease recurrence and survival was stopped. The percentage of patients taking at least 80% of the planned study medication was lower for the afatinib group (85.3%) than the placebo group (98.5%), almost certainly reflecting the appreciable toxicity associated with chronic administration of this pan-HER-targeted oral medication. Median disease-free survival (DFS) by investigator review was 43.4 months (95% CI 37.4–not estimable) with afatinib versus "not estimable" (95% CI 40.1–not estimable) with placebo (HR 1.13, 95% CI 0.81–1.57; stratified log-rank test p = 0.48). The DFS rate at 2 years was evaluated using the Kaplan-Meier method; the probability of being disease-free at 2 years was 67.2% in the afatinib group and 73.5% in the placebo group (estimated difference: −6.3%, 95% CI -15.0–2.5; p = 0.16). At the time of data cut-off for the futility analysis, overall survival data were immature. The effect of afatinib versus placebo on DFS was explored in pre-planned subgroup analyses based on stratification factors, biomarker status, demographics, baseline disease characteristics and prior anti-cancer chemotherapy. These subgroup analyses were generally consistent with the primary analysis and showed no clear trend of benefit in any subgroup, although there was a slight benefit for afatinib patients with nodal status N2b–N3 (HR 0.82, 95% CI 0.55–1.21).

Immune Checkpoint Inhibitors as Adjuvant Therapies in LA-SCCHN

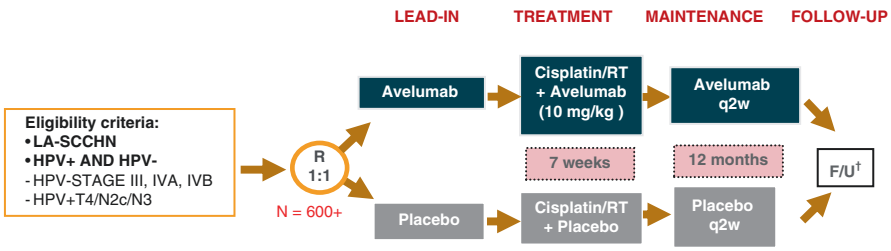
In recent years, immunotherapy has emerged as a new pillar in the treatment of many solid cancers [41]. This renewed interest in immunotherapy has been underscored by huge advances in our understanding of the fundamental biological principles that guide the activity of the immune system. In particular, specific immune checkpoints have been discovered that are integral components of normal immune responses. In normal health, these checkpoints function as negative regulators or "brakes" on the normal immune response and prevent T cells from becoming chronically activated or aberrantly targeted against normal tissues. Many cancers exploit these inhibitory pathways in order to escape from immunosurveillance.

Proteins that are expressed on activated T cells, such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed death-1 (PD-1), are key players

that allow many cancers to evade anti-tumour immunity by interfering with the activation and effector phases of immune responses, respectively. In the context of relapsed and/or metastatic head and neck cancer, we have clear evidence that blockade of signalling through the PD-1 pathway (mediated by programmed death ligand-1 (PD-L1) expressed on the surface of cancer and other cells) can yield significant clinical responses. Indeed, we now have positive phase III trial data, initially in the second-line and, more recently, in the first-line setting, to show that anti-PD-1-targeted therapies are capable of significantly improving overall survival in patients with relapsed and/or metastatic head and neck cancers [9–13]. As with chemotherapy and HER-family-targeted therapies, such data have spurred on investigators to investigate the potential value of immune checkpoint inhibitors (ICPI) as adjuvant therapies for cancer.

A number of lines of evidence can be invoked to support the hypothesis that adjuvant ICPI therapy might be beneficial in patients treated for LA-SCCHN. First, there are data in patients with melanoma that demonstrate that the "baseline tumour size" is an independent, statistically significant predictor of overall survival in patients treated with anti-PD-1 ICPI [42]. In addition, phase III clinical trials have shown improvement in progression-free and overall survival endpoints for tumour types such as melanoma and lung cancer [43–46]. As a guide to the management of SCCHN, the data from the PACIFIC trial in lung cancer are most compelling because the patient population comprised those with locally-advanced, stage III non-small-cell lung cancer who had not progressed on chemoradiotherapy delivered with curative intent [45, 46]. Interestingly, patients were required to commence adjuvant anti-PD-L1 therapy (durvalumab) within 42 days of completion of chemoradiotherapy.

At the time of writing, there are at least 3 major trials that have either completed recruitment or are still ongoing that address the question of adjuvant/maintenance ICPI in locally-advanced head and neck cancer. The designs of these trials are summarised in Figs. 14.2, 14.3 and 14.4.



Key: F/U – follow-up; HPV – human papillomavirus; LA-SCCHN – locally-advanced squamous cell cancer of the head and neck; q2w – every 2 weeks; R – randomisation; RT - radiotherapy

Fig. 14.2 Javelin Head and Neck-100 study design. Key: *F/U* follow-up; *HPV* human papillomavirus; *LA-SCCHN* locally-advanced squamous cell cancer of the head and neck; *q2w* every 2 weeks; *R* randomisation; *RT* radiotherapy

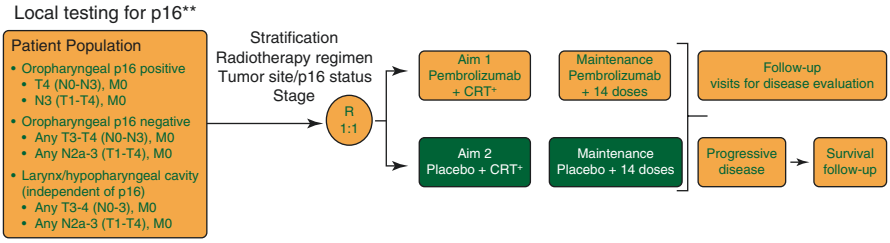


Fig. 14.3 KEYNOTE-412 clinical trial design. **Expression of P16 is highly correlated with HPV in oropharyngeal cancer. Tumors outside the oropharynx are considered HPV negative regardless of results of p16 staining. + *Chemotherapy* Cisplatin 100 mg/m^2 × 3; *RT* AFX – 200 cGY/6 per week, SFX – 200 cGy/5 per week. Key: *AFX* accelerated fractination; *p16-positive/negative* surrogate measure for human papillomavirus; *R* randomisation; *RT* radiotherapy; *SFX* standard fractionation

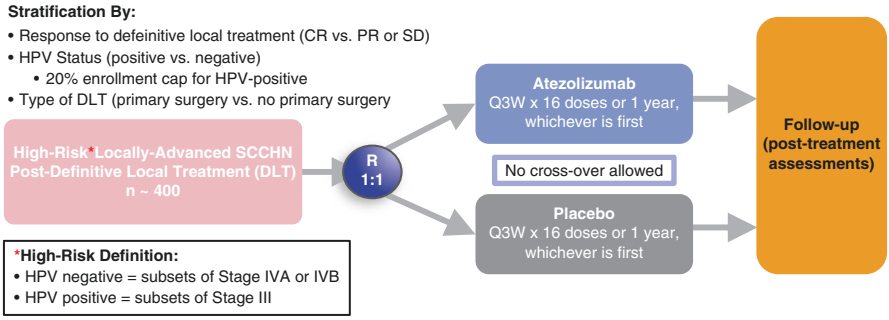


Fig. 14.4 IMvoke010 clinical trial design. Key: *CR* complete remission; *DLT* definitive local treatment; *HPV* human papillomavirus; *PR* partial remission; *Q3w* every 3 weeks; *R* randomisation; *RT* radiotherapy; *SD* stable disease

The Javelin Head and Neck 100 study was a randomized, double-blind, placebo-controlled, parallel-arm, superiority study of the anti-PD-L1 agent, avelumab, versus placebo. Patients with LA-SCCHN (oral cavity, oropharynx, larynx, or hypopharynx) who were eligible for definitive chemoradiotherapy were enrolled (details of patient groups are provided in Fig. 14.2). Patients were randomized to receive either avelumab or placebo plus standard-of-care chemoradiotherapy. Randomization was stratified by tumour stage (<T4 vs T4), nodal stage (N0/N1/2aN2b vs N2c/N3), and HPV status (positive vs negative). There were three treatment phases in the study: lead-in phase; treatment phase; and maintenance phase. The primary endpoint of Javelin Head and Neck 100 is the progression-free survival (PFS) per modified Response Evaluation Criteria in Solid Tumours

(RECIST) version v1.1 by investigator assessment. Secondary endpoints include overall survival, pathologic complete response, neck dissection, locoregional failure, objective response, distant metastatic failure, and duration of response, per modified RECIST v1.1 by investigator assessment. In March 2020, the study sponsors accepted the recommendation of the independent Data Monitoring Committee to terminate the JAVELIN Head and Neck 100 trial, as the study is unlikely to show a statistically significant improvement in the primary endpoint of PFS based on a pre-planned interim analysis [47]. A detailed analysis of the study findings are likely to be available for examination by the scientific community in 2021.

KEYNOTE-412 (ClinicalTrials.gov, NCT03040999) has a similar design to Javelin Head and Neck 100 (Fig. 14.3). It is a randomized, double-blind, placebo-controlled, phase III study of pembrolizumab 200 mg or placebo every 3 weeks in combination with chemoradiotherapy and as maintenance/adjuvant therapy for a total of 17 doses over one year [48]. Study recruitment has now closed, but patients remain in follow-up as the data mature. Eligible patients were randomly assigned 1:1 to pembrolizumab or placebo with stratification according to radiotherapy regimen (accelerated versus standard fractionation), tumor site, p16 status (oropharynx—p16 positive vs oropharynx—p16 negative or larynx/hypopharynx/oral cavity cancer), and tumour stage (III vs IV, AJCC TNM Version 7). Treatment is continued until centrally confirmed disease progression, unacceptable toxicity, intercurrent illness that prevents further administration of treatment, investigator's decision to withdraw the patient, non-adherence to treatment or trial procedures, administrative reasons requiring cessation of treatment, or the patient has received 17 administrations of pembrolizumab/placebo (approximately 1 year). The trial is split into three treatment phases. The first phase includes the pembrolizumab/placebo priming dose, followed by chemoradiotherapy in combination with two additional pembrolizumab/placebo doses given every 3 weeks (duration, 8 weeks). The second phase includes pembrolizumab/placebo maintenance/adjuvant dosing (14 doses over about a year) during post-treatment follow-up. The third phase includes post-treatment follow-up. The primary end point of the trial is event-free survival (EFS) using RECIST v1.1. EFS is defined as the time from the date of randomization to the date of first record of (1) progression per RECIST v1.1 by blinded independent central review ([a] locoregional progression or recurrence or [b] distant metastasis), (2) salvage surgery at the primary tumour site when invasive cancer is present, (3) neck dissection performed >20 weeks after completion of CRT when invasive cancer is present, or (4) death from any cause. The key secondary end point is overall survival, which is defined as the time from randomization to death from any cause. Other secondary end points include safety and patient-reported outcomes (PROs); PROs are assessed using the European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ) core 30 items (C30) and head and neck module (H&N35) as well as the EuroQoL-5D (EQ-5D). Exploratory end points include potential predictive biomarkers and immune dynamics in the subgroup of patients with oropharyngeal p16-negative or larynx/hypopharynx/oral cavity HNSCC and the overall population.

The ImVoke-10 study (ClinicalTrials.gov NCT03452137) is a phase III, multicentre, randomized, double-blind, placebo-controlled trial of the anti-PD-L1 ICPI, atezolizumab, as adjuvant therapy following definitive local therapy for high-risk LA-SCCHN [49]. Its design is depicted in Fig. 14.4. Approximately 400 patients will be randomised between atezolizumab and placebo given in a truly adjuvant setting (Fig. 14.4). The co-primary endpoints are independent review facility assessed EFS (per RECIST v1.1) or death from any cause and overall survival.

Summary and Conclusions

Despite relatively poor outcomes for many patients who present with LA-SCCHN, we have, thus far, failed to develop effective adjuvant therapies to prevent locoregional and/or metastatic relapse following definitive local therapy. Extensive effort has been exerted in attempting to develop adjuvant chemotherapy schedules, but to no avail. Similarly, attempts to exploit the concept of "oncogene addiction" by using adjuvant HER-family-targeted therapies have not been successful. There is no evidence that either small molecule inhibitors or monoclonal antibodies given in the adjuvant situation can favourably alter recurrence rates or survival outcomes. Currently, most effort is being channeled into studies that seek to evaluate the potential role of ICPI as adjuvant therapies. Considerations around these trial designs are complex, since both the Javelin Head and Neck 100 and the KEYNOTE-412 studies involve a combination of concomitant (with chemoradiotherapy) and adjuvant ICPI therapy. Neither study is designed to allow separate evaluation of the role of the concomitant versus the adjuvant components of the therapeutic package. Nevertheless, given the remarkable results in the context of relapsed and/or metastatic head and neck cancer, there is cause for optimism that we may be able to improve outcomes for our patients.

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Chapter 15
Optimal Supportive Measures during Primary Treatment

Paolo Bossi and Luigi Lorini

Introduction

Supportive care given during cancer treatment has several aims. Reducing the burden of toxicities and anticipating their appearance by adopting adequate preventative measures; improving quality of life by relieving symptoms induced by the treatment or the disease itself and allowing to maintain a correct dose intensity, therefore, giving the patient the optimal chance to be cured [1].

According to the principles of Multinational Association of Supportive Care in Cancer (MASCC), "supportive care makes excellent cancer care possible". Oncological treatments of head and neck cancer (HNC) performed with curative intent represent one of the most intensive therapies in terms of adverse events and of psychological distress [2]. Therefore, it is essential to accompany the curative treatment with all the measures that could relief patient's symptoms.

Reasons to Implement Supportive Care during Curative Treatment in Head and Neck Cancer

The importance of supportive care in HNC during curative approaches could be grouped into 6 main reasons:

1. Reduction of acute toxicity
2. Reduction of late effects
3. Increase of compliance—maintain dose intensity

P. Bossi (✉) · L. Lorini
Medical Oncology Unit, Department of Medical & Surgical Specialties, Radiological Sciences & Public Health, University of Brescia at ASST-Spedali Civili, Brescia, Italy
e-mail: paolo.bossi@unibs.it

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4. Improvement of quality of life
5. Reduction of costs
6. Homogeneity and consistency in clinical trials

First, the possible reduction of acute toxicities. The burden of acute toxicities during radiotherapy with or without chemotherapy, performed either in the definitive or the adjuvant setting is well-known [3]. The most frequent acute toxicities reported are represented by mucositis, dysphagia, weight loss, anorexia, infections, dermatitis, nausea and vomiting. The adoption of preventative actions to reduce the severity and duration of these toxicities may be beneficial. However, another way to indicate the consequences of the burden of toxicities induced by treatment in HNC is considering the rate of toxic deaths. Mortality due to therapies mirrors the toxicity of the treatment itself and it could be considered both in the acute (occurring during treatment) or in subacute period (in the period of 30 days after treatment completion). In Table 15.1, the rate of toxic death occurring in some clinical trials in HNC patients is depicted. It should be considered that clinical trials are often offered to the most "fit" patients, without severe comorbidities; therefore, the rate of death due to cancer treatment toxicities could be also higher in the real-life setting. Moreover, elderly cancer patients, even if treated with less intensive treatments, frequently avoiding chemotherapy or substituting cisplatin with less toxic carboplatin, are at a higher risk of acute toxicities and treatment-induced death. In a recent analysis, patients ≥70 years showed a higher rate of hospitalization, greater adverse events and a lower 3-month overall survival than their younger counterparts [4].

When assessing acute, as well as late toxicities, it should be acknowledged that while locoregional relapse, distant recurrence and second primary tumors are quite frequent events in advanced cancer stages, patients with HNC are at the same time

Table 15.1 Rate of acute deaths during radiation + systemic treatments

First Author	Treatment	Mortality due to treatment (%)	Ref.
Brizel	HFRT ± CT (cddp-5-FU)	2	[5]
Calais	RT vs CT (cddp) + RT	1–2	[6]
Adelstein	RT vs RT + CT (cddp) vs RT + CT (cddp+5-Fu)	2–3	[7]
Argiris	CTRT (5 trials)	5.5	[8]
Adelstein	RT + CT (cddp-5-FU)	1	[9]
Pfister	RT+ CT (cddp) + cetuximab	9	[10]
Bonner	RT ± cetuximab	No acute death	[11]
Givens	IMRT + CT	2–4	[12]
Lefebvre	Seq vs alt RT + CT (cddp)	3–6	[13]
Bourhis	CTRT vs accelerated RT + CT vs very accelerated RT	3–6	[14]
Ang	CTRT ± cetuximab	1–2	[15]

HFRT hyperfractionated radiotherapy; *CT* chemotherapy; *RT* radiotherapy; *cddp* cis-diammine-dichloroplatinum (II), cisplatin; *IMRT* intensity modulated radiotherapy; *CTRT* concurrent chemo-radiation; *Seq* sequential; *alt* alternating

at risk for mortality due to adverse treatment effects or comorbid diseases [8, 16]. Mell et al. were able to identify several risk factors for competing mortality in advanced HNC: age, comorbid disease, BMI, sex and the distance that needs to be traveled to the treating center [16]. Interestingly, they observed large subsets of patients with similar disease-free survival, but at a markedly different risk for competing events. Moreover, they confirmed that older patients and patients with comorbidities were more prone to suffer from toxicities and not obtaining benefit from intensive treatments. This would translate into the need of tailoring the intensity of treatment according to patient's functionality and frailty and, in parallel, activate personalized supportive care according to the identified needs.

The same group evaluated a large sample of patients from three randomized trials who were treated with radiation with or without systemic therapy [17]. They developed a nomogram to predict the group of patients who could selectively benefit from an intensive treatment. Factors involved in this definition were younger age, improved performance status, higher body mass index, node-positive status, p16-negative status, and oral cavity primary. These are the patients with a higher relative hazard for recurrence versus competing mortality (ω score positive ratio). On the flip side, we need to evaluate the best supportive intervention according to the patient's risk of complication. Further applications of this nomogram in this regard are strongly awaited.

Supportive care strategies are also useful in increasing patient's compliance to treatment and in allowing treatment dose intensity to be maintained. It is well known that interruptions in radiation therapy may jeopardize the outcome of the treatment itself. As the intensity of treatment escalates, adverse events also increase and along with the possibility of unplanned radiation treatment breaks and prolongationof the radiation treatment time [18]. These factors are associated with lower locoregional control rates. It has been estimated that tumor control rate is approximately 1% lower for every day of missed treatment [19, 20]. Similarly, dose intensity of concomitant chemotherapy is also of importance, as shown in several reports [21]. Cumulative cisplatin dose higher than 200 mg/m^2 concurrently with radiation has been shown to offer higher probability of disease control and overall survival, at least in the population of HPV-negative cancers [21]. In this regard, optimal supportive care may ensure treatment continuity and allow for the best chance of cure.

Concurrent treatments profoundly impact on quality of life (QoL) of HNC patients during the acute phase of treatment. The score of several domains and patient-reported outcomes worsen throughout the course of treatment and slowly recover in the weeks that follow [22]. All the measures able to contain and limit QoL worsening and to potentially allow quick recovery to baseline or even to increase overall QoL represent an important help for patients. Often, patients' burden of symptoms corresponds also to caregivers' psychological issues [23]. In addition, caregivers should be offered specific support and stressful conditions should be identified early.

Sometimes, costs also represent a leverage to be used in discussing the importance of supportive care. It is true that reimbursement of new drugs represents a challenge for healthcare systems, but it should be considered that adverse events,

due to treatment, may also lead to complications impacting on overall treatment costs. For instance, mucositis toxicity is associated with the adoption of preventive/therapeutic measures possibly increasing the overall treatment costs. The use of opioids to relieve mucositis-associated pain, the preventative or therapeutic placing of gastrostomy or nasogastric feeding tubes, and the increased risk of infections and the consequent need of antibiotics, antimycotic or antiviral drugs and resorting to hospitalization represent some of the interventions required to approach mucositis complications, all impacting on costs. When comparing patients experiencing severe (grade ≥ 3) vs non-severe (grade < 3) mucositis, costs of laboratory diagnostic tests, use of medications, imaging procedures, visits and inpatient hospitalizations were shown to be significantly higher for patients suffering of severe mucositis [24]. Therefore, cost–effectiveness of any new supportive care intervention should be an outcome that should be included in new clinical trials (see an example in [25] and in Fig. 15.1).

Lastly, supportive care needs to be standardized as much as possible to offer homogeneity and consistency into clinical trials. Zafar et al. reported the importance of defining what the best supportive care is when performing randomized

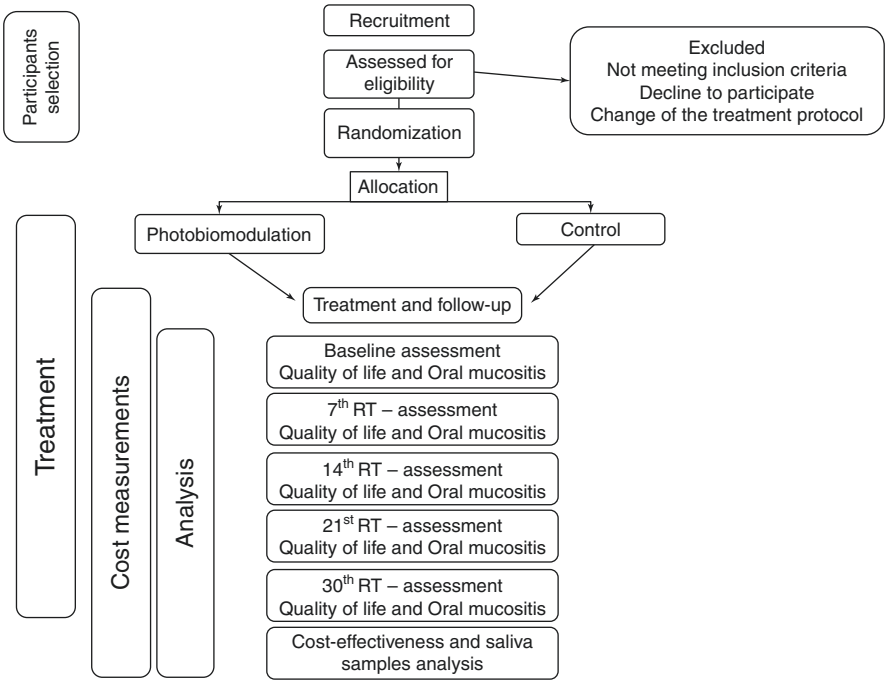

Fig. 15.1 Design of a new trial with photobiomodulation, which considers cost-effectiveness parameters. Reproduced with permission from Springer Nature [26], under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium

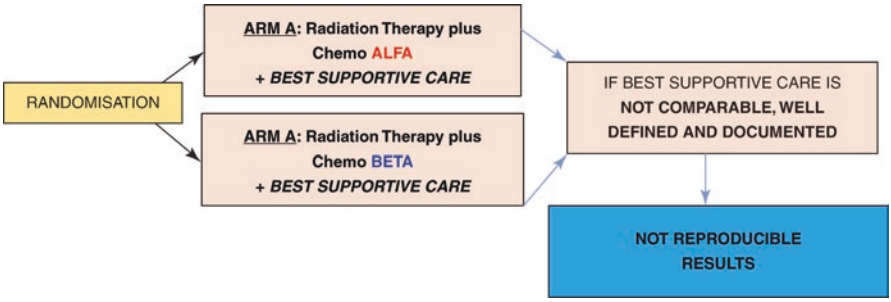

Fig. 15.2 The importance of clearly defining supportive care in curative treatment

trials in advanced disease patients, also considering no active oncological treatment [27]. This could easily be transposed to randomized clinical trials in the setting of HNC curative treatments. In fact, if we want to perform trials with the aim to evaluate new compounds to be integrated with radiation therapy for HNC patients, we should be able to strictly ensure that supportive care employed in the intervention and in the experimental arm are the same. If they are different, this could constitute a bias that could preclude the analysis of results.

We advocate that in each trial in HNC, the supportive care measures would be clear, well defined and documented (Fig. 15.2).

Multiple Interventions for Different Aspects of Support

It is difficult to limit the interventions for supportive care to specific domains or signs and symptoms, as the process of care of the patient is comprehensive and considers the person as a whole. However, a list of the most frequent issues related to the treatment of HNC which could be object of a supportive approach is presented in Table 15.2.

Supportive care needs of patients with HNC profoundly differ between the period of curative treatment, the subacute phase and the period of follow-up in long-term survivorship. Patients immediately post-treatment show larger number of unmet needs compared with those in extended survivorship [28]. Psychological issues represent the most prevalent unmet needs, followed by pain and other physical symptoms. Patients in longer-term survivorship need more support regarding anxiety, changes in sexual relationships, and fear of death and dying.

As a detailed description of all the interventions available to support the patients during HNC treatment is out of the scope of this chapter, we will provide hereafter the last information about mucositis prevention and treatment, as an example of how to implement the care of the patient according to the latest literature data.

Table 15.2 Most frequent issues of HNC patients requiring supportive care during treatment

• Mucositis
• Dysphagia
• Dermatitis
• Fibrosis
• Osteonecrosis
• Trismus
• Infections
• Pain
• Bleeding
• Xerostomia/sticky saliva
• Dysgeusia
• Voice troubles
• Nutrition
• Dyspnea
• Nausea and vomiting
• Anorexia
• Constipation/diarrhea
• Fatigue
• Anxiety/depression

The Example of Mucositis: An Early and up-to-Date Supportive Care Intervention

Mucositis is one of the most distressing symptoms the patients are complaining about during curative treatment with radiation with or without systemic therapy. MASCC/ISOO developed the Clinical Practice Guidelines for the management of mucositis, with the first edition published in 2004 and periodically updated [29]. This represents the result of a systematic review of the literature, with studies rated according to the presence of major and minor flaws; the final guidelines are then developed into different levels of evidence [30]. We will present hereafter the changes in the guidelines, derived from the accurate revision of the literature; the other recommendations or suggestions remained unchanged since the previous version [29].

Basic oral care has been considered a key strategy in preventing mucositis. Specifically, implementation of multi-agent combination of oral care protocols has been shown to prevent mucositis in different settings of treatment, namely, with chemotherapy, radiation and hematopoietic stem cell transplantation [31]. Bland rinses should be employed, as they allow to increase oral clearance of debris, promote oral hygiene, and improve patient comfort during cancer therapy. The use of saline or sodium bicarbonate rinses may help improving oral clearance. Even if no guideline was possible to consistently suggest professional oral care due to lack of solid data, a dental evaluation and treatment is indicated prior to cancer therapy. In fact, the professional intervention may increase dental and oral cavity hygiene, removing possible causes of infections from odontogenic sources, which could be

the door to systemic spread. In the process of oral care, patient (and caregivers) education has an important role, as it could ensure compliance to preventative and therapeutic suggestions.

In patients undergoing radiation or chemoradiation for HNC, the use of benzydamine mouthwashes is suggested, based on the results of several randomized clinical trials; at the moment, benzydamine is the only anti-inflammatory mouthwash with sufficient evidence in the guidelines [32]. On the contrary, the panel who evaluated the literature suggested not to use chlorhexidine as prevention of oral mucositis during radiotherapy for HNC.

The use of *photobiomodulation* (low-level laser therapy) has increased over the last few years and several studies have been reported with this tool with both preventive and therapeutic aims. Guidelines has recommended the use of intra-oral photobiomodulation in the prevention of mucositis during HNC radiation with or without chemotherapy [33]. The anti-inflammatory properties of low-level laser therapy may support its use, even if some concerns regarding facility requirements, trained personnel, and local regulatory requirements may limit its application. Moreover, standardization of protocols is required to expand the use of this tool. However, this approach may represent another weapon in the therapeutic armamentarium for prevention and treatment of mucositis and pain associated with mucositis.

Concerning treatment of pain due to mucositis, topical morphine 0.2% mouthwash is suggested as per indications coming from randomized clinical trials [34]. It has been shown that opioid receptors are present at the surface of injured mucosa and topical morphine could directly act on them. In this regard, further trials are needed to evaluate how to integrate topical and systemic opioid therapy and the impact of morphine mouthwashes in reducing the need of systemic administration of the same class of drugs.

There is also a suggestion in favor of the use of *per os* glutamine for the prevention of oral mucositis in patients with HNC treated with concurrent chemoradiation [35, 36]. Only a caution has been given, as the use of parenteral glutamine in another setting (hematopoietic stem cell transplantation) showed a higher mortality rate.

Guidelines also report negative suggestions and recommendations, as the results of clinical trials do not always support the use of a specific medication or treatment. For a complete picture of the new guidelines we refer to the full paper that has just been approved [37].

Conclusion

Supportive care needs to be integrated early in the course of treatment for HNC patients. Implementation of specific protocols is strongly recommended, therefore giving a comprehensive view on all the aspects of patient's care. A tailored assessment of patient's needs could help in identifying the aspects that should be sustained the most and the actions to be taken before starting the treatment itself. In this regard, the model of "simultaneous care" advocated at the beginning of the pathway

of care of advanced cancer patients could also be used in the approach to HNC patients before starting curative treatment with radiation (with or without systemic therapy), in both the definitive and the adjuvant setting.However, the logistical organization and the possible benefit of simultaneous care embedded in HNC treatment have not been investigated yet and deserve to be assessed in well-conducted clinical trials.

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Part III
Recurrent and/or Metastatic Disease

Chapter 16
Salvage Surgery in Head and Neck Cancer

Stijn van Weert, Sat Parmar, and C. René Leemans

Introduction

Salvage surgery (SS) for head and neck cancer is a much-addressed issue due to its complexity and high stakes for the individual patient. Since the introduction of organ preservation strategies and the rise of concomitant chemoradiation (CCRT) in advanced disease, challenges in SS have grown substantially due to toxicity and a tendency to poor healing. Radicality, which greatly determines success, is often difficult to foresee after previous treatment. Major complications postoperatively have to be anticipated and dealt with.

Realistic expectations should be discussed with the patient as well as the best treatment strategy in each individual patient. Salvage surgery should not be considered a fallback option as the outcome is significantly worse than after primary surgery regardless of adjuvant therapy. Active physician driven surveillance is paramount in early detection of residual or recurrent disease to increase salvage rates.

The dynamic field of head and neck cancer treatment, with developments as increasing incidence of HPV- positive oropharyngeal carcinoma (OPSCC) and related treatment paradigm shifts, has a significant impact on the role of SS [1–6].

S. van Weert (✉)
Department of Otolaryngology – Head and Neck Surgery, Amsterdam University Medical Centers, location VUmc, Amsterdam, The Netherlands
e-mail: s.vanweert@amsterdamumc.nl

S. Parmar
Department of Oral and Maxillofacial Surgery, University Hospital Birmingham, Birmingham, UK

C. R. Leemans
Department of Otolaryngology – Head and Neck Surgery, Amsterdam University Medical Centers, Vrije Universiteit, Amsterdam, The Netherlands
e-mail: cr.leemans@amsterdamumc.nl

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Evolution of Salvage Surgery

Since the emergence of organ preservation in advanced head and neck cancer there has been an increase in need for salvage surgery with various results. The addition of chemotherapy (CT) to primary radiotherapy (RT) has a reported survival benefit of 4–8% but also increases toxicity leading to a more complication prone course if SS is needed. Goodwin in 2000 commented on salvage surgery as—"the double-edged sword"—in the head and neck addressing these issues posing the key question whether the ends justify the means [7]. Despite the use of modern techniques and the increased use of free tissue transfer, the 5-year overall survival after SS does not exceed 40% [8].

Cisplatin (CP) is widely used as radio sensitizer in combined modality treatment in patients with head and neck squamous cell carcinoma (HNSCC).

Bonner et al. recommended cetuximab (an epidermal growth factor receptor inhibitor) as an alternative for CP in patients in whom CP was contra-indicated and its use grew substantially [9]. The side effects were different from those caused by CP and were mainly a cutaneous rash. In the event of residual disease after cetuximab/RT, so called "bioradiation", SS seemed to meet the same setbacks as in combined modality treatment with CP. In clinical practice, cetuximab also significantly added to toxicity and poorer healing tendency in SS [10]. More recently de-escalation trials have shown that cetuximab/RT results in poorer survival outcome in treating HPV-positive OPSCC as compared to CP/RT and has thus been abandoned in this setting. The focus in de-escalation of HPV-positive disease is now on lowerRT or CCRTdoses, induction chemotherapy with definitive treatment based on the response and on up-front minimally invasive surgery with tailored adjuvant treatments [6]. All of these novel approaches will likely influence the field of SS.

History has shown that any non-surgical treatment prior to salvage surgery is associated with a degree of toxicity, determined by the type of treatment as well as individual patient variation. SS may be needed not only for residual or recurrent disease but also for toxicity related functional loss of the aerodigestive tract. The latter is usually seen in advanced hypopharyngeal or laryngeal cancers. These patients may need a (mostly total) laryngectomy due to recurrent aspiration and pneumonia, dyspnea and cartilage necrosis.

Tumor Factors

The surgeon performing SS in HNSCC has to consider both the tumor stage and site. Early stage tumors are obviously better salvageable than advanced stage tumors. Laryngeal recurrence has the best outcome after SS, in contrast to an isolated neck recurrence with adverse features in the previously treated neck, which is on the other end of the spectrum [7, 11], ·(Table 16.1) Goodwin also showed that the 2- year DFS after SS was 24–55% in recurrent neck disease compared to 58% in

Table 16.1 Survival rate per site [7]

Site (all stages)	Survival (%)
Oral cavity	26
Pharynx	47
Larynx	58
Neck	25
Total	44

Goodwin WJ Jr. Salvage surgery for patients with recurrent squamous cell carcinoma of the upper aerodigestive tract: when do the ends justify the means?. *Laryngoscope*. 2000;110(3 Pt 2 Suppl 93):1–18

Table 16.2 Stage related outcome [7]

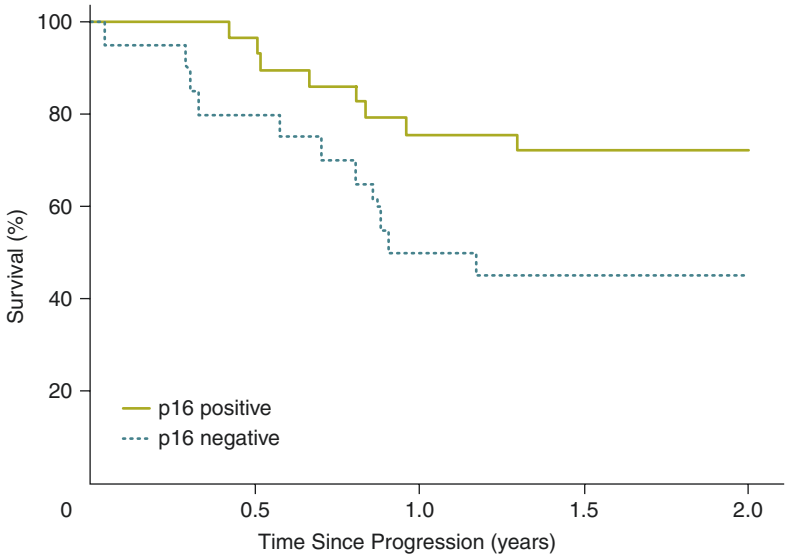
Stage (initial)	I&II	III	IV
2-year survival (%)	70	33	<25
Good QoL(%)	60–85	40	30
Surgical complications	6	30	30
Death related to surgery	Rare	<2%	<2%

Goodwin WJ Jr. Salvage surgery for patients with recurrent squamous cell carcinoma of the upper aerodigestive tract: when do the ends justify the means?. *Laryngoscope*. 2000;110(3 Pt 2 Suppl 93):1–18

recurrent laryngeal carcinoma [7]. Stage is of critical importance as illustrated by a dramatic drop in 2-year post salvage DFS with increasing initial stage (I-II vs. III and IV: 70% vs. 33% and < 25% respectively). Advanced stage disease has a negative impact on quality of life, surgical complications and surgery related death [7]. (Table 16.2) Primary advanced stage disease makes up for the majority of salvage candidates as these tumors show a higher incidence of primary treatment failure. These stage III-IV tumors have a relatively high complication rate with SS. Besides advanced stage disease and positive margins, a short disease-free interval and previous chemotherapy have a negative impact on outcome [12]. Lymph node metastasis at the time of SS and in particular the presence of multiple nodes and/or extracapsular spread (ECS) should be considered as a negative prognostic indicator whereas regional, non- extracapsular single node recurrence outside the previously treated field may result in 5- year disease free survival (DFS) up to 60% [13, 14].

An important factor to consider is the role of HPV in salvage treatment. A 3-year 25% recurrence rate has been reported by different authors. Both Fakhry et al. and Zenga et al. showed that outcome of SS in HPV-positive OPSCC was superior to other sites of HNSCC recurrences. Recurrences in HPV-positive OPSCCs can currently often be treated non-surgically because more patients with HPV(+) OPSCC are being treated nowadays with primary surgery, without adjuvant therapy. Although both HPV+ and HPV- patients benefit from SS with improved overall survival (OS), the outcome of HPV+ patients is superior. (Fig. 16.1) [17–19].

The only independent prognosticator on multivariate analysis is surgical margins. However, achieving clear margins in SS is demanding and extensive



No. at risk					
p16 positive	29	28	22	21	21
p16 negative	20	16	10	9	9

Fig. 16.1 Survival after salvage surgery relative to p16- status [15]. Fakhry C, Zhang Q, Nguyen-Tan PF, et al. Human papillomavirus and overall survival after progression of oropharyngeal squamous cell carcinoma. *J Clin Oncol*. 2014;32 [16]:3365–3373. Reprinted with permission©

submucosal growth makes the delineation of proper margins difficult. This in turn may lead to disappointing histopathological results with only limited (due to previous (chemo) radiation) adjuvant treatment options being available [7, 12, 18].

The best salvageable HNSCC recurrence is laryngeal cancer (2-year DFS 58%, Goodwin) and is the commonest surgically salvaged tumor [20]. Early stage laryngeal cancer is often irradiated or operated on by transoral laser surgery (TLM) as an initial treatment with good results. While the majority of laryngeal SCCs are so called "in the (voice)box" tumors and so surgical margins are relatively easy to achieve in cases of recurrence by performing a laryngectomy (usually total but partial laryngectomy may be feasible in select cases). Van der Putten et al. analyzed outcome of salvage laryngectomy after primary CCRT treatment failure and found a 5- year OS of 27%- Fig. 16.2- and a disease specific survival of 35% [22]. In contrast, advanced OPSCC and hypopharyngeal carcinoma show the poorest outcome in SS. Previous systematic reviews conclude however, that the predominant subsite in head and neck SS is the larynx rendering these subsite differences questionable because of scarce data on advanced non-laryngeal salvaged patients [20]. In oral cavity cancer, primary treatment is essentially surgical. In case of no adverse features after primary surgery adjuvant (chemo) radiation can be avoided. This would leave room for adjuvant treatment in case SS is needed.

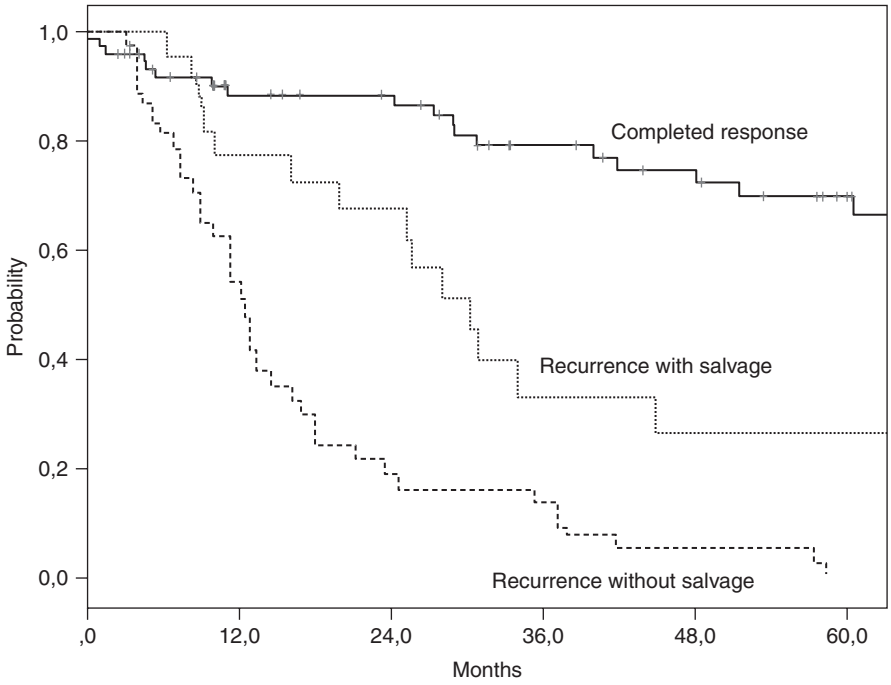


Fig. 16.2 Overall survival after most recent treatment for advanced laryngeal cancer [21]. Putten L, Bree R, Doornaert PA, et al. Salvage surgery in post-chemoradiation laryngeal and hypopharyngeal carcinoma: outcome and review. *Acta Otorhinolaryngol Ital*. 2015;35 [3]:162–172

The key tumor factors thus determining a more favorable course after SS for HNSCC are early stage disease of the tumor, low tumor burden in the neck, no ECS, clear surgical margins, laryngeal site, HPV positivity in OPSCC, no previous chemotherapy and a long disease free interval after initial treatment (>6 months) (Table 16.1) [7, 12–14, 18].

Patient Factors

Patient performance status is equally important for eventual outcome in SS. If considering SS, each case has to be considered individually and be discussed in a multidisciplinary team (MDT). Previous reports have shown irrefutable evidence that MDT discussion leads to an optimal treatment proposition [23, 24]. The definitive decision should not be made by the treating surgeon individually. The patient wishes should be paramount provided that the patient has been thoroughly informed and has a complete understanding of the options available.

Functional status presalvage is a strong indicator for postsalvage outcome. If patients have a relatively poor quality of life (QoL) after primary treatment with

regards to speech and swallowing, further deterioration of these vital functions after SS is likely. Patients should be informed about possible long-term complications like permanent feeding tube dependency and tracheostomy [25–28]. In salvage laryngectomy for toxicity induced sequalae the intention is to restore swallowing and the airway by tracheostomy for improvement of QoL. Whether this expected QoL is accurately predictable and acceptable for the patient will differ in each individual case. Shared decision making is key in this respect and has been more highlighted over the past years with growing attention to value based healthcare [29].

Comorbidities play an important role in the expected outcomes of SS. Is the patient safely able to undergo extensive surgery and is his/her vascular status sufficient for possible use of free flaps? Is the feeding status sufficient to minimize post-operative wound healing problems and other complications? If adjuvant systemic treatment is expected then there should be no medical contra-indication for that (e.g. poorly controlled diabetes or extensive cardiovascular disease). There should at least be a realistic aim to optimize the patient's condition prior to SS to allow for a non-eventful recovery. Kim et al. have advocated the use of the Charlson-Age Comorbidity Index (CACI) as prognostic model for outcome prediction in SS [15, 16, 30–32].

Lastly, the patient's family should not be overlooked. The impact of head and neck cancer on family life is significant and patients considered for SS have been in this situation with their relatives already during the course of their primary treatment. Residual or recurrence of a tumor is devastating and SS brings uncertainties and anxiety for everyone involved. It is of utmost importance to involve the patient's network and offer psychosocial support for those in need [21].

Reconstructive Surgery after Resection for Salvage

The use of pedicled flaps such as the pectoralis major myo(cutaneous) (PMM(C)) flap and the latissimus dorsi (LD) flap have been reported since decades. Ariyan was the first (1979) to describe the PMMC flap in head and neck reconstruction [33]. Today, the PMM(C) is still considered one of the more versatile flaps for reconstruction as well as a preventive measure for wound healing problems such as wound dehiscence or pharyngocutaneous fistula after laryngectomy.

While pedicled flaps are still very useful, free flaps have gained a predominant place in SS over the last decades. It is advantageous to bring healthy, well vascularized tissue in an irradiated environment without having to use local tissue with potential limited geometry.

In SS, the neck is invariably vessel depleted due to sacrifice of the vessels at the time of previous surgery or due to the effects of chemoradiotherapy. Scarring may make identification and isolation of vessels difficult. These factors make reconstructive and in particular free flap surgery a challenge. Pre-operative assessment in terms of reviewing previous operation notes and imaging is essential. A dual phase CT-scan or MR angiogram will predict what neck vasculature may be used for reconstruction

and thus aid planning of the surgery. Vessels within the radiation field, especially after 60–70 Gy, have been shown to have significant intimal changes in arteries [34]. Thus it may be advisable to avoid the use of vessels exposed to high levels of radiation. Previous radiation may also adversely affect the success of microvascular reconstruction [35, 36]. Other studies showed equivalent free flap success rates but an increased incidence of complications [37, 38]. Care must be taken to choose the correct flap for reconstruction and ensure that the flap has adequate pedicle length. Soft tissue flaps with good pedicle length are the radial forearm, anterolateral thigh, latissimus dorsi and rectus abdominis flaps. Composite flaps with good pedicle lengths are the fibula and tip of scapula. (Fig. 16.3) Flaps with poor pedicle length are the Deep circumflex iliac artery and the scapula flap. (Fig. 16.4).

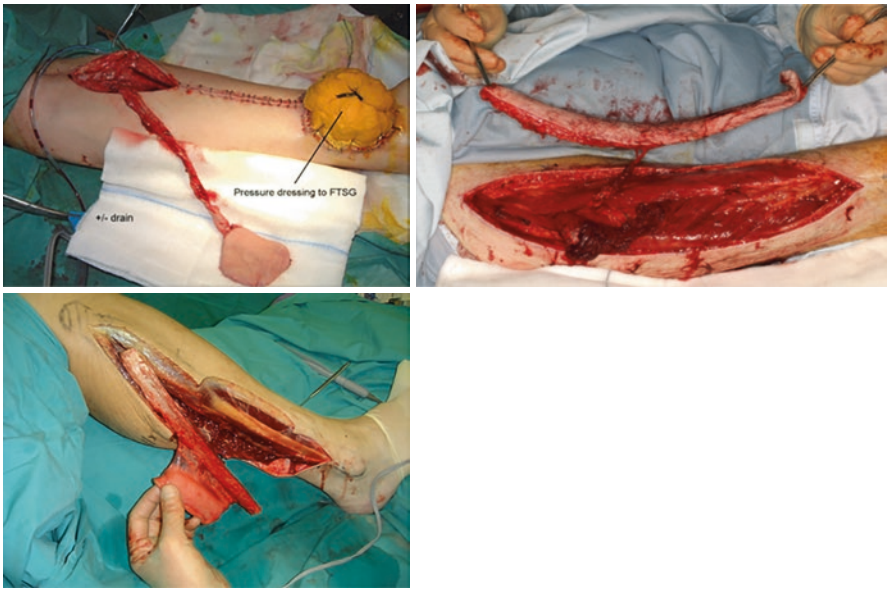


Fig. 16.3 Flaps with adequate pedicle length—radial, anterolateral thigh and fibula

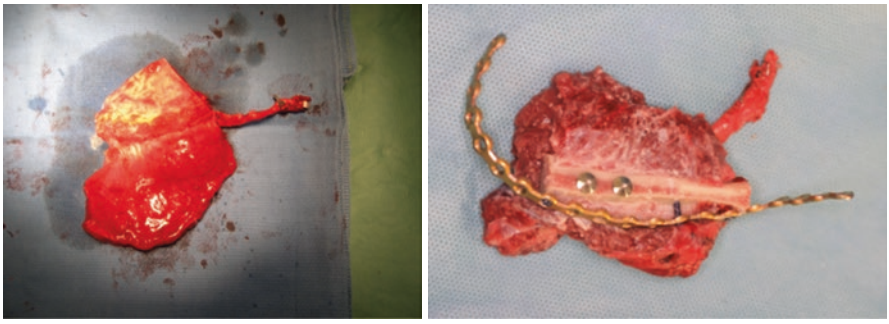


Fig. 16.4 Flaps with poor pedicle length- Deep Circumflex Iliac Artery bone flap and Scapula flaps

Arterial and Venous Options for Reconstruction in a Salvage Neck

Normally branches of the external carotid artery are used if found and patent. If no branches are found the external carotid artery can be harvested at its distal end and end to end anastomosis carried out but there is often a discrepancy in vessel size.

Arteries from the contra-lateral neck can be used but require the flap pedicle length to be long or need vein grafts. End to side anastomosis on the carotid artery has also been described, with no neurological deficit. The transverse cervical artery and vein are vessels located at the base of level IV. They have a reasonable calibre but a flap with a long pedicle is often required. The artery is more reliable then the vein. The Internal mammary vessels are located on the under surface of the upper 6 ribs just lateral to the sternum. Studies show that with careful dissection 85% of internal mammary pedicles can reach the mandibular angle [39]. A corlett loop uses the cephalic vein that is mobilised and detached distally and this is anastomosed to an artery in the contra-lateral neck to create a fistula. This is then divided and provides a longer artery and vein for anastomosis to the flap. Vein grafts can be utilised to lengthen the pedicle length for both arteries and veins. However, vein grafts require two anastomoses for each vessel and thus have a higher rate of failure in several studies [40].

Venous Options for Reconstruction in a Salvage Neck

The use of vein grafts, transverse cervical vessels, the corlett loop and internal mammary vessels have already been described above. The cephalic vein can be harvested, detached distally and rotated into the neck for the venous anastomosis. The vein can be easily found in the deltopectoral groove, detached distally and rotated either under or above the clavicle (Figs. 16.5 and 16.6).

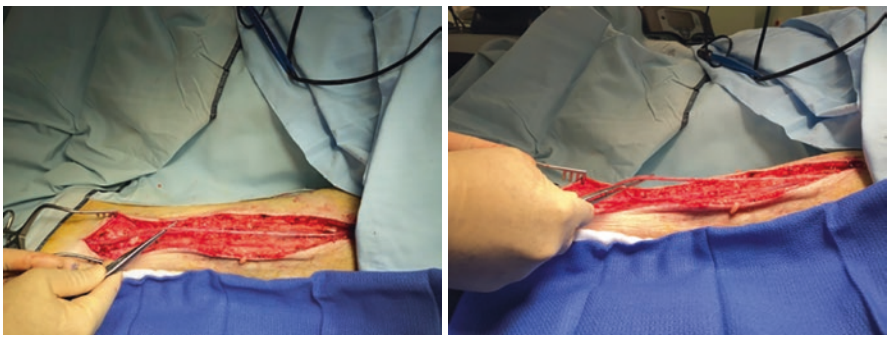


Fig. 16.5 Harvest of long saphenous vein

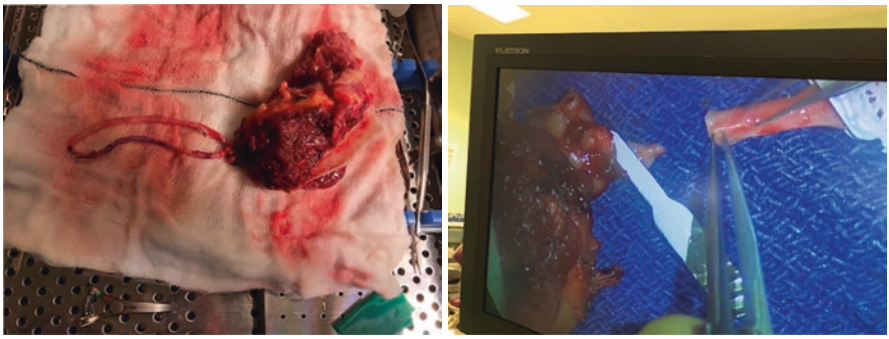


Fig. 16.6 Vein grafts anastomosed to a scapula flap to lengthen the pedicle

Advanced Options for Reconstruction in a Salvage Neck

The pedicle from a previous reconstruction may be used for a new reconstruction but makes the assumption that the former flap has developed an alternate vascularisation. Extracorporeal perfusion of microvascular reconstruction has been described by Wolff for reconstruction in vessel depleted necks. They were able to use the devices for up to two weeks to allow flap autonomization and become independent of the ECMO (Extracorporeal Membrane Oxygenation) machine [41].

Complications in Salvage Surgery

The reported complication rates in SS for recurrent HNSCC can be 67% illustrating that SS is not easily embarked on [16]. In order to improve uniformity and reproducibility in reporting surgical complications, the use of the Clavien Dindo classification for head and neck surgical oncology has been adopted [42]. The addition of neck dissection (ND) to SS for the primary tumor site increases the risk of complications [43–45]. Complications after SS after primary CCRT have been identified as an independent predictor for poor prognosis [46]. Besides the perioperative complications, long term complications as progressive fibrosis, feeding tube dependency and permanent tracheostomy are frequently observed after SS [25–28].

Ideal Candidates

The crucial question to be posed is which patients are amenable for SS with realistic chances of cure and acceptable functional outcome. Ideally, these would be non-smoking and non- drinking young patients with no comorbidities and where initial treatment was for an early stage head and neck cancer. In the past these types of

patients were rare but since the increase in HPV-positive OPSCC they are regularly seen. Primary treatment may be transoral robotic surgery (TORS) combined with ND in case of nodal disease and CCRT in advanced cases of OPSCC or in the presence of ECS. De-escalation of primary and adjuvant treatment is an ongoing subject of multiple trials on the brink of reporting like PATHOS and ECOG E-3311 [3, 47]. Fakhry et al. reported on a significantly better outcome in SS for p16 positive OPSCC (72% 2-y OS) than for p16 negative OPSCC (45% 2-y OS) [28].

In practice, ideal candidates are however seldom encountered as described by Zafereo et al. [32]. They concluded that 3-and 5 year- OS in SS for recurrent OPSCC is only 42% and 28% respectively. Young patients (representing a mere 7% of the total group of recurrent OPSCC) with a prolonged disease free interval and small recurrent tumors had 3- and 5-year OS were 74% and 44% respectively. This poses the question whether prognostic modelling could be of help in decision making. Since several prognosticators have been identified, tools are available for guidance in treatment strategy. Hamoir and Tan for example have proposed a decision model based on comorbidity index, local recurrence vs. loco-regional recurrence, larynx vs. non- larynx and early vs. advanced stage disease. In cases of an early stage laryngeal local recurrence, 2- year DFS up to 96% may be possible. However, as soon as one negative prognosticator was added, the rate dropped dramatically to around 60% and even to 28.6% in cases of an advanced, non-larynx loco-regional recurrence [37, 38]. Other studies have proven that young age as a positive factor and that the presalvage Charlson-Age Comorbidity Index (CACI) can be applied in prognostic modelling [36, 40].

There should be a realistic chance of achieving a R0 resection before proceeding with SS since positive margins in SS have been reported in up to 22% of cases due to reasons already mentioned (submucosal spread, perineural invasion). Positive but even close margins have been identified as an independent factor for re-recurrence. This should be put in perspective since 5- year OS is reportedly around 40% even in cases of clear margins [34, 40, 41]. Positive margins status and/or ECS after SS should be an incentive for enrolment in clinical trials for adjuvant treatment with chemo- or immunotherapy. Table 16.3 summarizes the prognosticators in head and neck salvage surgery.

Table 16.3 Prognosticators in salvage surgery

Positive prognosticators	Negative prognosticators
Early stage disease	Advanced stage disease
Clear surgical margins	Positive surgical margins
Laryngeal site	Non- laryngeal site
HPV positive in OPSCC	HPV negative in OPSCC
No previous chemotherapy	Previous chemotherapy
>6 months DFS	<6 months DFS
N0–1 without ECS	N > 1 or ECS present

Conclusion

SS is the preferred rescue modality after primary treatment failure in HNSCC. Despite the evolution in surgical techniques, improvement of pre- and postoperative care, treatment in designated head and neck centers, an increasing incidence of HPV-associated OPSCC, improved patient selection and an ongoing understanding of prognosticators, 5- year OS is in the range of 30–50% to date [48].

The decision to proceed with SS should therefore not be taken lightly and always in careful discussion with the patient and in the MDT meeting after meticulous analysis of the tumor and patient factors. Expectations should be realistic and communicated in that manner with everyone involved.

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Chapter 17
Re-Irradiation for Local Relapses or Second Primaries: When and how?

Volker Budach and Alexander Thieme

Introduction

Radiotherapy (RT), alone or in combination with surgery and chemotherapy, is a mainstay of curative treatment of patients with head and neck cancer (HNC). Despite advances in treatment and intensification of regimens, e.g. chemoradiation (CRT) [1] or alternative fractionated RT [2, 3], locoregional recurrence as the predominant pattern of failure occurs in 15–50% of patients and represents the most common cause of death in this patient population [4–7]. Most recurrences emerge during the first 2 years after initial RT and 80% occur in-field of formerly irradiated volumes [8]. Furthermore, a second primary HNC in the previously irradiated volume is frequently encountered [9]. It may arise from field cancerization, radiation-induced changes, or de novo from past or continued tobacco or alcohol abuse. Whenever feasible, salvage surgery is the treatment of choice. However many patients are not surgical candidates due to comorbidities, disease progression to an unresectable stage, or patient preferences.

Re-irradiation (Re-RT) is a potentially curative treatment option but represents a challenging problem and carries a poor prognosis. Re-RT with conventional radiation techniques (2D or 3D conformal RT) carries a serious risk of treatment-related toxicities, including treatment-related deaths [10]. With conventional RT-techniques only, small gains of Re-RT compared with chemotherapy alone could be seen in oncological outcome which did not justify the high-grade toxicity experienced by

V. Budach (✉)
Departments for Radiation Oncology and Radiotherapy, Charite University Medicine Berlin, Berlin, Germany
e-mail: volker.budach@charite.de

A. Thieme
Department of Radiation Oncology and Radiotherapy, Charité–Universitätsmedizin Berlin, Berlin, Germany
e-mail: alexander-henry.thieme@charite.de

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the patients [11, 12]. Modern radiation techniques like intensity-modulated radiotherapy (IMRT), stereotactic body radiotherapy (SBRT), and proton therapy (PT) have shown improved disease control compared with conventional 2D or 3D Re-RT by more precisely delivering high radiation doses to target volumes while reducing toxicities. These improvements open up the possibility to ask the question again which strategy of Re-RT in the setting of HNC is the most successful. Many studies on Re-RT with modern radiation techniques have already been conducted. However, interpretation of their results is ambiguous, especially due to the low number of patients per study, a variety of treatment regimens used, an inherent heterogeneity of patient characteristics, and possible biases resulting from mostly retrospective evaluations. This chapter will focus on patient selection strategies, choice of an adequate treatment regimen, expected oncological outcome, and toxicities after Re-RT.

When to re-Irradiate?

With a careful patient selection, Re-RT can achieve a longer progression-free survival (PFS) and disease-free survival (DFS); however, severe acute and late side effects must be taken into account. Therefore, Re-RT's adequate patient selection criteria are crucial to avoid unnecessary toxicities, and a further reduction of quality of life (QoL) in patients whose life expectancy already is limited. Several prognostic factors for survival after HNC Re-RT have been reported. An appropriate algorithm for treatment selection needs to consider factors related to the disease, comorbidities, and organ dysfunction. A simplified decision tree can be found in Fig. 17.1. Ideally, Re-RT should always be based on a therapeutic decision of an interdisciplinary tumor board. Comprehensive informed patient consent regarding toxicities and expected benefits from the treatment are necessary prerequisites for joint decision-making. Ideally, patients should be included in prospective randomized clinical trials to generate better models on individually predictive factors, which would allow a more precise treatment selection in this extremely vulnerable patient population.

Previous Toxicity and Patient-Related Considerations

Limiting toxicity and maintaining organ function should be the major priority when considering Re-RT. There is no consensus concerning the cumulative dose of organs at risk (OARs) when performing Re-RT. Due to a known heterogeneity of radiosensitivity in patients [14], it is important to consider the treatment-related toxicity of the initial RT. Patients with higher-grade toxicities from initial RT such as osteoradionecrosis, severe fibrosis, or dysphagia should be excluded. Tanvetyanon et al. found that pre-existing organ dysfunction and comorbidities belong to the most

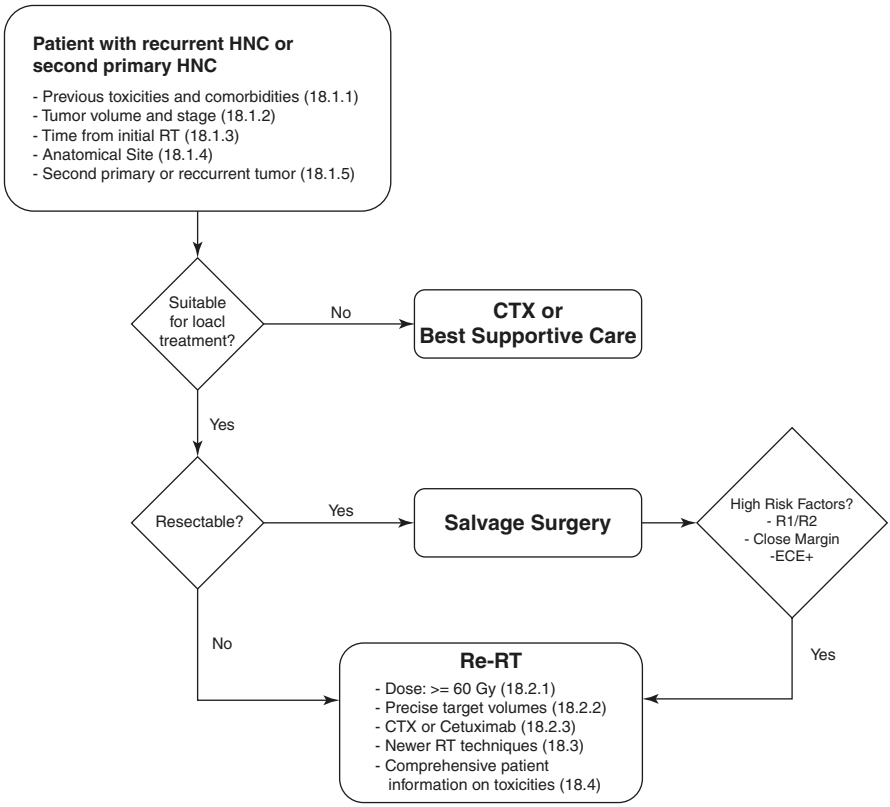

Fig. 17.1 Algorithm for treatment selection for patients with HNC recurrence or second primary HNC in a previously irradiated location. Modified from Strojan et al. [13]. Abbreviations: *RT* radiotherapy, *CTX* chemotherapy, *Re-RT* re-irradiation, *ECE+* extracapsular extension, *R1* microscopic resection margin, *R2* macroscopic residual tumor

important factors of long-term outcome. A nomogram was created on the basis of these findings to predict the 24 months survival probability after Re-RT [15].

Treatment Volume and Recurrent Stage

Various studies have found that patients with smaller treatment volumes have higher locoregional control (LRC) and overall survival (OS) rates after Re-RT with thresholds of gross tumor volume (GTV) <15 cm^3 [16] and < 25 cm^3 [17–19] and of planning target volume (PTV) <27 cm^3 [20] and ≤ 40 cm^3 [21]. In a retrospective study with 91 patients receiving Re-RT for locally recurrent nasopharyngeal carcinoma (NPC), the 3-year local failure-free survival rates for rT1, rT2, and rT3 were 64%, 61.5%, and 18.4% [22], respectively. Additionally, it has been reported that a GTV

>25 cm^3 was predictive for acute toxicities in a series of SBRT treatments [18, 19]. With IMRT, a clinical target volume (CTV) ≥40 cm^3 was associated with increased late toxicity [23, 24]. Consequently, Re-RT for patients with bulky tumors in a curative approach should only be offered with caution [25].

Time Interval since Initial Radiotherapy

Several studies suggest that the time interval since initial RT is prognostic for OS. In a phase II trial (RTOG 9610), patients (n = 86) with recurrent HNC or second primary HNC arising in a previously iradiated field were enrolled to receive CRT with 1.5 Gy twice-daily and 4 cycles of 5-fluorouracil (5-FU) and hydroxyurea. Patients who received Re-RT less than 1 year from initial RT had a significantly worse OS than patients with an interval of more than 1 year (median OS 7.7 vs 9.8 months, p = .033) [12]. More studies support the finding that the time interval since initial RT is an independent factor for OS [26, 27]. While no minimum time interval between Re-RT and the previous RT is established, most trials require intervals of at least 6 months with a longer interval preferred. A study by Chen et al. found a higher risk of toxicity in patients receiving Re-RT with a shorter time interval than 1 year [25].

Anatomical Site

Outcomes also correlate with the site of recurrence with nasopharyngeal and laryngeal cancer revealing a better prognosis [23, 28–32]. Generally, nasopharyngeal tumors have a higher radiosensitivity which could be also an explanation for its favorable prognosis with Re-RT. For early-stage rT1/rT2 cases, brachytherapy is effective to achieve a 5-year local control rates of 85% and OS of 61.3% [33]. In a retrospective series of 90 patients treated with SBRT with a median dose of 18 Gy in 3 fractions or 48 Gy in 6 fractions, a 3-year local failure-free survival of 89.4% could be achieved. Also, for selected patients with laryngeal carcinoma good clinical outcomes have been described. Patients with recurrent early stage I and II laryngeal carcinomas were treated with Re-RT and a cumulative dose ranging from 60 to 70 Gy. Five-year local control and OS rates of 60% and 93%, respectively, were observed while the majority of patients had a functional larynx [31].

Second Primary vs Recurrent Tumors

It is plausible to assume that second primary cancers in a previously radiated volume could respond better to Re-RT compared with recurrent HNC due to the inherent radioresistence of recurrent tumor cells. Several studies support this assumption.

In RTOG 96–10, patients with a second primary had a 1-year OS rate of 54% and median survival time of 19.8 months compared with 38% and 7.7 months for patients with recurrent HNC [34]. In another study conducted by Stevens et al., 100 patients treated with Re-RT alone had a 5-year LRC of 60% and 37% OS for secondary primaries compared with 27% and 17% for patients with recurrent HNC [35].

Re-Irradiation after Salvage Surgery

A multicenter phase III randomized controlled trial (RCT) compared salvage surgery and re-chemoradiation (Re-CRT) with salvage surgery only [36]. Patients (n = 130) were recruited at 16 French and Belgian sites. Eighty-four percent of the patients underwent lymph node dissection. Higher risk factors were evaluated by histopathological examination, namely positive or close margins, extracapsular extension, or metastases in more than one lymph node. In the Re-CRT-arm, patients received 6 cycles of 2 Gy fractions for 5 days (60 Gy total) concomitant with hydroxyurea and 5-FU over the course of 11 weeks. A significant improvement in LRC (HR 4.51, $p < 0.001$) and DFS (HR 1.68, $p < 0.01$) in favor of the Re-CRTarm were observed (Fig. 17.2). However, no difference in OS could be noticed which might relate to treatment-associated death, distant metastases, and second primary tumors in the Re-CRT group. Also, severe toxicity (grade 3 and 4) at 2-years could

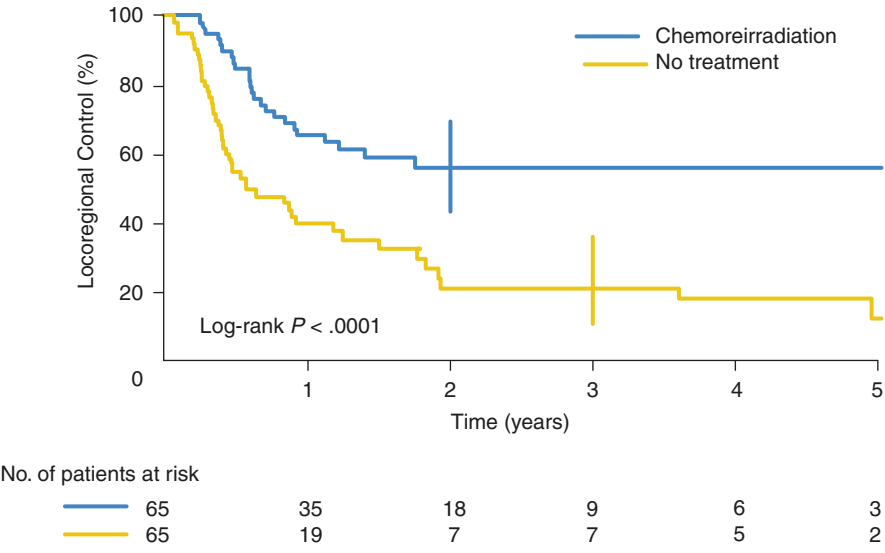

Fig. 17.2 Kaplan-Meier pots for LCR in patients with recurrent HNC treated with postoperative Re-CRT. LRC was significantly improved in the Re-CRTarm vs salvage surgery alone (HR 4.51, $p < 0.001$) [36]

have contributed in the Re-CRT arm with 39% vs 10% in the surgery alone arm (p = 0.06).

Take Home Message for when to re-Irradiate?

- Careful patient selection for Re-RT remains paramount.
- Appropriate patient selection criteria comprise factors related to the patient and disease.
- Favorable factors are no comorbidities and adequate organ functions, small tumor volumes <40cm^3, time interval from initial RT to Re-RT (>6 mos.) and nasopharyngeal or laryngeal tumors.
- Decision for or against Re-RT should be based on the consensus of an interdisciplinary tumor board.
- A comprehensive informed consent of patients concerning the benefits and risks of the treatment is a key issue for joint decision-making.

How to re-Irradiate?

Re-Irradiation Dose

Generally, there is no consensus on the radiation dose for target volume, a particular fractionation scheme, or allowed cumulative doses for OARs. Recurrent HNC after initial RT suggests the presence of radiation-resistant tumor cells which implies the need for a high dose Re-RT. This might be in particular true for patients who received CRT as initial treatment. Indeed, several studies have reported a dose-response relationship for improved LRC [15, 17, 23, 37]. In a study conducted by Salama et al., patients with a total dose of ≥58 Gy had a 3-year OS of 30% compared with 6% for patients receiving less than 58 Gy [10]. Other investigators use a slightly higher target dose of 60 Gy as found in several Re-RT protocols [38, 39]. In an SBRT study with 85 patients, local control (LC) was significantly higher in patients receiving ≥35 Gy compared with a lower dose (p = 0.014) [40]. Since all of these reports are retrospective, it is important to consider possible selection biases like patients with better performance status and smaller tumor volumes might have received higher radiation doses.

Re-Irradiation Volume

In the setting of Re-RT for local tumor recurrence, there is no debate about the need for a maximally tolerable total dose to the macroscopically recurrent tumor. However, elective irradiation to the neck is controversially discussed [39]. It can be argued that in most cases the elective neck did receive a lower total dose than the

target volume of the primary during initial RT and could consequently tolerate an additional elective irradiation series, but it is evident that the hazards of toxicities increase with the size of the treatment volume. Moreover, in a multi-institutional retrospective analysis comprising 505 patients, elective nodal irradiation was not associated with an improved locoregional failure or OS but with increased risks of acute toxicities [41]. Based on the current study situation and opposed to RT in the primary setting, radiation of elective nodal volumes cannot be recommended.

Multiple studies have reported that the most common pattern of failure is local [23, 42, 43]. From recent studies with image-guided radiotherapy (IGRT) and computer-assisted RT planning, the clinical target volume (CTV) should include the gross tumor volume (GTV) or tumor bed plus a minimal safety margin [20, 23, 26, 44, 45] (Table 17.1). Target delineation based on computed tomography (CT) or positron emission tomography (PET)-CT scans, modern immobilization, and radiation techniques including IGRT, allow for a more precise Re-RT with smaller margins accounting for microscopic disease and positioning uncertainties.

Concurrent Systemic Therapy

The role of concurrent systemic therapy is not clearly defined because prospective randomized studies with a head to head comparison of Re-CRT with Re-RT alone are still lacking. Concurrent systemic therapies can be beneficial in terms of radiosensitization and harmful in terms of increasing toxicities [46], two factors that have to be carefully balanced in the setting of Re-RT. In a series of IMRT studies, Re-CRT has been administered at least to a part of the study population [11, 23, 42–45, 47–50]. Takiar et al. showed an improved LRC for patients receiving platinum-based Re-CRT, particularly when Re-RT was given adjuvantly [23]. There have also been studies reporting adverse outcomes with Re-CRT, although the findings might be biased by patient selection with advanced tumor stages and higher risk features [48, 49]. In a prospective phase II trial conducted by Tao et al., 53 patients were randomized after salvage surgery to receive either split course 60 Gy in 11 weeks with concomitant 5-FU/hydroxyurea or 60 Gy in 5 weeks with 1.2 Gy twice daily and cetuximab, which was found to be tolerable without significant acute toxicity [51].

The still dismal prognosis in pre-irradiated locally recurrent HNC with radiotherapy alone is a challenge and should lead to a combination of irradiation with appealing new drugs like the immune checkpoint inhibitors (CPIs). While the early experience with SBRT was gained without the addition of systemic therapies, recent studies have proved the safety of concurrent cetuximab with this approach [18, 52]. Results on CPIs in the setting of recurrent and metastatic HNC targeting cytotoxic lymphocyte antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1), and programmed cell death ligand-1 (PD-L1) have recently changed treatment paradigms and might have the potential to play a role in the Re-RT setting as well. There are currently several studies underway investigating this topic, e.g. the RTOG

Table 17.1 Selection of IMRT studies for Re-RTof recurrent and second primary HNC

Author/ year/ reference	Study Type	No. patients	Re-RT Median Total Dose/ Fractionation Schedule/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and 4/ Treatment related death	Outcome at 2 yr	Conclusion
Chen et al/2002/ [54]	Retrospec.	12	60 Gy/2 Gy per fx/NS	Surgery 25% CTX 42%	15.5 (4–55)	NS/8%	8 patients alive at 3–16 mo post re-RT	IMRT offers viable re-RT for recurrent HNCs
Lee et al/2007/ [28]	Retrospec.	74	59.4 Gy/1.8-2Gy per fx/ PTV = GTV/TB + 10–20 mm	Surgery NS CTX NS	38 (5–380)	15%/NS	LRC 52%	IMRT predicted better LRC
Biagioli et al/2007/ [44]	Retrospec.	41	60 Gy/1.8-2Gy per fx every other week/ PTV = GTV/TB + 5-20 mm	Surgery 41.5% ICT 31.7% CRT 100%	25 (6–240)	10%/5%	OS 48.7%	IMRT with CRT given every other week appears to be both well tolerated and feasible
Langer et al/2007/ [11]	Prospec.	105	60 Gy/1.5 Gy bid every other week/PTV = GTV + 20 mm (or more)	CRT 100%	39.6 (6.1–317.9)	33.8%/8%	LRC 30% OS 26%	Despite a high incidence of grade 5 toxicity, OS exceeded results seen with CTx alone
Sulman et al/2009/ [37]	Retrospec.	74	60 Gy/1.8–2 Gy per fx/ CTV = GTV/TB + 10-20 mm (+elective lymph nodes) PTV = CTV + 3-5 mm	Surgery 27% CTX 49%	46 (23–445)	20%/1.4%	LRC 64% OS 58%	IMRT morbidity was significant but may be lower compared with CRT
Duprez et al/2009/ [26]	Retrospec.	84	69 Gy/2–2.5 Gy per fx/ CTV = GTV/TB + 5-15 mm (+ elective lymph nodes) PTV = CTV + 3 mm	Surgery 23% CTX 20%	49.5 (5–298)	21%/2%	LRC 48% OS 35%	20% long-term survival in a non-selected patient

Author/ year/ reference	Study Type	No. patients	Re-RT Median Total Dose/ Fractionation Schedule/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and 4/ Treatment related death	Outcome at 2 yr	Conclusion
Popovtzer et al/2009/ [42]	Retrospec.	66	68 Gy/2 Gy or 1.25 Gy bid/ PTV = GTV/TB + 5 mm	Surgery 33% CRT 71%	37 (6–184)	29%/2%	LRC 27% OS 35%	Almost all locoregional failures occurred within the re-irradiated GTV
Sher et al/2010/ [43]	Retrospec.	35	60Gy/1.8-2Gy per fx/ CTV = GTV + 10–15 mm PTV = CTV + 5 mm	Surgery 49% ICT 28% CRT 100%	30 (NS)	46%/11%	LRC 67% OS 48%	Improved LRC but significant risk of developing late complications
Zwicker et al/2011/ [47]	Retrospec.	43	49 Gy/1.8–2 Gy per fx/ CTV = GTV + 5–10 mm	Surgery 34% CRT 50%	43 (NS)	20%/3%	LC 53% LRC 45% OS 34%	Acceptable toxicity and encouraging rates of LC and OS
Chen et al/2011/ [20]	Prospect.	21	66 Gy/NS/ CTV = GTV + 5 mm PTV = CTV + 3 mm	No	14 (6–132)	NS/0%	LC 65% LRC 77% OS 40%	IMRT with daily IGRT has effective disease control and low morbidity
Kharofa et al/2012/ [45]	Retrospec.	38 (76% IMRT)	60 Gy/2 Gy per fx/ PTV = GTV/TB + 10-20 mm	Surgery 34% CRT 100%	28 (3–228)	7%/0%	OS 40%	Re-RT with weekly paclitaxel and carboplatin has an acceptable toxicity profile and offers a potentially curative option

(continued)

Table 17.1 (continued)

Author/ year/ reference	Study Type	No. patients	Re-RT Median Total Dose/ Fractionation Schedule/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and 4/ Treatment related death	Outcome at 2 yr	Conclusion
Duprez et al/2014/ [48]	Retrospec.	60	70 Gy or 69.12 Gy/2 Gy or 2.16 Gy per fx/ CTV = GTV + 5–15 mm (+ elective lymph nodes) PTV = CTV + 3 mm	Surgery 22% CRT 33%	27 (6–240)	30%/6.7%	LRC 48% OS 32%	IMRT offers 5-year disease control and OS in recurrent HNC for 1/3 and 1/4 patients, respectively
Takiar et al/2015/ [23]	Retrospec.	206	Primary 66 Gy and adjuvant60 Gy/1.8 or 2 Gy per fx/ CTV = GTV + 8–15 mm PTV = CTV + 3–5 mm	Surgery 51% CRT67%	Primary 45.3 (2.8–756) Adjuvant 27.4 (5.0–388.7)	50%/1.4%	LRC 65% OS 57%	IMRT has promising local control and survival in selected patients. Treatment-related toxicity remains significant
Curtis et al/2016/ [49]	Retrospec.	81	Primary 69.6 Gy and adjuvant 60 Gy/NS/NS	Surgery 52% CRT 74%	33.2 (NS)	NS/NS	LRC 50% OS 50%	OS appears superior to the published literature
Vargo et al/2018/ [55]	Retrospec.	217	60 Gy/33 fx (median)/NS	CTX 84%	37.2/(2–408)	56.7%/1.8%	OS 35.4%	Re-RT both with SBRT and with IMRT appears relatively safe
Ward et al/2019/ [50]	Retrospec.	505	66 Gy/NS/(40–80)	Surgery 49.1% CRT 77.5%	21.5/ (0–128.1)	16.7%/NS	NS	The risk of late toxicity may be more dependent on patient and disease factors than modifiable treatment factors

Abbreviations*: Retrospec.* retrospective, *Prospec.* Prospective, *RT* radiotherapy, *Re-RT* Re-Irradiation, *IMRT* intensity-modulated RT, *IGRT* image guided radiotherapy, *bid* two fractions per day, *CTX* chemotherapy regimen, *CRT* concurrent chemoradiotherapy, *ICT* induction chemotherapy, *NS* not specified, *TB* tumor bed, *PTV* planning target volume, *CTV* clinical target volume, *GTV* gross tumor volume, *OS* overall survival, *LRC* locoreginal control, *LC* local control, *HNC* head and neck cancer, *yr*. years

KEYSTROKE randomized phase II trial investigating the addition of Pembrolizumab to SBRT in patients with unresectable recurrent or second primary HNC.

Take Home Message for how to re-Irradiate?

- Higher re-irradiation doses seem to be associated with better local tumor control.
- Techniques for reduction of target volumes should be used, since larger treatment volumes are associated with increased toxicities.
- After Re-RT, the predominant pattern of failure is in-field, therefore elective nodal irradiation is not recommended.
- Concurrent systemic therapy should be offered to selected patients.

Radiation Techniques

Intensity-Modulated Radiotherapy (IMRT)

IMRT is a form of a radiation technique that uses multiple angled radiation fields or treatment arcs and intensity modulation to generate highly complex dose distributions. This enables to irradiate the target volume with conformality at higher doses while allowing for a more precise sparing of OARs. There is also the possibility to treat target volumes with an inhomogeneous dose. This technique is called "simultaneous integrated boost" which can deliver different dose levels to multiple target volumes. IMRT has already demonstrated its benefits in reducing toxicity for adjacent healthy tissue in the primary disease of HNCs [53]. Especially with Re-RT it is crucial to minimize the radiation exposure and cumulative dose of previously irradiated healthy tissues to reduce the risk of high grade toxicities. Likewise, it is important to deliver a high, tumoricidal dose to the target volume. Several studies have been conducted to evaluate the efficacy of IMRT for Re-RT of HNCs or second primary HNCs (Table 17.1). Most of these studies are retrospective with exception of the phase II trial RTOG 99–11 [11] and a prospective single institution registry trial [20]. All studies vary widely by different RT treatment regimens regarding the total dose and fractionation schedule, the application of concurrent chemotherapy, and the patient populations.

One of the first larger studies on the topic of Re-RT and IMRT that could show an improved oncological outcome was conducted by Lee at al [28]. Patients (n = 105) with recurrent HNC underwent Re-RT with 74 patients receiving IMRT and 31 patients 3D-conformal RT with a median dose of 59.4 Gy. An improved 2-year LRC of 52% could be observed with IMRT compared with 20% for 3D conformal RT ($p < 0.001$) (Fig. 17.3). A recurrence at the nasopharynx was associated with an improved LRC. Median OS was 15 months with a 2-year OS rate of 37%. Severe grade 3 and 4 late toxicities were reported in 15% of cases with a median onset of 6 months after Re-RT. Predictors of superior OS were non-squamous cell

carcinoma (SCC) histology, recurrence at the nasopharynx site, and a Re-RT dose of ≥50 Gy. A retrospective study with a larger number of patients (n = 206) has been reported by Takiar et al. [23]. Patients were treated with IMRT and doses of 66 Gy in the definitive and 60 Gy in the adjuvant setting, and factors were correlated with oncological outcome. The 2-year OS and LRC rates were 57% and 65% respectively. SCC was associated with a worse prognosis compared with a non-SCC histology. Nasopharynx site and a 70 Gy Re-RT dose were associated with an improved outcome. Grade 3 toxicities and higher were reported in 32% after 2 years and 48% at 5 years and were associated with larger treatment volumes (>50 cm^3). No grade ≥ 3 toxicities were observed for treatment volumes <25 cm^3. Similar 2-year OS and LRC rates of 50% and 60% were reported by another IMRT study without grading of toxicities [49]. Duprez et al. reported the worst outcomes of IMRT studies, with 2-year OS and LCR of 32% and 48%, respectively [26]. Twenty percent of the patients developed grade 4 or higher toxicities. In a subset of patients, IMRT can offer a durable local control, however, severe late toxicities are not uncommon and treatment related-deaths can be observed in up to 11% of the cases.

Stereotactic Body Radiotherapy (SBRT)

SBRT is a highly precise RT which delivers hypofractionated doses of radiation to the tumor volume in a limited number of fractions. SBRT has already become the standard for several cancer treatments, e.g. brain metastases, early-stage lung cancer, or prostate cancer. Fewer fractions result in shorter overall treatment time from 5–7 weeks for standard IMRT regimes to 1 day to 2 weeks for SBRT. Additionally, there might be biological advantages to the ablative doses delivered, since other mechanisms of cell kill are activated than with lower doses used in conventionally fractionated RT. It could also be shown that shorter treatment times can result in better treatment outcomes, probably by overcoming the well-known repopulation effect found in HNC [56]. Sublethal damage repair, one mechanism of healthy tissue tolerance for conventionally fractionated RT, is lacking for SBRT. Therefore, another strategy with other definitions of precision and conformality of dose distribution has to be implemented to prevent toxicities.

There are two prospective studies supporting SBRT as Re-RT for recurrent HNC [52, 57]. The study of Comet et al. comprised 40 patients who received SBRT with a dose of 36 Gy in 6 fractions with a treatment interval of 11–12 days [52]. The authors reported a median OS of 13.6 months with a 79% response rate and grade 3 or higher toxicities in only 10% of the patients. Lartigau et al. conducted a multi-institutional phase II study with 56 patients and the same SBRT dose regimen but with the addition of cetuximab [57]. The authors reported a median OS of 11.8 months, 1-year OS of 48%, and a median PFS of 7.1 months. Treatment-related toxicities grade 3 or higher were observed in 32% of patients and one death from hemorrhage occurred.

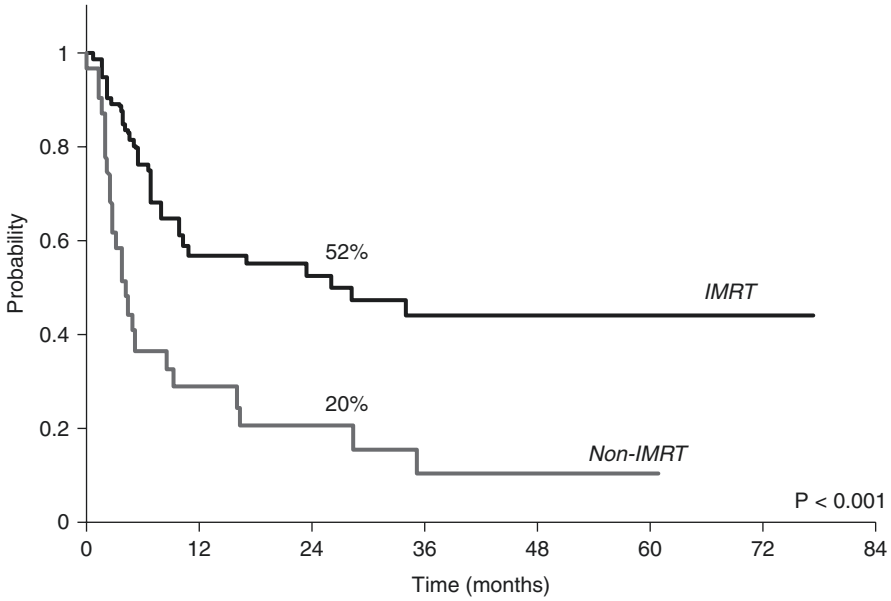

Fig. 17.3 Kaplan-Meier plots for LCR of patients with recurrent HNCs treated with either IMRT or non-IMRT (3D-conformal RT). LRC was significantly improved in the IMRT-arm vs the Non-IMRT-arm (2-year LRC 52% vs 20%, $p < 0.001$) [28]

Most other studies on the topic of SBRT in the setting of recurrent HNC published in the last couple of years have been retrospective. The study by Vargo et al. [55] is a pivotal multi-institutional study comprising a larger number of IMRT and SBRT cases with the aim to identify prognostic factors in both treatment modalities. The study found an improved OS associated with IMRT vs SBRT in the unadjusted model with a 2-year OS of 35.4% for IMRT and 16.3% for SBRT ($p < 0.001$). However, multivariable analysis accounting for other known prognostic factors did not show any significant difference between IMRT vs SBRT.

SBRT should be applied with caution if recurrences are located nearby critical organs like neurological structures or the carotid artery. One must be aware that normal tissues located partially inside or very close to the target volume receive the same ablative doses as the tumor itself potentially leading to impaired damage repair and consequential late damage [17]. Roman et al. developed a treatment selection algorithm for IMRT and SBRT based on the tumor location and ability of the patient to be treated with CTx [58]. A study by Yazici et al. [59] recommended using IMRT instead of SBRT if the maximum carotid artery dose exceeds 34 Gy if or more than 180° of the carotid artery is invaded. The same author also describes a reduced risk of severe toxicity by utilizing an every-other-day radiation protocol (Table 17.2).

Table 17.2 Selection of SBRT studies for Re-RTof recurrent and second primary HNC

Author/ year/ reference	Study Type	No. patients	Re-RT Median Dose/ Fractionation Schedule/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and4/ Treatment related death	Outcome at 2 yrs	Conclusion
Siddiqui et al/2009/ [60]	Retrospec.	21	16 Gy/1 fx or 18 Gy/1 fx or 36 Gy/6 fx or 48 Gy/6 fx/ PTV = GTV	No	19 (3–200)	24%/0%	LC 40.4% OS 14.3%	SBRT in single or fractionated doses offers a viable treatment option for selected patients
Roh et al/2009/ [61]	Retrospec.	36	30 Gy/3–5 fx/ PTV = GTV + 2-3 mm	Adj. CTX 17%	24 (3–253)	8%/3%	LC 52.2% OS 30.9%	SBRT is an effective treatment modality as a salvage treatment with good short-term local control
Heron et al/2009/ [62]	Prospec.	25	25–44 Gy/5 fx/ PTV = GTV	No	13 (5–94)	NS	1-yr OS 16%	Reirradiation up to 44 Gy using SBRT is well tolerated in the acute setting
Unger et al/2010/ [63]	Retrospec.	65	30 Gy/5 fx/ PTV = GTV + 2-10 mm Elective lymph node in 34%	Surgery 29% CTX 54%	26 (2–318)	9%/1.5%	LRC 30% OS 41%	Encouraging response rates with acceptable toxicity
Cengiz et al/2011/ [64]	Retrospec.	46	30 Gy/5 fx/ PTV = GTV	No	38 (4–306)	13.3%/15.6%	1-yr OS 47%	Good local control with considerable 1-year survival is achieved with a relatively high rate of morbidity and related mortality

Author/ year/ reference	Study Type	No. patients	Re-RT Median Dose/ Fractionation Schedule/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and4/ Treatment related death	Outcome at 2 yrs	Conclusion
Kodani et al/2011/ [16]	Retrospec.	21	30 Gy/5 fx/ PTV = GTV	No	51 (2–360)	19%/10%	OS 50%	Re-RT has significant risk of severe and even fatal late complications in the form of necrosis and hemorrhage in re-irradiated areas
Comet et al/2012/ [52]	Prospec.	40	36 Gy/6 fx/ CTV = GTV + 5 mm PTV = CTV + 1 mm	Con Cetux 37.5% Con CTX 2.5%	32 (8–263)	7.5%/0%	OS 24%	Short SBRT with or without Cetux is an effective salvage treatment with good response rate
Iwata et al/2012/ [65]	Retrospec.	51	20 Gy/1 fx or 30 Gy/3 fx or 35 Gy/5 fx PTV = GTV + 0-5 mm	Adj. CTX 33%	18 (3–132)	23%/0%	LC 40% OS 40%	CyberKnife is feasible and effective for local recurrences of nasal and paranasal carcinomas
Shikama et al/2013/ [65]	Retrospec.	28	30 Gy/1–7 fx/NS	Con CTX 11%	9 (3–40)	4%/10.7%	OS 21.7%	Tanvetyanon's nomogram accurately estimates the survival probability
Lartigau et al/2013/ [57]	Prospec.	60	36 Gy/6 fx/ CTV = GTV + 5 mm PTV = CTV + 1 mm	Con Cetux 100%	38 (mean)/ NS	30%/1.7%	1-yr OS 47.5%	Short SBRT with Cetux is an effective salvage treatment with good response rate
Vargo et al/2014/ [18]	Prospec.	50	40 Gy or 44 Gy/5 fx/ PTV = GTV + 3-5 mm	Con Cetux 100%	18/(3–423)	6%/NS	1-yr LRC 37% 1-yr OS 40%	SBRT with concurrent Cetux appears to be a safe salvage treatment

(continued)

Table 17.2 (continued)

Author/ year/ reference	Study Type	No. patients	Re-RT Median Dose/ Fractionation Schedule/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and4/ Treatment related death	Outcome at 2 yrs	Conclusion
Vargo et al/2014/ [19]	Retrospec.	132	44 Gy/5 fx/NS	Con Cetux 45%	23/(4–423)	7%/NS	1-yr LRC 48% 1-yr OS 38%	SBRT ± Cetux achieves promising tumor control and survival with low rates of acute/late toxicity even for recurrences > 25 cc
Kress et al/2015/ [66]	Retrospec.	85	30 Gy/5 fx	Surgery 29% CRT 61%	32/(1–324)	5.9%/NS	LRC 28% OS 24%	SBRT reirradiation results in limited toxicity
Yamazaki et al/2016/ [21]	Retrospec.	107	30 Gy/5 fx	Surgery 43%	14.5/ (0.7–1180)	21%/8.4%	LRC 64% OS 35%	Ulceration is an important prognostic factor for adverse events and survival
Vargo et al/2018/ [55]	Retrospec.	197	40 Gy/5 fx	CTX 55%	14.4/(1–420)	31.2%/0.5%	OS 16.3%	Re-RT both with SBRT and with IMRT appear relatively safe

Abbreviations: *Retrospec*. retrospective, *Prospec*. Prospective, *RT* radiotherapy, *Re-RT* Re-Irradiation, *CTX* chemotherapy regimen, *Con* concurrent, *Adj* adjuvant, *Cis* cisplatin, *Cetux* cetuximab, *NS* = not specified, *PTV* planning target volume, *CTV* clinical target volume, *GTV* gross tumor volume, *OS* overall survival, *LRC* locoreginal control, *LC* local control, *yr*. year, *HNC* head and neck cance

Proton Therapy

Proton therapy (PT) might lead to additional benefits for a selected group of patients with recurrent HNC. The profile of energy deposition has potential dosimetric advantages to spare OARs since protons release most of their energy in the characteristic "Bragg peak" at the end of the rays. Beyond this peak, a steep dose fall-off occurs which can be exploited for precise OAR sparing. When considering one field, the PT beam's entry path receives a lower integral dose than with photons which further facilitates an improved OAR sparing. With PT, two different treatment techniques are used: (1) passive scattering proton therapy (PSPT) which uses scattering devices to broaden the proton beam and a range-modulation device to create a spread-out Bragg peak, and (2) intensity-modulated proton therapy (IMPT) taking advantage of bundles of scanning beams for further improvements of dose conformality compared with PSPT. PT Re-RT schemes for recurrent HNC have the potential of a substantial reduction in the integral dose of healthy tissues with decreased treatment-related toxicities. An in silico study comparing IMPT and IMRT could demonstrate that IMPT can significantly reduce OAR dose in the setting of Re-RT of recurrent HNC [67].

Four studies have been published with the exclusive use of PT for recurrent HNC (Table 17.3). In a study by Romesser et al. [68], 92 patients received a median dose of 60.6 Gy relative biological effectiveness (RBE) via PSPT. One-year oncological outcomes were reported with OS of 65% and LRC of 75%. The majority of locoregional recurrences (77%) were in-field. Severe late toxicity (grade 3 or higher) was mostly related to the skin (9%) and to dysphagia (7%), and there were two patients (3%) with treatment-related death due to bleeding. Phan et al. conducted a study on 60 patients receiving IMPT, in 75% of the cases with a median dose of 66 Gy RBE [24]. The 2-year OS and LRC were 70% and 73%, respectively. The 2-year actuarial rate of severe grade 3 or higher late toxicity was 26% and was associated with a Re-RT treatment volume of >50 cm^3. Two patients (3%) receiving PT Re-RT to the pharynx (3%) died of potentially treatment-related toxicities. In another series of 61 patients by McDonald et al., PSPT was used to deliver a median of 66 Gy RBE for patients with a microscopic and 70.2 Gy RBE for patients with a gross disease. The median reported OS was 16.5 months with a 2-year OS of 32.7% and LRC of 80.3%. Local failure was associated with larger tumor volumes and lower Re-RT doses (continuous).

Overall, Re-RT taking advantage of PT with a reported 2-year LRC in the range from 50% to 80% and severe late toxicities between 20% and 25% seems promising. Further studies are warranted to get a better understanding of this treatment modality and to compare the results with IMRT trials.

Table 17.3 Selection of proton therapy studies for Re-RTof recurrent and second primary HNC

Author/ year/ reference	Study Type	No. patients	Re-RT Median Dose/ Fractionation Schedule/ Proton Therapy Technique/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and4/ Treatment related death	Outcome at 2 yrs	Conclusion
Lin et al/1999/ [69]	Retrospec.	16	62.8 Gy RBE/1.8–2.0 Gy RBE per fx/PSPT/NS	Surgery 19% CTx 75%	20 (NS)	NS/NS	LRC 50% OS 50%	Adequate tumor coverage was found to be the most important variable influencing LRC and survival
Phan et al/2016/ [24]	Retrospec.	60	61.5 Gy RBE adjuvant and 66 Gy RBE definitive/NS/ PSPT 25% and IPMT 75%/ CTV = GTV/TB + "high risk areas" PTV = CTV + 3 mm	Surgery 58% CRT 73%	47.1 (7.3–438.2)	26%/3%	LRC 73% OS 65%	PT can be a safe and effective curative re-RT strategy, with acceptable rates of toxicity and durable disease control
Romesser et al/2016/ [68]	Retrospec.	92	60.6 Gy RBE/2.0 Gy RBE per fx/PSPT/ PTV = CTV + 3 mm	Surgery 39.1% CRT 47.8%	34.4 (14.2–92.5)	Skin 9% and dysphagia 7%/2%	1-yr LRC 75% 1-yr OS 65%	PT re-RT can provide effective tumor control with acceptable acute and late toxicity profiles

Author/ year/ reference	Study Type	No. patients	Re-RT Median Dose/ Fractionation Schedule/ Proton Therapy Technique/ Irradiated Volume	Additional Therapy	Median time between last RT to Re-RT in months (range)	Late Toxicity Grade 3 and4/ Treatment related death	Outcome at 2 yrs	Conclusion
McDonald et al/2016/ [70]	Retrospec.	61 (SCC 32, non-SCC 29)	70.2 Gy RBE definitive and 66 Gy RBE adjuvant/1.8–2 Gy RBE/ PSPT/ CTV = GTV/TB + 10 mm	SCC: Surgery 34.4% ICT 3.1% CRT 50% Non-SCC: Surgery 62.1% ICT 3.4% CRT 6.9%	SCC: 17.5 (2–318) Non-SCC: 32 (4–298)	24.6%/4.9%	LRC 80% OS 33%	Re-RT with PT provided reasonable LRC, toxicity profiles, and survival outcomes for advanced-stage disease and heavily pretreated population

Abbreviations: *Retrospec*. retrospective, *Prospec*. Prospective, *RT* radiotherapy, *Re-RT* Re-Irradiation, *IMRT* = intensity-modulated *RT*, *IGRT* image guided radiotherapy *bid* = two fractions per day, *CTx* chemotherapy regimen, *CRT* concurrent chemoradiotherapy, *ICT* induction chemotherapy, *NS* not specified, *TB* tumor bed, *PTV* planning target volume, *CTV* clinical target volume, *GTV* gross tumor volume, *OS* overall survival, *LRC* locoreginal control, *LC* local control, *HNC* head and neck cancer, *RBE* relative biological effectiveness, *PSPT* passive scatter proton therapy, *IPMT* intensity-modulatedproton therapy, *PT* proton therapy

Brachytherapy

Brachytherapy (BT) is a kind of internal radiotherapy that involves the placement of short-range radiation sources inside body cavities or interstitially. BT provides advantages over external beam radiotherapy by focussing high radiation doses to tumor volumes while minimizing the radiation exposure of healthy tissue by its steep dose gradients. BT can be differentiated by the dose rate of the used radiation source: (1) Low-dose rate (LDR) with a dose rate of up to 2 Gy/h, (2) Medium-dose rate with 2–12 Gy/h, and (3) High-dose-rate with >12 Gy/h. Possible radiation sources are the radioisotopes iodine-125 (^{125}I) or iridium-192 (^{192}Ir).

BT can be used for isolated nodal relapses in the neck, which occur in 10% of patients following curative treatment of HNC [71–73]. In a systematic review by Tselis et al., 686 patients from 12 retrospective studies have been analyzed who received BT with a median dose in the range of 30–70 Gy (Table 17.4) [74]. All studies except one used ^{192}Ir as a radiation source. In this patient population, a 2-year OS of 13%–57% and LC of 26%–67% could be achieved while the observed grade 3 or higher late toxicity was in the range of 4–14%. The authors concluded that CT-guided HDR-BT is a treatment modality that can play an important role in the management of inoperable recurrent neck disease providing palliation and acceptable tumor control. However, the caveat of this review is the low number of patients per study (range 17–164) and the possible biases arising from the retrospective evaluation in terms of patient heterogeneity and unbalanced competing risk factors.

Take Home Message for Radiation Techniques

- All modern radiation techniques allow an effective sparing of healthy tissue which is an important strategy to reduce significant toxicities observed with Re-RT.
- In selected patients, durable tumor control can be achieved.
- SBRT should be used with caution if recurrences are located within critical healthy tissues.
- PT compared with photons has potential advantages for toxicity reduction which should be evaluated in RCTs.
- Brachytherapy can play an important role in local control and palliation of recurrent disease of small volume irrespective of the site.

Toxicities of re-Irradiation

Acute and in particular late toxicities experienced by patients after Re-RT have a significant impact on the QoL and can even endanger their lives. Data on tolerance doses for Re-RT are scarce. However, prognostic factors predicting toxicities are

Table 17.4 Selection of interstitial brachytherapy studies for treatment of isolated neck nodal relapses in a previously irradiated volume [74]

Author	Patients	Neck treatment technique/ BKT-dose	Toxicity Grade III–IV	Results/comments
Kupfermann [7]	22	Surgery + ^{192}lr-LDR-BRT/ median 00 Gy (20–60 Gy)	27%	1 y OS = 82%, 2y OS = 57%, 5 y OS = 46%, 2y LC = 67%
Cornes [41]	39	Surgery + ^{192}lr-LDR-BKT/ mean 49.6 Gy	23%	2 y OS = 38%, 1 y LC = 63%
Pellizzon [50]	21	Surgery + ^{192}lr-HDR-BRT + EBRT/median 35 Gy (12–48 Gy)	19%	5 y OS = 50%, 8 y OS = 42.9%, 5 y LC = 42.5%, 8 y LC = 28.6%
Zelefsky [40]	100	(a) Surgery + LDR-BRT (51 implants)/mean 40 Gy (b) ^{125}l-LDR-seeds (66 implants)/MPD 171 Gy	(a) na (b) na	1 y OS = 44%, 2 y OS = 20%, 1 y LC = 65%, 2 y LC = 26%
Nutting [8]	72	Surgery + ^{192}lr-LDR-BRT/60 Gy	15%	2 y OS = 31%, 5 y OS = 23%, 2 y LC = 37%, 5 y LC = 23%
Bollet [5]	84	(a) ^{192}lr-LDR-BRT (n = 72)/mean 56.5 Gy (30–112 Gy) (b) ^{192}lr-LDR-BRT + EBRT (n = 12)/mean 38Gy (23.6–50 Gy)	30%	1 y OS = 33%, 2y OS = 13%, 3y OS = 6%, 1 y LC = 49%, 2 y LC = 31%, 3 y LC = 0%/7% Grade V toxicity
Housset [4]	23	Split course ^{192}lr-LDR-BRT/65 Gy	36%	1 y OS = 26%, 2 y OS = 13%/4% Grade V toxicity
Choo [1]	17	(a) ^{192}lr-LDR-BRT (n = 8)/ mean 57 Gy (50–60 Gy) (b) Surgery + ^{192}lr-LDR-BRT (n = 9)/40–60 Gy	(a) 25% (b) 11%	(a) 25% alive at 9 months (b) 33% alive at 10 months
Syed [21]	21	^{192}lr-LDR-BRT/50–60 Cy	19%	OS = 28% at 18 months, LC = 28% at 18 months/14% fatal necroses
Wibault [22]	164	(a) ^{192}lr LDR-BRT (n = 138)/mean 7000 rad (5000–7500 rad) (b) ^{192}lr-LDR-BRT + EBRT (n = 26)/mean 5250 rad	26%	Mean overall survival 8,8 months/11% Grade V toxicity
Kolotas [24]	49	^{192}lr-HDR-BRT/median 30 Cy (30–36 Cy)	4%	1 y OS = 52%, 2 y OS = 31%, 3 y OS = 6%
Present study	74	(a) ^{192}lr-HDR-BRT (n = 69)/median 30.0 Gy (10.0–36.0 Gy) (b) ^{192}lr-HDR-BRI + EBRT (n = 5)/median 30Gy (24.0–36.0 Gy)	13%	1 y OS = 42%, 2 y OS = 19%, 3 y OS = 6%, 1 y LC = 67%, 2 y LC = 67%, 3 y LC = 67%

LDR low dose rate; *HDR* high dose rate; *BRT* brachytherapy; *EBRT* external beam radiotherapy; *OS*. overall survival; *LC* local control; *^{192}lr* iridium-192; *^{125}l* iodine-125; *y* year; *MPD* matched peripheral dose; *na* not available

known and have been previously discussed in this chapter (18.1). Dionisi et al. conducted a pooled analysis of 39 studies comprising 3766 patients on the topic of treatment-related side effects and organ tolerances after Re-RT [75]. Studies included were mostly retrospective (n = 31, 79.5%) but also of randomized (n = 3, 7.7%) and prospective (n = 5, 12.8%) designs. Regarding the treatment modality, the analysis comprised heterogeneous treatment modalities as CRT (n = 10, 25.6%), IMRT (n = 26, 66.6%), SBRT (n = 5, 12.8%), and brachytherapy (n = 2, 5.1%). Data on acute toxicities could be analyzed from 38 studies. Grade 3 or higher acute toxicities were observed in 32% of the patients (n = 1193) with 0.9% treatment-related deaths (n = 37) due to neutropenia, fatal hemorrhages, and aspiration pneumonia. No difference in the rate of acute toxicities grade 3 or higher could be observed depending on radiation techniques, Re-RT, and cumulative dose or fractionation. Severe grade 3 or higher late toxicities were observed in 29.3% (95 CI [23.5–36.4%]) of the patients (Fig. 17.4). In the pooled analysis, the risk for treatment-related death was generally low (<5%), but some series reported rates >20%. A common cause of Re-RT-related deaths was fatal hemorrhage caused by a carotid blowout.

A model for prediction of grade 3 or higher toxicities after Re-RT has been developed by Ward et al. based on a retrospective study from nine institutions [50]. Patients (n = 505) received Re-RT with IMRT with a median dose of 66 Gy and outcomes were analyzed to generate a multivariable competing-risk model. A nomogram for a 2-year severe late toxicity prediction has been created, which can be integrated into the informed decision-making process of individual patients (Fig. 17.5). An additional aim was to assess whether the risk of late toxicities outweighs the risk of progression or death. Severe late toxicity with grade ≥ 3 had a 2-year incidence of 16.7% (95% CI 13.2–20.2%), while the risk for tumor progression or death was 64.2% (95% CI 59.7–68.8%). The risk of tumor progression or death is approximately four times higher than the risk of developing grade 3 or higher late toxicities after Re-RT, so ultimately, patients have to prioritize their needs based on this information.

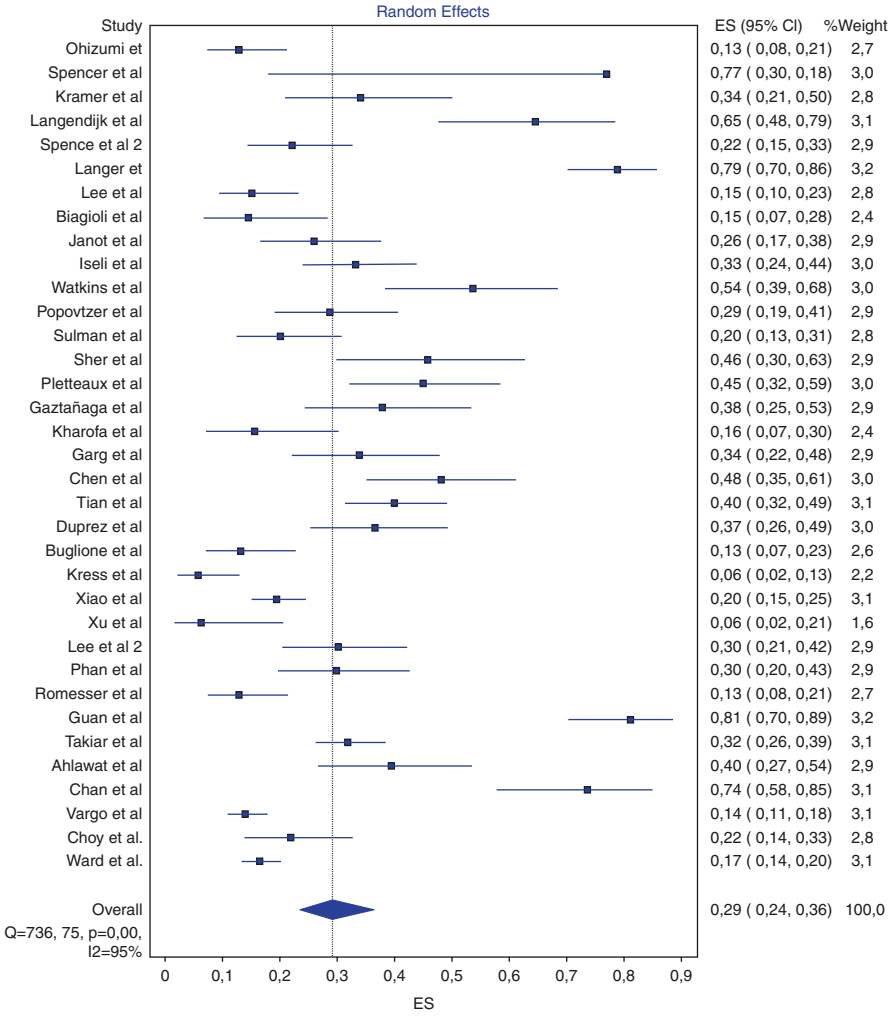

Fig. 17.4 Pooled analysis of 35 Re-RT studies on severe grade 3 or higher toxicities revealing an average rate of 29.3% and showing a general picture of expected toxicities after re-treatment [75]**.** Abbreviations: ES effect size

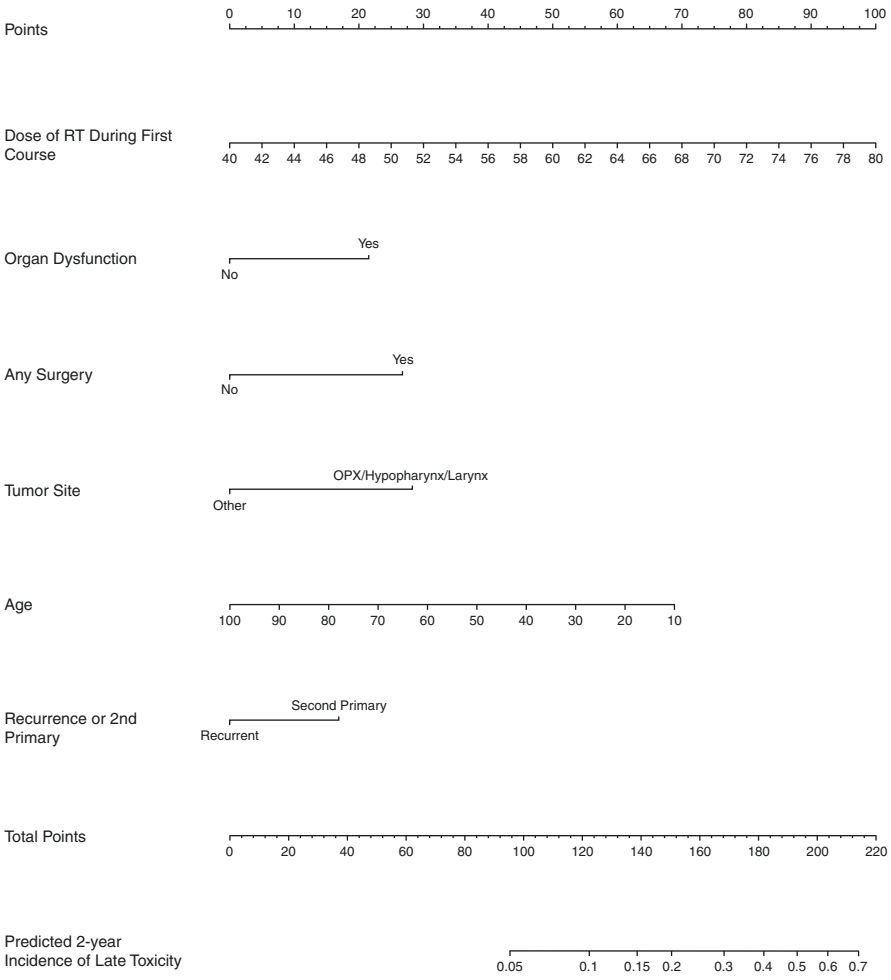

Fig. 17.5 Nomogram for prediction of grade 3 or higher late toxicities at 2 years after completion of Re-RT with IMRT [50]

Take Home Message for Toxicities of re-Irradiation

- There is a significant risk in the range of approximately 30% for developing severe grade 3 late radiation toxicities from Re-RT including treatment related death.
- However, the majority of patients will experience tumor progression or death before encountering severe sequels.
- Nomograms for prediction of severe late toxicity can be integrated into the patient education.

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Chapter 18
New and Promising Targeted Therapies in First and Second-Line Settings

Dylan F. Roden, Jennifer M. Johnson, Petr Szturz, Paolo Bossi, and Athanassios Argiris

Introduction

The increasing efficiency and decreasing cost of next generation DNA sequencing (NGS) has allowed for a better understanding of the complex molecular pathways that contribute to carcinogenesis [1]. Utilizing these improved techniques, cancer genomes can now be systematically studied. Unfortunately, separating the "driver" mutations responsible for carcinogenesis from the "passenger genes" is not straightforward, and the clinical relevance of certain mutations continues to be debated.

Several large scale projects across the globe have accomplished the characterization of cancer genomes. The Cancer Genome Atlas (TCGA) Program is a joint venture between the National Cancer Institute and the National Human Genome Research Institute representing 20 institutions across the US and Canada. Since its inception in 2006 it has molecularly characterized over 20,000 cancer genomes with matched normal samples spanning 33 cancer types. The International Cancer

D. F. Roden
Department of Otolaryngology- Head and Neck Surgery, New Jersey Medical School, Rutgers University, Newark, NJ, USA
e-mail: Dylan.Roden@Rutgers.edu

J. M. Johnson · A. Argiris (✉)
Department of Medical Oncology, Thomas Jefferson University, Philadelphia, PA, USA
e-mail: Jennifer.M.Johnson@Jefferson.edu; Athanassios.Argiris@Jefferson.edu

P. Szturz
Department of Oncology, Lausanne University Hospital (CHUV), Lausanne, Switzerland

P. Bossi
Department of Medical Oncology, University of Brescia, Brescia, Italy
e-mail: Paolo.Bossi@Unibs.it

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Genome Consortium and the COSMIC database have also helped to establish baseline mutational profiles in many cancer pathologies [2].

A genomic characterization of HNSCCwas published in *Nature* based on 279 tumor included in TCGA [3]. It showed high genomic instability with a mean copy number alteration of 141 and a relative paucity of gene fusion mutations that are implicated in other solid tumors [3]. A genomic difference in Human Papilloma Virus (HPV) negative and HPV positive tumors was seen, with most HPV negative tumors having loss of p53 (84%) and deletion of CDKN2A (58%) whereas HPV positive tumors more commonly had amplification mutations in PIK3CA (56%). Consistent with the effects of tobacco exposure, HPV negative tumors harbor a much larger number of chromosomal alterations and amplifications compared to HPV positive tumors [4].

HNSCC's multiple mutations in a genetically complex landscape makes it difficult for one targeted therapy to have sustained efficacy. Cancers defined by carcinogen-induced genomic chaos, such as UV-induced melanoma or tobacco-related HNSCC, are driven by a multitude of competing molecular pathways, and are thus some of the most challenging to address with targeted therapies [5]. Efficacy of targeted therapies may be confounded by the presence of coexisting tumor cell populations (clones), each with its own related but genetically distinct profile [6].

Nevertheless, potentially targetable (actionable) genomic alterations are constantly being discovered and investigated [7]. Current efforts are directed at understanding not just single gene alterations within tumor types but multi-gene expression signatures to identify functionally relevant and potentially actionable pathways. The incorporation of RNA sequencing and proteomic techniques may one day add another layer of complexity and understanding. As more tumors are sequenced, drug development expands, and our understanding of molecular pathways improves new targets and drugs will doubtless be identified. Even in the absence of actionable alterations, genetic analyses can produce a list of predictive biomarkers that can provide important prognostic information.

This review focuses on targeted therapies aimed at molecular pathways most frequently perturbed in HNSCC that have been investigated or are of potential interest in the treatment of recurrent/metastatic (R/M) HNSCC.

EGFR Pathway

EGFR is a member of the ErbB family of receptor tyrosine kinases that includes EGFR (ErbB-1), HER2/neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4). Activation of these receptors initiates a signal transduction cascade via two primary pathways: RAS/RAF/MEK/ERK (MAPK/ERK) and PI3K/AKT/mTOR. Through complex mechanisms reviewed elsewhere, perturbed activation leads to dysregulation of the cell cycle and decreasing apoptosis while increasing DNA synthesis and cellular proliferation, leading to uncontrolled growth.

EGFR is overexpressed in >90% of HNSCC tumors [8]. EGFR gene amplification or high polysomy is common, seen in 58% of HNSCC as evaluated by fluorescence in situ hybridization (FISH) [9]. Other studies report a 17% rate of increase copy number of EGFR [10]. Studies indicate that an increased copy number of EGFR correlates with poor prognosis [9, 10]. EGFR overexpression is linked to worse outcomes including shorter relapse-free and overall survival (OS) [11]. However, increased expression does not necessarily predict response to EGFR-directed therapy [12].

Targeted therapies against EGFR include monoclonal antibodies (mAb) that block the extracellular ligand-binding domain and tyrosine kinase inhibitors (TKIs) that prevent activation of these receptors within the cytoplasm. Cetuximab has been the most widely used targeted therapy in HNSCC. In addition to cetuximab, monoclonal antibodies targeting the ErbB pathway include panitumumab, zalutumumab, nimotuzumab, and trastuzumab whereas EGFR TKIs include gefitinib, erlotinib, lapatinib, and afatinib (Table 18.1).

Cetuximab

Cetuximab is a chimeric monoclonal antibody against EGFR. Until the introduction of immune checkpoint inhibitors cetuximab was the only molecularly targeted therapy with FDA approval for HNSCC. It was first approved for colorectal cancer in

Table 18.1 ErbB Pathway Targeted Therapies and FDA approval

Drug	Mechanism	Cancer Type	FDA approval
Cetuximab	EGFR Ab	HNSCC, colorectal	2006
Panitumumab	EGFR Ab	Colorectal	2006
Zalutumumab	EGFR Ab	–	None
Nimotuzumab	EGFR Ab	HNSCC	None *
Matuzumab	EGFR Ab	–	None
Trastuzumab	Her2/neu Ab	Breast	1998
Duligotuzumab	EGFR + HER3 Ab	–	None
Patritumumab	HER3 Ab	–	None
Dacomitinib	TKI against EGFR + HER2 + HER4	–	–
Gefitinib	TKI against EGFR (reversible binding)	NSCLC	2003–2005, 2015
Erlotinib	TKI against EGFR (reversible binding)	NSCLC, pancreatic	2004
Lapatinib	TKI against EGFR + Her2/neu (reversible binding)	Breast	2007
Afatinib	TKI against EGFR + Her2/neu + Her4 (irreversible binding)	NSCLC	2013

*approved for HNSCC in other countries

2004. In March 2006 cetuximab was approved to be used concomitantly with radiation in the definitive treatment of locally advanced HNSCC or as a single agent in patients who had failed previous platinum-based chemotherapy [13]. In 2011, it obtained approval for first-line use in the R/M setting in combination with chemotherapy. The EXTREME study investigated standard of care chemotherapy with cisplatin (or carboplatin) plus 5-fluorouracil with or without cetuximab. It demonstrated prolonged OS with the addition of cetuximab (10.1 months to 7.4 months, HR 0.8, p = 0.04) as well as improved progression-free survival (PFS) and objective response rate (ORR) [14]. In 2019, data was published from the RTOG 1016 trial comparing concurrent chemoradiation with cisplatin versus cetuximab in HPV-positive oropharyngeal carcinoma. After a median follow up of 4.5 years cetuximab did not meet the pre-specified non-inferiority OS endpoint. Five-year OS was significantly worse for the cetuximab arm 77.9% than for the cisplatin arm 84.6% (two-sided 95% CI 1.03–2.05,log rank p = 0.0163) [15]. The De-ESCALaTE phase III trial randomized HPV positive low risk oropharyngeal cancer patients (non-smokers or like time smokers with a smoking history of less than 10 pack years) to receive either cetuximab or cisplatin with radiation therapy. The primary outcome of overall severe toxicity events at 24 months did not differ significantly between the 2 groups (mean number of events per patient 4.8 with cisplatin versus 4.8 with cetuximab p = 0.49). Efficacy outcomes favored cisplatin: 2-year OS 97.5 vs 89.4% HR 5 (1.7–14.7) and 2-year recurrence 6% vs 16.1% HR 3.4 (1.6–7.2) [16]. Further development of cetuximab in HNSCC may be in combination regimens rather than alone as a radiation sensitizer. In R/M HNSCC, the combination of cetuximab with immunotherapy or other novel approaches remains of interest.

Other ErbB Antibodies

Panitumumab, another monoclonal antibody against EGFR, did not produce efficacy results sufficient to gain regulatory approval in HNSCC. Panitumumab had low single-agent activity in recurrent/metastatic HNSCC [17, 18], whereas its addition to cisplatin and 5-FU did not result in statistically significant OS benefit in a phase III trial (SPECTRUM). The phase III SPECTRUM trial was similar in design to the EXTREME trial with some notable differences: carboplatin was allowed in SPECTRUM only after renal impairment or neurologic toxicity, maintenance panitumumab was not mandatory, EXTREME was conducted in Europe while SPECTRUM was a global trial, and the minority of patients had undergone prior treatment for locally advanced HNSCC in EXTREME (38%) while they represented the majority in SPECTRUM (81%).The addition of panitumumab to cisplatin and fluorouracil improved PFS (median PFS 5.8 vs 4.6 months, HR 0.78, p = 0.004) but not OS (median OS 11.1 months versus 9.0 months, HR 0.873, p = 0.1403), which was the primary endpoint [19]. In subset analysis, panitumumab improved OS in patients with p16 negative tumors (11.7 vs 8.6 months, HR 0.73, p = 0.0115) [19].

TKIs against ErbB

Tyrosine Kinase Inhibitors (TKIs) against ErbB have been approved for other solid tumor types, but have had disappointing efficacy in HNSCC (erlotinib, gefitinib, lapatinib). Oral afatinib was compared to intravenous weekly methotrexate as second-line treatment for patients with recurrent/metastatic HNSCC after platinum-based therapy in two phase III clinical trials. In the global LUX-Head & Neck 1 study, there was a modest but significant improvement in median PFS favoring afatinib (2.6 vs 1.7 months, $p = 0.03$) that did not translate into an OS benefit [20]. Similar results were obtained in the subsequent LUX-Head & Neck 3 study that compared afatinib with methotrexate in Asian patients [21].

Future Research into ErbB Targeting

At present, there are no robust predictive biomarkers of response to ErbB targeted therapies in HNSCC [22]. Despite frequent overexpression of the receptor protein, mutations in the EGFR gene occur with low frequency (16% in HPV negative HNSCC according to TCGA) and sequencing of HNSCC tumors has not demonstrated recurrent EGFR mutations. As opposed to NSCLC where clusters of mutations within exons 18–21 (tyrosine kinase domain) are seen, the mutations in HNSCC are more dispersed [23]. This may potentially explain the modest benefit of ErbB targeted therapies seen in HNSCC as compared to NSCLC [5].

Another hypothesis for limited efficacy of these agents is the presence of EGFRvIII mutation that leads to constitutive activation of the receptor independent of ligand binding. These patients would not be responsive to ErbB targeted therapy. This mutation was previously reported to be as high as 42% in HNSCC, although more recent reports suggests this is uncommon (<1%) [3, 24]. This discrepancy may be attributable to difficulty in EGFRvIII detection using RNA sequencing [25].

Increased EGFR copy number is generally acknowledged as a poor prognosticator in HNSCC. In a post hoc analysis of the EXTREME trial, EGFR copy number was elevated in 40% of patients but was not found to be a predictive biomarker for the efficacy of cetuximab [12].

In colorectal cancer, RAS mutations are a predictive biomarker for cetuximab resistance, and wild type RAS status is confirmed prior to delivery of cetuximab for colorectal cancer [26]. In HNSCC RAS mutations are uncommon (4%) in cetuximab-naive HPV negative HNSCC. However, acquisition of RAS mutations during cetuximab treatment may be common and associated with progressive disease [27]. In addition, multiple other mechanisms of resistance to ErbB targeted therapies have been described, such as downstream mutations in the PI3K/AKT/mTOR pathway [28].

Given the lack of predictive biomarkers, choosing which patients will derive the most significant benefit from ErbB targeted therapy is difficult. Further research

into predictive biomarkers of cetuximab efficacy may help to appropriately select patients that can benefit from this agent in future trials. Cetuximab-mediated tumor immunogenicity through antibody-dependent cellular toxicity (ADCC) has led to enthusiasm with combined approaches involving immunotherapy, e.g. combination regimens with avelumab or monalizumab [29].

PI3K/AKT/mTOR Pathway

The PI3K/AKT/mTOR pathway is a critical intracellular cascade important in cell cycle regulation, proliferation, motility, and survival. It is the most frequently dysregulated pathways in HNSCC on both a genomic and proteomic level [3, 30]. HPV positive oropharyngeal squamous cell carcinoma (OPSCC) commonly has helical domain mutations in PIK3CA. PIK3CA is an oncogene that encodes one subunit of the enzyme PI3K, a protein kinase that phosphorylates many downstream signaling proteins including AKT. PTEN is a tumor suppressor gene that encodes the PTEN protein which is a phosphatase that antagonizes PI3K signaling.

mTOR inhibitors were the first agents in this pathway to be investigated. Rapamycin, everolimus and temsirolimus are non-selective inhibitors that demonstrated limited efficacy as solo agents with significant toxicity. Preclinical studies demonstrated that mTOR inhibitors may overcome resistance to EGFR blockade and improve the efficacy of ErbB pathway agents [29]. A phase II study of temsirolimus and erlotinib for platinum-refractory R/M HNSCC was closed early because 6 out of 12 patients withdrew within 6 weeks due to toxicity or death [31]. Another phase II study of everolimus plus erlotinib failed to demonstrate a benefit in platinum-resistant R/M HNSCC despite a reasonable toxicity profile [32]. A multicenter randomized phase II study of temsirolimus with or without cetuximab randomized 80 patients with R/M HNSCC who failed a previous EGFR-based therapy (MAESTROHN, NCT01256385). There was no difference in the primary outcome of mPFS (3.5 vs 3.5 months) [33].

Buparlisib is a pan-class 1 phosphoinositide 3-kinase (PI3K) inhibitor. In a multicenter, double blind, placebo controlled phase II trial of 158 patients (BERIL-1), buparlisib + paclitaxel was superior to paclitaxel alone in the 2nd line treatment of R/M HNSCC (median PFS 4.6 vs 3.5 months, HR 0.65, one sided $p = 0.01$) [34]. There was also a benefit in the secondary endpoint of OS (10.4 vs 6.5 months, one-sided $p = 0.04$). This trial demonstrated that response to buparlisib was not contingent on dysregulation of the PI3K/AKT/mTOR pathway via PIK3CA mutations or loss of PTEN expression (both of which were infrequent 1–13%). Thus, a phase II trial of buparlisib monotherapy was conducted in patients with refractory HNSCC who had progressed after both platinum and cetuximab. Patients were enrolled in parallel cohorts based on the presence or absence of PIK3CA mutations in exons 9 or 20. The PIK3CA mutated cohort was prematurely closed due to slow accrual and limited activity was seen in either group (median PFS 1.8 months for mutated and

1.7 months for nonmutated cohorts) [35]. A phase III trial is underway evaluating buparlisib plus paclitaxel versus paclitaxel alone for R/M HNSCC who have progressed on platinum-based chemotherapy with or without prior anti-PD1/PDL1 treatment (BURAN study). Based on this trial, this agent may potentially become a treatment of choice for those who do not respond to immunotherapy.

PX-866 is another oral, irreversible, pan-isoform inhibitor of PI3K that has been investigated in separate phase II clinical trials in combination with docetaxel or cetuximab [36, 37]. These trials have not yielded promising results so far.

Cell Cycle Regulation

Alterations in cell cycle regulatory mechanisms are common in HNSCC, especially in HPV negative tumors. Cyclin-Dependent Kinases (CDK) help regulate progression through the cell cycle. Mutations in TP53 (the most commonly mutated gene in HNSCC), CCND1 amplification, CDKN2A deletion, and p16 inactivation enable evasion of typical mitotic checkpoints. Aberrant cyclin D-dependent kinase activation leads to unregulated cell proliferation. Oral CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) have been evaluated in several phase 1 trials. These agents are under investigation as monotherapy or in combination with other drugs such as cetuximab or gedatolisib (PI3K/mTOR inhibitor).

A phase I trial of palbociclib in combination with cetuximab demonstrated an acceptable toxicity profile with no dose limiting toxicities and 5/9 patients showing measurable decreases in tumor target lesions [38]. A subsequent phase II trial (PALATINUS, NCT02499120) evaluated palbociclib + cetuximab and placebo + cetuximab in R/M HPV negative HNSCC. The study did not meet its primary endpoint. The median OS was 9.7 with palbociclib vs 7.8 months with placebo ($p = 0.18$). There was no difference in PFS (median PFS 3.9 vs 4.6 months, $p = 0.5$), but there were more hematologic adverse events with the addition of palbociclib [39]. Currently palbociclib is only approved for HR-positive, HER2-negative breast cancer.

Prexasertib (LY2606368) is a small molecule checkpoint kinase inhibitor against Chk1/2 which is involved in the S-G2 phase checkpoint. In a phase I trial that investigated this agent as monotherapy in 45 patients with advanced or metastatic nonhematologic cancerof whom 5 had HNSCC, there were 2 partial responses, one of which was in a patient with HNSCC [40]. There were 7 dose-limiting toxicities, all hematologic, most often transient grade 4 neutropenia. This prompted further evaluation in advanced squamous cell carcinomas [41]. Out of the 101 patients enrolled 57 had HNSCC. Median PFS was only 1.6 months for this group though there were 3 patients with a partial response. Later trials of prexasertib with chemotherapy and radiation were terminated (NCT02555644). Patient selection for CDKN2A/p16 loss may offer a route for further exploration of this target [42].

DNA Repair Inhibitors

DNA damage repair (DDR) inhibitors are medications that interfere with DNA repair mechanisms. These medications have been investigated as monotherapy, in combination with other cytotoxic chemotherapeutics (cisplatin), and with RT. This combination is mechanistically logical, as the initiation of DNA damage by these traditional treatments may have a more profound impact on tumor death when the repair of their damage is prevented by DDR inhibitors. As opposed to many other targeted therapies that have been used in the R/M setting, some of these agents have been investigated in the definitive setting.

Poly (ADP-ribose) polymerase (PARP) is an enzyme involved in the repair of single-stranded DNA breaks. PARP inhibitors are a class of medications that prevent the repair of this form of DNA damage. If cells with unrepaired single strand breaks proceed through mitosis, double strand DNA breaks develop, which can lead to cell death. Olaparib (AZD2281) was the first PARP inhibitor approved by the FDA in December 2014 for germline BRCA mutated ovarian cancer who had failed 3 previous chemotherapies. Rucaparib, niraparib, and talazoparib have more recently been granted FDA approval for other tumor types. No PARP inhibitors have approvals in HNSCC. Olaparib was combined with cetuximab and RT in a phase 1 trial for definitive treatment of locoregionally advanced inoperable HNSCC in smokers, and is in trials in combination with cisplatin plus RT (NCT02308072, ORCA-2) or olaparib and RT alone (NCT02229656) for definitive treatment [43].

ATM and ATR are protein kinases involved in the recognition and repair of double strand DNA breaks. ATM plays a crucial role in the G1/S cell cycle checkpoint as well as intra-S phase checkpoint. Downstream targets of ATM include CHK2 and p53 [44]. ATR is activated by single strand DNA structures that may arise at resected DNA double strand breaks or at stalled replication forks. ATR is the principal mediator of the G2/M cell cycle checkpoint as well as the intra-S phase checkpoint. Downstream targets of ATR include CHK1. Both ATM and ATR inhibitors are in clinical development: ATM- KU559403, KU60019, and KU55933 and ATR- VX970 also known as M6620, VE821, VE822, and AZD6738 also known as ceralasertib. These agents have sensitized tumor cells to radiation *in vitro*, but there is limited data on their efficacy *in vivo* [42, 45, 46]. AZD6738 was combined with the PD-L1 inhibitor durvalumab in a multicohort trial. Twenty five patients with either non-small cell lung cancer or HNSCC were enrolled in the trial and 1 response was seen in a HNSCC patient [47]. A phase 1 trial of the ATR inhibitor VX970 also known as M6620 in combination with cisplatin and radiation is currently underway enrolling clinical stage III or IV HNSCC (NCT02567422).

WEE1 is a tyrosine kinase involved in the phosphorylation and inactivation of cyclin dependent kinase 1 (CDK1) – bound cyclin B which results in G2 cycle arrest. AZD1775 is a WEE1 inhibitor hypothesized to target p53-mutant tumors being investigated in NCT01748825. *In vivo* assays have shown WEE1 inhibitor sensitizes head and neck cancer cells to NK cell lysis, potentially indicating a future role for combination with immunotherapy [48].

DDR inhibitors are under study as part of combination therapies in the definitive setting. Moreover, novel combinations of DDR inhibitors with immunotherapy are of interest. Future trials may bring DDR inhibitors to the forefront of HNSCC treatment.

Antiangiogenesis

Vascular endothelial growth factor (VEGF) and its tyrosine kinase receptors are involved in angiogenesis and proliferation. Treatments against this pathway include both antibodies against VEGF as well as tyrosine kinase inhibitors against VEGFR and are summarized in Table 18.2. Tumor VEGF overexpression is common in HNSCC and is an independent negative prognostic factor for survival in locoregionally advanced HNSCC [49, 50]. Unfortunately, investigation into this class of agents for HNSCC has demonstrated limited efficacy with considerable toxicity. Mutations in the VEGF pathway have prognostic relevance, but are not predictive with regard to response to therapy.

There are currently multiple anti-angiogenic agents that have been approved by the FDA (Table 18.2). These range from ligand-directed antibodies to receptor-directed antibodies to small molecule inhibitors to immunomodulatory agents. Research in HNSCC has focused on monoclonal antibodies and tyrosine kinase inhibitors (TKIs). Development of these antibodies and TKIs has occurred through monotherapy as well as though combinations with other modalities and therapeutic agents: chemotherapy, radiotherapy, molecularly targeted therapy, and more recently, immunotherapy.

Table 18.2 Selected FDA approved anti-angiogenic agents for the treatment of solid tumors

Agent	Molecular Targets
Monoclonal antibodies	
Bevacizumab	VEGF
Ramucirumab	VEGFR2
Fusion protein	
Ziv-Aflibercept	VEGF, VEGF-B, PlGF
Multi-kinase inhibitors	
Sorafenib	RAF/MEK/ERK, VEGFR 1–3, PDGFR- β, c-KIT, FLT3, RET
Sunitinib	VEGFR1 and 2, PDGFR-α and -β, c-KIT, RET, CSF1R, FLT3
Vandetanib	VEGFR2 and 3, EGFR, RET
Pazopanib	VEGFR 1–3, PDGFR-α and -β, FGFR-1 and − 3, c-KIT
Axitinib	VEGFR 1–3, PDGFR-α and -β, c-KIT
Regorafenib	VEGFR1–3
Lenvatinib	VEGR1–3, FGFR1–4, PDGFR- α, c-KIT, RET
Cabozatinib	VEGFR2, AXL, RET, MET, c-KIT, FLT-3

When used as monotherapy in previously treated R/M HNSCC overall responses have been disappointing [51–53]. When used in combination with other therapies, such as cetuximab plus sorafenib or docetaxel plus vandetanib, there was no additional benefit in phase II randomized trials [54, 55], however, two single arm trials (one with cetuximab plus pazopanib and another with carboplatin, paclitaxel plus sorafenib) reported promising results [62, 63] (Table 18.3).

Bevacizumab is an antibody against VEGF-A that has been studied in the definitive setting as well as in combination with chemotherapy in R/M HNSCC (Table 18.4). In the definitive setting, the addition of bevacizumab to cetuximab, pemetrexed, and RT did not demonstrate any additional survival or disease control benefit, but did have more hemorrhagic complications [64].

E1305 was a phase III randomized trial that investigated the addition of bevacizumab to platinum doublet therapy as first-line treatment in patients with R/M

Table 18.3 VEGFR tyrosine-kinase inhibitors studied as monotherapy or combination therapy in HNSCC

Agents	Study Design/ Phase	N	Response Rate	mPFS (months)	mOS (months)	Reference
Sorafenib 400 mg BID	Single arm, phase II	27	4%	1.8*	4.2	Elser, 2007 [56]
Sorafenib 400 mg BID	Single arm, phase II	41	2%	4	9	Williamson, 2010 [57]
Sorafenib 400 mg BID	Single arm, phase II	23	38%**	3.4	8	Lalami, 2016 [52]
Sunitinib 37.5 mg QD	Single arm, phase II	38	3	2	3.4	Machiels, 2010 [58]
Sunitinib 50 mg QD 4/6 weeks	Single arm, phase II Cohort A: PS 0–1 cohort B: PS 2	22	A: 8% B: 0%	A:2 * B: 2.5*	A: 4.9 B: 4.5	Choong, 2010 [59]
Sunitinib 50 mg QD 4/6 weeks	Single arm, phase II	17	0	2.3*	4	Fountzilas, 2010 [60]
Axitinib 5 mg BID	Single arm, phase II	30	7%	3.7	10.9	Swiecicki, 2015 [53]
Semaxinib 145 mg/m^2 twice per week	Single arm, phase II	35	3%		6.25	Fury, 2007 [61]
Cetuximab + Pazopanib	Single arm, phase I	31	35%	5.3	9.5	Adkins, 2019 [62]
Cetuximab +/− sorafenib	Randomized, phase II	55	8% v 8%	3 v 3.2	9 v 5.7	Gilbert, 2015 [54]
Docetaxel +/− vandetanib	Randomized, phase II	29	7% v 13%	0.75 v 2.1	6.3 v 5.6	Limaye, 2013 [55]
Carboplatin, paclitaxel, sorafenib	Single arm, phase II	48	55%	8.5	22.6	Blumenschein, 2012 [63]

**TTP* time to progression; **Metabolic response rate by Fluorodeoxyglucose Positron Emission Tomography; *BID* bis in die (two times per day); *QD* quaque die (one a day); *mPFS* median Progression-Free Survival; *mOS* median Overall Survival, *PS* Performance Status

Table 18.4 Bevacizumab-containing combination therapies in R/M HNSCC

Agents Combined with Bevacizumab	Study Design	Phase	N	Primary Efficacy Endpoint	Reference
Chemotherapy					
Pemetrexed + bevacizumab	Single arm	II	47	mTTP 5 months	Argiris, 2011 [65]
Investigator's choice: Cisplatin+5-FU, Cisplatin+docetaxel, Carboplatin+5-FU, or Carboplatin+ docetaxel +/− Bevacizumab	Randomized	III	403	mOS 12.6 months with bevacizumab 11 months without bevacizumab (p = 0.13)	Argiris, 2019 [66]
EGFR inhibitor					
Cetuximab + bevacizumab	Single arm	II	46	RR 16%	Argiris, 2013 [67]
Erlotinib + bevacizumab	Single arm	I/II	10 / 48	mPFS 4.1 months	Cohen, 2009 [68]

mTTP median Time To Progression, *mOS* median Overall Survival, *RR* overall Response Pate, *mPFS* median Progression-Free Survival

HNSCC. A total of 403 patients were enrolled in multiple centers. With the addition of bevacizumab there was an improvement in objective response rate (36% vs 25%, p = 0.01) and PFS (median PFS 6.1 months with bevacizumab vs 4.4 months without bevacizumab, p = 0.001) but not OS (median OS 12.6 months with bevacizumab vs 11 months without bevacizumab, p = 0.13), which was the primary endpoint [66]. Although the primary endpoint of the study was not met, there was a numerical overall survival advantage at 2, 3 and 4 years in the bevacizumab arm (25% vs 18% at 2 years, 16% vs 10% at 3 years, and 12% vs 6% at 4 years for chemotherapy plus bevacizumab versus chemotherapy alone). Patients experienced more treatment-related toxicities with bevacizumab, particularly grade 3–5 bleeding. While this study provided evidence of improved antitumor activity with the addition of an anti-angiogenic agent to chemotherapy, no randomized trials have shown survival benefit with this approach in HNSCC. Studies with better-tolerated anti-angiogenic agents in combination with chemotherapy or other targeted agents should be considered. It is likely that better patient selection based on molecular biomarkers will optimize outcomes. Moreover, combination of anti-angiogenic agents with immunotherapy could improve anti-tumor efficacy due to synergist effects on the immune response. A randomized phase III trial of pembrolizumab with or without lenvatinib as first-line treatment for R/M HNSCC is currently ongoing (ClinicalTrials.gov Identifier: NCT04199104).

RAS-RAF-MEK-ERK Pathway

The RAS-RAF-MEK-ERK pathway (synonymous with MAPK/ERK pathway) is a mitogenic signal transduction cascade that leads to progression through the cell cycle and mitosis. The RAS family of genes (HRAS, KRAS, NRAS) encode

GTPase proteins that are involved in cellular signal transduction leading to cell growth, differentiation, and survival. RAS signals upstream of the PI3K and MAPK pathways. In human cancers RAS are commonly mutated oncogenes. However, in HNSCC RAS is mutated in only 4–6% of tumors [69, 70]. RAS mutations are associated with high levels of EGFR resistance (EGFR acts upstream of RAS). *In vitro*, PI3K inhibitors (which act downstream of RAS) have demonstrated efficacy in HRAS mutant HNSCC [69].

Tipifarnib is a farnesyltransferase inhibitor. This enzyme catalyzes the binding of farnesyl groups to RAS proteins, enabling them to localize to the cell membrane where they can exert their oncogenic effects. A phase II trial of tipifarnib in patients with HRAS mutant HNSCC reported partial responses in 9/18 evaluable patients (objective response rate of 50%) [71–73]. The median duration of response was 14.7 months and the median PFS was 5.9 months. Interestingly, the enrolled patients had an estimated median PFS of 2.8 months on the prior line of therapy. Additional studies are ongoing with this agent as monotherapy and in combination with chemotherapy.

Agents also in this pathway include dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) which have been most commonly used in BRAF mutant melanoma. BRAF mutations are not common in HNSCC, as low as 3% [74] but have proven to be highly targetable including in cancers traditionally refractory to other treatments [75]. The specific efficacy of these agents in HNSCC is as yet unproven.

FGFR

Targeting the Fibroblast Growth Factor Receptor (FGFR) pathway has very recently been generating enthusiasm in several cancer types, including HNSCC. FGFR is made up of five isoforms, FGFR1–4 being RTKs and FGFR5 lacking an intracellular domain. Downstream signaling of FGFR occurs through several pathways including MAPK/ERK, PI3K/AKT/mTOR, PLCγ, and STAT leading to proliferation, survival, angiogenesis, and migration [76]. Rogaratinib is an adenosine triphosphate (ATP) competitive inhibitor of FGFR 1–4 [77]. Erdafitinib, another pan-FGFR inhibitor, has been used to treat urothelial cancer, where FGFR mutations are present in 32% [78].

FGFR1 mutations are present in about 5–10% of HPV negative HNSCC while FGFR3 are present in 1–12% HPV positive HNSCC. Bayer, the manufacturer of the rogaratinib, recommends using a mRNA based FGFR assay (RNA scope) to preselect patients that may derive benefit. This assay is being used to determine eligibility for rogaratinib treatment in the EORTC UPSTREAM trial, discussed later. FGFR1–3 mRNA positivity was found in 56.5% of a cohort of 46 HNSCC patients [79]. However, patients with high mRNA levels do not necessarily have genetic FGFR alterations [80]. There is preclinical data that demonstrates that FGFR signaling may mediate cisplatin resistance in HNSCC [81].

Neurotrophic Tyrosine Kinase Receptor Family

The Neurotrophic Tyrosine Kinase Receptor (NTRK) family is synonymous with Tropomyosin receptor kinase (Trk). The NTRK1 gene encodes the Tropomyosin receptor kinase A (TrkA) which binds neurotrophin (nerve growth factor). This signaling pathway is important for neuronal differentiation and avoidance of programmed cell death. Tropomyosin-related kinase B (TrkB) serves as a receptor for brain-derived neurotrophic factor (BDNF) and for neurotrophic factor 4 (NT4), and has been found to be a potentially important mediator of the invasive properties of HNSCC and a mediator of the epithelial-mesenchymal transition (EMT). In particular, TrkB and BDNF are expressed in >50% of HNSCC tumors, and stimulation of this pathway increases the migratory and invasive properties of HNSCC [82]. The BDNF-TrkB signaling pathway has been implicated in platinum resistance in HNSCC [83].

It is now appreciated that fusions of NTRK1, NTRK2, and NTRK3 represent oncogenic alterations in multiple tumor types. The FDA granted accelerated approval for larotrectinib, an oral TRK inhibitor, in November 2018 for patients with solid tumors harboring an NTRK gene fusion. This approval was based on pooled results of three trials (LOXO-TRK-14001, SCOUT, and NAVIGATE) that included a combined 55 adults and pediatric patients with NTRK gene fusions [84]. The associated cancers spanned many pathologies including soft tissue sarcoma (20%), salivary gland cancer (22%), infantile fibrosarcoma (13%), thyroid cancer (9%), lung cancer, melanoma, colon cancer, gastrointestinal stromal tumor, appendix cancer, breast cancer and pancreatic cancer. Results from this pooled analysis showed a 75% overall response rate, a 22% complete response rate and a 53% partial response rate across these various tumor types. This approval was unique in that it was the second histology agnostic approval ever granted by the FDA, and the first ever for a specific genomic aberration. The first histology agnostic approval was pembrolizumab in May 2017.

Entrectinib is another exciting agent in this class [85]. In a pooled analysis integrating data from three ongoing phase 1 or 2 clinical trials (ALKA-372-001, STARTRK-1 and STARTRK-2) 57% of patients had an objective response, 7% of which was a complete response. LOXO-195 and TPX-0005 are 2nd generation TRK inhibitors that are being investigated in patients who have developed resistance to other TRK therapies [86].

These agents are unlikely to have significant impact in HNSCC, as NTRK fusion mutations are rare in HNSCC. However, the development of this targeted therapy for this specific genomic aberration, its markedly profound efficacy, as well as the approval process and indication represents an exciting precedent for future drug development and clinical trial design.

Implications for Clinical Trial Design

Clinical trial design has adapted to the changing landscape of cancer genomics. The disappointing efficacy of targeted therapies in HNSCC may be due to the lack of molecular selection. Refinement in research strategy may lead to improved outcomes. Basket trials include patients from multiple different cancer pathologies and organ systems that are all united by a common mutation. These trials test the effect of one targeted therapy designed to counteract this specific mutation that is shared by all eligible patients, such as the larotrectinib in LOXO-TRK-14001 trial. These studies greatly increase the number of patients who are able to receive and potentially benefit from new drugs. Umbrella trials, on the other hand, include patients all of the same tumor type (i.e. HNSCC). Patients are screened for genomic aberrations, and may be eligible for different treatment arms of the study depending on the genetic profile of their tumor. Umbrella trials are designed to test the impact of different drugs on different mutations in a single type of cancer. This strategy allows for biomarker enrichment in each study arm. Finally, "super umbrella" trials are umbrella trials that include patients with multiple histologies.

The National Cancer Institute's (NCI's) Molecular Analysis for Therapy Choice (MATCH) (NCT02465060) initiative is an ongoing phase II super umbrella trial. This tissue of origin agnostic trial has 35 possible treatment subprotocols based on the genetic abnormality specific to a patient's tumor. New subprotocols can be added as targets and drugs become available. The American Society of Clinical Oncology (ASCO) has a likeminded trial entitled Targeted Agent and Profiling Utilization Registry (TAPUR). This nonrandomized trial is also openly recruiting and uses molecular profile testing to decide which FDA-approved targeted therapy may provide clinical benefit to patients who have failed standard first-line treatment. These trials can help enroll large number of patients to assess efficacy as well as develop hypotheses for future clinical trials.

EORTC 1559 (UPSTREAM) is the first European biomarker driven umbrella trial in R/M HNSCC which opened in December, 2017 [61]. This trial enrolls patients with R/M SCC progressing after first-line platinum-based chemotherapy. Patients are tested for 13 oncogenes and tumor suppressor genes: EGFR, HER2, TP53, PIK3CA, CCND1, NRAS, KRAS, HRAS, PTEN, FGFR1, FGFR2, FGFR3, and cMET. Based on the molecular alterations identified in the tumor, patients may be eligible for one of six different biomarker driven treatment cohorts. They may be eligible for targeted therapies including afatinib (ErB TKI), palbociclib (CDK 4/6 inhibitor), niraparib (PARP inhibitor), or rogaratinib (FGFR inhibitor). In patients without any actionable mutation, they are enrolled in an immunotherapy cohort (monalizumab ± durvalumab). Upfront selection of patients/tumors with actionable targets and matching them with the appropriate targeted therapies may improve patients' outcomes. This strategy of designing trials with molecularly enriched patient populations will hopefully demonstrate improved efficacy for molecularly targeted therapies.

In November 2017 the Centers for Medicare and Medicaid Services (CMS) in the US released a position statement on Next Generation Sequencing (NGS). NGS will be covered by insurance as a diagnostic laboratory test for patients with recurrent, metastatic, or advanced stage IV cancer who are seeking further cancer treatment. There are several predefined reporting and registry criteria that both the test and the testing center must adhere to in order to receive payment for NGS. This decision helps push forward a major shift in the exploration of further treatment for patients who may have limited and/or disappointing treatment options. NGS enables a more in depth understanding of specific drivers of a patient's cancer, and allows for opportunities to employ targeted therapies directed at these mutated pathways.

Even when an actionable mutation is discovered, treatment response is often seen for a finite amount of time. Cancers are heterogeneous populations of cells and may evolve under pressures of drug treatment [6]. Testing and retesting of the genomic composition of refractory cancers will be necessary in order to understand how and why resistance mechanisms to targeted therapies develop. When discordant treatment responses are seen in different metastatic lesions within the same patient, biopsies can reveal a different genetic make-up in these separate tumors. New strategies, and in some cases common pathway dual inhibitor therapies, will need to be engineered in order to best prevent mutational escape.

Conclusions

The molecular landscape of HNSCC is complex and has yielded relatively few targetable mutations. Our current understanding has led to clinical investigation of several agents targeting EGFR, PI3K, VEGF/VEGFR, RAS and other pathways with variable success. Careful patient selection may provide a path forward. Recent successes with tipifarnib monotherapy in selected patients harboring HRAS mutant tumors as well as the combination of buparlisib plus paclitaxel in unselected tumors underscore that the study of novel targets, targeted agents, and biomarkers must continue in HNSCC.

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Chapter 19
Update of Immune Therapies in Recurrent/ Metastatic Head and Neck Cancer

Danny Rischin

Background: Prior to the Emergence of Immune Therapies

It is now over 30 years since single agent cisplatin was demonstrated to be active in recurrent/metastatic mucosal head and neck squamous cell carcinoma (R/M HNSCC) with trials suggesting improved survival [1, 2]. However, subsequent progress was slow, with the widely accepted use of platinum-based doublets shown to increase response rates without impacting on survival [3, 4]. The Extreme trial was a significant advance with the addition of the anti-EGFR monoclonal antibody, cetuximab to platinum and 5-Fluorouracil improving overall survival compared to chemotherapy alone [5]. The median overall survival improved from 7.4 months in the chemotherapy-alone arm to 10.1 months in the arm that received chemotherapy plus cetuximab (hazard ratio, 0.80; 95% confidence interval, 0.64 to 0.99; P = 0.04). Based on these results, the Extreme regimen was approved in many jurisdictions and became the standard of care for first-line treatment of R/M HNSCC. No treatment had been shown to improve survival in the second-line or beyond R/M HNSCC setting.

Emergence of Immune Therapies in HNSCC

Monoclonal antibodies directed against the PD-1 or PD-L1 receptors have transformed the treatment of many cancers, after initial success in melanoma. The first major report in R/M HNSCC was at the Annual Meeting of the American Society of

D. Rischin (✉)
Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia

Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, Australia
e-mail: danny.rischin@petermac.org

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Oncology in 2014 where the results of the head and neck cohort of Keynote 012 treated with the anti-PD1, pembrolizumab were reported. The key findings have held up over time: the response rate was 18%, responses were durable, similar activity was seen in patients with HPV positive and negative tumours and the response rate in patients with PD-L1 positive tumours was higher [6]. Over the last 5 years we have seen unprecedented clinical trial activity in HNSCC, with the role of immune checkpoint inhibitors as part of standard of care established.

Second-Line Randomised Trials of Immune Checkpoint Inhibitors

Three immune checkpoint inhibitors have been tested in randomised phase 3 trials. (see Table 19.1). The first trial was the Checkmate 141 that compared nivolumab 3 mg/kg 2-weekly to investigators choice of standard of care (methotrexate, docetaxel or cetuximab) in a 2:1 randomisation [7]. Eligibility included R/M HNSCC oral cavity, pharynx or larynx, progression <6 months after last dose of platinum and no limit on prior lines of therapy. The median overall survival (OS) improved from 5.1 months to 7.5 months with a hazard ratio of 0.68, P = 0.01. 2-year survival improved from 6.0% to 16.9% [8]. Nivolumab delayed time to deterioration in patient reported quality of life outcomes compared to standard of care [9]. Based on the results of this trial nivolumab was approved throughout the world for use in platinum refractory patients. In a similarly designed trial of pembrolizumab compared to standard of care, Keynote 040, median OS improved from 6.9 months to 8.4 months with a hazard ratio of 0.80 [10]. Pembrolizumab was initially approved in the US based on Keynote-012 and later in Europe based on Keynote-040 restricted to patients with PD-L1 Tumour Proportion Score (TPS) ≥ 50%. In contrast to the nivolumab and pembrolizumab trials, the anti-PD-L1 durvalumab did not meet its primary endpoint when compared to standard of care in a phase 3 trial [11].

Table 19.1 Randomised trials of immune checkpoint inhibitors in ≥2nd-line recurrent/metastatic HNSCC

Trial	Anti-PD1/ anti-PD-L1	Control arm	Hazard ratio (95%CI) Overall survival	2 year survival	Median OS (months)
Checkmate 141 [7, 8]	Nivolumab	Methotrexate, docetaxel or cetuximab	0.68 (0.54–0.86), P = 0.01	16.9% v 6.0%	7.5 v 5.1
Keynote-040 [10]	Pembrolizumab	Methotrexate, docetaxel or cetuximab	0.80 (0.65–0.98), nominal P = 0.016		8.4 v 6.9
Eagle[11]	Durvalumab	Methotrexate, taxane, fluoropyrimidine or cetuximab	0.88 (0.72–1.08), P = 0.20	18.4% v 10.3%	7.6 v 8.3

First-Line Randomised Trials of Immune Checkpoint Inhibitors

The Keynote-048 trial evaluated the role of pembrolizumab alone (200 mg 3 weekly) or in combination with platinum-5-FU chemotherapy compared to the standard of care, the Extreme regimen of platinum, 5FU and cetuximab [12]. The rationale for combining chemotherapy with immunotherapy included possible disruption of the tumour architecture that might overcome tumour exclusion, enhancement of antigen shedding and more rapid control than immunotherapy alone. In both chemotherapy arms a maximum of 6 cycles of chemotherapy was administered, but patients could stay on pembrolizumab for up to 35 cycles and could continue with cetuximab. Key eligibility criteria included SCC of the oropharynx, oral cavity, hypopharynx and larynx, no prior systemic therapy for R/M disease, > 6 months since completing chemoradiation, ECOG performance status 0–1, tissue sample for PD-L1 assessment available and known p16 status if oropharyngeal primary.

The primary study endpoints were overall and progression-free survival (PFS) in the PD-L1 combined positive score (CPS) ≥ 20, CPS ≥ 1 and the total populations. Secondary endpoints included PFS at 6 and 12 months, response rate, quality of life and safety. Duration of response was an exploratory endpoint. The trial had a complex statistical design that allowed for several hypotheses about OS and PFS to be determined in parallel first either in the CPS ≥ 20 or total population. Subsequent testing in other populations e.g., CPS ≥ 1 only took place if the first hypothesis was positive. The pre-specified analysis plan allowed alpha from successful hypotheses to be passed to other hypotheses.

882 patients were randomised in <2 years from 206 sites in 37 countries. The arms were well balanced for baseline characteristics. The median age was approximately 61, > 80% were male, and 21% had p16 positive oropharyngeal cancer. With regard to PD-L1 status approximately 22% had TPS ≥ 50%. 40–45% had CPS ≥ 20, 85% had CPS ≥ 1.

Pembrolizumab when compared to Extreme improved OS in the CPS ≥ 20 and CPS ≥ 1 populations, and was non-inferior in the total population (Table 19.2). In the CPS ≥ 20 population the HR was 0.61 (95%CI 0.45–0.83, p = 0.0007), with medians of 14.9 versus 10.7 months and 2 year survival rates of 38.3% versus 22.1%. In the CPS ≥ 1 population the HR was 0.78 (95%CI 0.64–0.96, p = 0.0086), with medians of 12.3 versus 10.3 months and 2 year survival rates of 30.2% versus 18.6%. In the total population the HR was 0.83 (95%CI 0.70–0.99, p = 0.0199 which did not meet the superiority threshold for statistical significance), with medians of 11.5 versus 10.7 months and 2 year survival rates of 19.7% versus 10.0%. The progression-free survival curves crossed with more early progressions in the pembrolizumab arm. The response rate for the CPS ≥ 20 was 23.3% and for the CPS ≥ 1 it was 19.1%, while in the Extreme arm it was approximately 35%. The duration of response was markedly prolonged in the pembrolizumab arm, median 22.6 months versus 4.2 months. The safety profile was favourable for the pembrolizumab monotherapy arm when comparing treatment-related adverse events with

Table 19.2 Keynote-048: Overall Survival

		Hazard ratio (95% CI)	2 year survival (months) pembro arm versus Extreme	Median OS (months) pembro arm versus Extreme
Pembrolizumab versus Extreme[a]				
	PD-L1 CPS[b] ≥ 20	0.61 (0.45–0.83); p = 0.0007	38.3% v 22.1%	14.9 v 10.7
	PD-L1 CPS ≥ 1	0.78 (0.64–0.96), P = 0.0086	30.2% v 18.6%	12.3 v 10.3
	Total population	0.83 (0.70–0.99), P = 0.0199[c]	27.0% v 18.8%	11.5 v 10.7
Pembrolizumab + platinum/5FU Versus extreme				
	PD-L1 CPS ≥ 20	0.60 (0.45–0.80), P = 0.0004	35.4% v 19.4%	14.7 v 11.0
	PD-L1 CPS ≥ 1	0.65 (0.53–0.80), P < 0.0001	30.8% v 16.8%	13.6 v 10.4
	Total population	0.77(0.63–0.93), P = 0.0034	29.0% v 18.7%	13.0 v 10.7

[a]Extreme—cisplatin or carboplatin, 5-Fluorouracil and cetuximab
[b]CPS—combined positive score
[c]non-inferior but did not meet superiority threshold

incidence ≥15% in the total population: any grade 58.3% v 96.9%, grade 3–5 16.7% v 69.0%, led to death 1.0% v 2.8%, and led to discontinuation 4.7% v 19.9%. Subgroup analyses for OS revealed that the pembrolizumab arm was favoured in most comparisons.

Pembrolizumab + chemotherapy when compared to Extreme improved OS in the CPS ≥ 20, CPS ≥ 1 and in the total population (Table 19.2). In the CPS ≥ 20 population the HR was 0.60 (95%CI 0.45–0.82, p = 0.0004), with medians of 14.7 versus 11.0 months and 2 year survival rates of 35.4% versus 19.4%. In the CPS ≥ 1 population the HR was 0.65 (95%CI 0.53–0.80, p = 0.0086), with medians of 13.6 versus 10.4 months and 2 year survival rates of 30.8% versus 16.8%. In the total population the HR was 0.77 (95%CI 0.63–0.93, p = 0.0034), with medians of 13.0 versus 10.7 months and 2 year survival rates of 29.0% versus 18.7%. The progression-free survival curves favoured the pembrolizumab arm but did not reach the designated superiority threshold. Unlike the monotherapy arm, there was no increase in early progression in the pembrolizumab-chemotherapy arm relative to Extreme. The response rate for the CPS ≥ 20 was 42.9% versus 38.2%, and for the CPS ≥ 1 it was 36.4% versus 35.7%, in the pembrolizumab-chemotherapy and Extreme arms respectively. The duration of response was prolonged in the pembrolizumab-chemotherapy arm e.g., in the CPS ≥ 20 median was 7.1 months (range 2.1+ − 39.0+) versus 4.9 months (1.2+ − 31.5+). The safety profile was similar in terms of number

of adverse events, grade 3–5 events, deaths due to adverse events and adverse events that led to discontinuation.

Based on the results of Keynote-048 both pembrolizumab monotherapy and pembrolizumab and chemotherapy have been established as new first-line therapies for R/M HNSCC, and have been approved in many jurisdictions. In the US, the FDA approved pembrolizumab monotherapy for patients whose tumours express PD-L1 CPS $\geq$ 1, and the pembrolizumab-chemotherapy combination for all patients. In Europe, the EMA has approved monotherapy and the combination in patients with CPS $\geq$ 1. The pre-specified analysis plan did not permit evaluation of efficacy in the CPS 1–19 and CPS < 1 subgroups separately, though these exploratory analyses will be presented at a later date. However, there is sufficient information available to suggest that pembrolizumab monotherapy would not be recommended in the CPS < 1 population.

Overall, the results do not suggest synergy between platinum/5FU and pembrolizumab with similar numbers of longer-term survivors in the combination and monotherapy arms. The combination offers the benefit of more rapid response and less risk of early progression than monotherapy. Patient selection will be important with the combination favoured for patients with high symptom burden and/or rapidly progressive disease and/or disease with imminent risk of complications e.g., airway compromise. On the other hand, patients who do not have these features could be treated with monotherapy that is associated with a much more favourable toxicity profile. Although the response rate is higher with Extreme than monotherapy, the durability of pembrolizumab responses has translated into a major survival advantage in the CPS $\geq$ 20 and $\geq$ 1 populations. The long-term survival benefit in the pembrolizumab arms appears to be greater than can be explained by the long term responders alone. It is possible that exposure to an immune checkpoint inhibitor alters the tumour microenvironment and in turn changes the natural history of R/M HNSCC and the response to subsequent therapies. The Extreme regimen or platinum/taxane-cetuximab combinations [13] will continue to have a role in the CPS <1 population and in patients with a contraindication to immunotherapy. The role of cetuximab/chemotherapy regimens for 2nd-line R/M HNSCC is worthy of study.

Combination of Other Treatments with Anti-PD1 or PD-L1 Agents

As the role of anti-PD1 and anti-PD-L1 agents have become established in many cancers, there has been increasing focus on combinations with other agents. There has been a rapid expansion in the number of combination immunotherapy trials since 2011. It has been increasing significantly year on year, with 467 new trials in 2017 [14]. HNSCC was the sixth most common tumour type targeted for combination immunotherapy trials. Across all tumour types the most common strategy being

tested in trials was combination with anti-CTLA-4 agents, followed by chemotherapy and radiotherapy [15]. There are many rational combination strategies including agents involved in a) T cell priming e.g., anti-CTLA4, vaccines, oncolytic viruses, b) T cell activation and homing e.g., anti-OX40, TIM3/LAG3 inhibitors, targeted therapies, c) Tumour antigen release e.g., chemotherapy, radiotherapy, oncolytic virus, targeted therapy and d) Improving the tumour microenvironment e.g., TGF beta inhibitor, adenosine antagonist [16]. The sheer number of potential strategies, agents and combinations poses a major drug development challenge. Detailed discussion of combination strategies and development pathways for combinations is beyond the scope of this chapter. The focus will be on combinations that have yielded promising results in R/M HNSCC and in particular on combinations investigated in randomised trials.

Anti-PD1/PD-L1 with Anti-CTLA4 Combinations

The combination of the anti- cytotoxic T-lymphocyte-associated protein (CTLA4), ipilimumab with nivolumab is well established as the standard of care in melanoma [17]. This has led to investigation of this combination in several other malignancies. In R/M HNSCC two anti-CTLA 4 agents have been studied, ipilimumab and tremelimumab. Two trials combining tremelimumab with durvalumab have failed to show benefit for the combination over single agent durvalumab or when compared to chemotherapy. In the Condor randomised phase 2 trial, in patients deemed to have low or no PD-L1 expression, the response rate for durvalumab was 9.2%, durvalumab + tremelimumab 7.8% and for tremelimumab monotherapy 1.6% [18]. In the Eagle phase 3 trial neither the durvalumab monotherapy arm nor the durvalumab + tremelimumab arm improved OS compared to single agent chemotherapy [11]. The durvalumab + tremelimumab combination did not appear to be any better than durvalumab monotherapy, though the trial was not designed to conduct this comparison.

Ipilimumab and nivolumab was compared to nivolumab alone in the randomised phase 2 Checkmate 714 trial in 1st-line R/M HNSCC. There has been a press release that it did not meet its primary endpoints (https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-reports-first-quarter-financial-results-1).

Two phase 3 trials in the 1st-line R/M HNSCC setting are awaited. The Checkmate 651 trial that is comparing ipilimumab and nivolumab to Extreme, and the Kestrel trial comparing durvalumab +/− tremilumumab versus Extreme.

Other Combinations

There has been considerable interest in combining VEGF inhibitors with immune checkpoint inhibitors. Anti-angiogenic agents may decrease immunosuppression and increase CD8 infiltration when combined with immune checkpoint inhibitors.

Lenvatinib is a multikinase inhibitor of VEGFR1, VEGFR2 and VEGFR3 that is widely used in recurrent/metastatic papillary thyroid cancer. In endometrial cancer the combination with pembrolizumab achieved a response rate of 40% leading to accelerated approval by the FDA [19]. Preliminary results from an expansion cohort of the phase 1 trial of lenvatinib and pembrolizumab in R/M HNSCC reported responses in 8/22 patients (36%) [20]. A phase 3 trial in R/M HNSCC is planned.

The inducible T-cell co-stimulatory receptor (ICOS) is highly upregulated upon T-cell receptor stimulation and expressed on tumour infiltrating lymphocytes. HNSCC has high ICOS expression. The inducible T-cell co-stimulatory receptor agonist, GSK3359609 has been combined with pembrolizumab. In a HNSCC expansion cohort of the phase I trial, there were responses in 8/34 patients (24%), and the toxicity profile was similar to pembrolizumab monotherapy [21]. The combination of GSK3359609 with pembrolizumab, platinum and 5FU has also been tested and found to be safe. Induce 3 is a randomised phase 2/3 trial of pembrolizumab +/− GSK3359609 in 1st line R/M HNSCC. Induce 4 is a planned randomised trial of pembrolizumab, platinum and 5FU +/− GSK3359609.

SD-101 is a synthetic cytidine-phospho-guanosine (CpG) oligonucleotide agonist of Toll-Like Receptor 9. It stimulates dendritic cells to release interferon-alpha and mature into antigen presenting cells, in turn activating T-cell anti-tumour responses. In a phase 2 trial of intra-tumoural SD-101 in combination with pembrolizumab in immune checkpoint inhibitor naïve R/M HNSCC, responses were observed in 12/50 patients (24%) [22]. Responses were seen in injected and non-injected lesions and in 'cold' tumours. Treatment was reported to be well tolerated.

The NKG2A receptor is expressed on natural killer (NK) cells and some CD8+ tumour infiltrating lymphocytes. HLA-E, the NKG2A ligand, is upregulated in many cancers including HNSCC. NKG2A blockade with monalizumab promotes innate anti-tumour immunity mediated by NK and $CD8^+$ T cells and enhances human NK cell antibody-dependent cell-mediated cytotoxicity (ADCC) induced by cetuximab [23]. In a phase 2 trial of monalizumab and cetuximab, responses were seen in 11/40 (28%), with 36% response rate in immune checkpoint inhibitor naïve patients and 17% in patients previously treated with an immune checkpoint inhibitor [24]. The median duration of response was 5.6 months and the median overall survival was 8.3 months. A phase 3 trial is planned.

Finally, there are strategies targeting the human papillomavirus (HPV), which is now the predominant cause of oropharyngeal cancer in many countries. There are several HPV therapeutic vaccines under development. Results have been reported for the ISA 101 HPV 16 vaccine targeting E6 and E7 given in combination with nivolumab [25]. 24 patients were treated (22 had oropharyngeal cancer). The response rate was 33%, with median duration of response of 10.3 months and median OS of 17.5 months [25]. There is also considerable interest in developing cellular therapies for HPV associated cancers. In a preliminary report of T-cell receptor gene therapy for HPV associated cancers, autologous genetically engineered T cells expressing a T-cell receptor directed against HPV 16 E6 was administered to patients, and there was evidence of anti-cancer activity [26]. In addition, tumour-infiltrating lymphocyte therapy for HPV associated cancers has been

studied. With this adoptive T cell therapy TIL cultures from resected metastasis were selected for HPV E6/E7 reactivity and administered to patients [27]. Responses were observed in 7/29 patients (24%).

Conclusion

Immune checkpoint inhibitors have had a major impact on the management of R/M HNSCC. Based on Keynote-048, pembrolizumab +/− chemotherapy in HNSCC represents the new first-line standard of care for the majority of patients with R/M HNSCC. Many rational combinations of agents with immune checkpoint inhibitors are under investigation, but it is difficult to evaluate single arm trials of combinations, and the selection of the best combinations for study in randomised trials remains very challenging. In a rapidly evolving area the awaited results of completed trials of immune therapies in earlier stages of HNSCC may ultimately affect the optimal management options for R/M HNSCC.

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Part IV
Rare Head and Neck Cancers

Chapter 20
Patients with Rare Head Neck Cancers: Do They Need a Different Approach?

Carla M. L. van Herpen

Introduction on Rare tumors

Definition and Frequency of Rare Cancers

Rare cancers are the rare diseases in oncology needing specific approaches by the cancer community and national health systems [1]. Rare cancers are defined as malignancies whose incidence is less than 6 per 100,000 inhabitants. The reason why the definition is based on incidence and not on prevalence is, among others, that incidence does not change on other factors than frequency, i.e. not on survival. At this moment rare molecular subgroups of common cancers are not included in the rare cancers list. In absolute numbers more than 500,000 patients per year are diagnosed with a rare cancer, and 4,300,000 rare cancer patients are prevalent in Europe. The definition is widely adopted among the different scientific international societies like the European Society of Medical Oncology (ESMO) and the European Society for Radiotherapy and Oncology (ESTRO). This means that 22% of all diagnosed cancers are rare and out of the 260 cancer types identified [2], 223 (86%) are rare. The European Network for Rare Solid Cancers (EURACAN) uses this definition to create a reference network in order to improve rare cancer care (Table 20.1).

C. M. L. van Herpen (✉)
Department of Medical Oncology, Radboud University Medical Center, Nijmegen, The Netherlands
e-mail: carla.vanherpen@radboudumc.nl

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Table 20.1 Rare cancers in the head and neck. The rate is the incidence per 100,000 inhabitants per year in Europe [2]

Tumor	Rate
Nasal cavity and sinuses	0.44
Nasopharynx	0.44
Salivary glands major/minor	1.31
Epithelial cancers of Hypopharynx	1.19
Larynx	4.64
Oropharynx	2.58
Oral cavity	3.28
Lip	1.22
Soft tissue sarcoma	0.29
Bone sarcoma	(0.8)[a]
Merkel cell	(0.13)[a]

[a]The mentioned incidences of bone sarcoma and Merkel cell carcinoma is in the whole body and not exclusive in the head and neck region

Poor Prognosis of Rare Cancers

In Europe rare cancer patients have poorer survival as compared to common cancer patients. In 2011 the 5-year overall survival of rare cancers was 49% versus 63% in common cancers [3].

Moreover, the survival of rare cancer patients in the Netherlands has barely increased over time (from 50% in 1995–2000 to 56% in 2012–2016), in contrast to the common cancers (from 59% in 1995–2012 to 72% in 2012–2016) [4].

Problems and Challenges in Rare Cancers

Common challenges posed in rare cancers are a late or incorrect diagnosis [5], less experience and a limited number of experts in the field, limited number of clinical studies (i.e., *a limited number* of phase III studies), less registered medications, a limited number of guidelines and less registries and biobanks. Patients suffering from rare cancers report higher levels of distress compared to common cancer patients due to increased anxiety and uncertainty correlated with the delayed diagnosis and feelings of isolation invoked by limited disease specific support systems [6].

Rare Cancers in the Head and Neck Region

Rare cancers of the head and neck are epithelial cancers of the larynx, hypopharynx, nasal cavity and sinuses, nasopharynx, major salivary glands and salivary-gland type tumors, oropharynx, oral cavity and lip, eye and adnexa and the middle ear. Besides these malignancies specific for the head and neck region also other rare malignancies can be located in the head and neck region, such as soft tissue sarcoma, bone sarcoma and Merkel cell carcinoma.

Phase III studies performed in the head and neck cancer field frequently include the epithelial cancers of the larynx, hypopharynx, oropharynx and oral cavity together, thereby making them 'less rare'.

Salivary Gland Cancers

Salivary gland cancer (SGC) is a distinct but heterogeneous group of malignancies comprising approximately 6.5% of cases within head and neck malignancies. This makes it a rare cancer, with an estimated age-standardized annual incidence of less than 2/100,000 in most countries. The most recent World Health Organization (WHO) classification of Head and Neck Tumors distinguishes 22 histopathological subtypes of SGC, which makes each subtype even rarer. Recognition of, and differentiation between these different subtypes is notoriously difficult and different subtypes exhibit different clinical features adding up to the complexity of the disease. For localized and resectable disease, surgical resection with or without postoperative radiotherapy is the cornerstone of treatment. In case of local recurrent or metastatic (R/M) disease, systemic treatment is challenging, but urgent given the prognosis of this disease stage. Lumped for all types of SGC with distant metastases (71% of the patients will develop recurrent/metastatic disease) the median overall survival is 15 months with overall survival rates at 1, 3 and 5 years of 54.5%, 28.4% and 14.8%, respectively. This, however, varies widely between different subtypes. For example, in adenoid cystic carcinoma median overall survival of several years in patients with distant metastases has been reported. This contrasts with salivary duct carcinoma (SDC), an aggressive subtype of SGC, in which median overall survival for R/M disease receiving best supportive care was only 5 months in the past [7].

The clinicopathological diversity of the disease justifies therapy tailored to the specific SGC subtype, highlighting the importance of adequate pathological examination (e.g. subtype, stage, growth pattern), preferably performed by a salivary gland expert pathologist. However, rarity of SGC and its extensive heterogeneity

hinders large-scale patient accrual in prospective trials and difficulties in correct histopathological subtyping of SGC endanger homogeneity of cohorts. Therefore, performance of clinical trials in SGC is challenging. This is reflected in the limited amount of studies performed with classical chemotherapeutic agents, targeted agents or immunotherapy in SGC.

Survival rates and limited benefit of chemotherapy emphasize that there is an unmet need for new therapeutic strategies for patients with R/M SGC. The paucity of treatment options may be reduced by mapping tumor characteristics and unraveling genetic aberrations in search for possible targets for systemic therapies. By doing so, SGC patients could also share in the benefits of the therapeutic advances made in more common malignancies, especially since the body of evidence for presence of such targets in different histological subtypes is increasing.

Salivary Duct Cancer (as Example)

SDC is an aggressive subtype of SGC, representing 4–10% of all SGCs. Overall survival at 3, 5 and 10-years is poor: 70.5%, 43% and 26%, respectively. Of the patients with SDC treated with curative intent, 54% will develop locoregional recurrences and/or distant metastases. In patients with distant metastases, spread to lungs (54%) and bones (46%) is seen most, and a remarkably high percentage of brain metastasis has been observed (18%). Given the dismal prognosis and high prevalence of distant metastasis, systemic therapy is often required.

The androgen receptor (AR) and the HER2 receptor (encoded by *the HER2 gene*) are frequently expressed in SDC, respectively in 78–96% and 29–46% of cases. Targeting AR and/or HER2 is promising and are the best studied therapies in SDC patients.

A prospective phase 2 trial evaluating the effect of combined androgen blockade (CAB) with leuprorelin acetate and bicalutamide in 36 SGC patients (of which 34 were SDC patients), showed partial or complete responses in 41.7% [95%-CI 25.5–59.2%] and stabilization of disease in 44.4% [95%-CI 27.9–61.9]. The median progression-free survival was 8.8 months [95% CI, 6.3–12.3 months] and the median overall survival was 30.5 months [95% CI, 16.8 months to not reached] [8]. Especially given the low rate of observed grade 3 or 4 toxicity, CAB plays an important role in the palliative treatment of AR positive SDC patients. Besides its role in palliative treatment, androgen deprivation therapy (ADT) may also be beneficial in the adjuvant setting. Based on retrospective data, adjuvant ADT results in significantly improved 3-year disease free survival (DFS) in patients with stage 4A AR-positive SDC (48.2% [95%-CI 14.0–82.4%] versus 27.7% [95%-CI 18.5–36.9%] in the control group who did not receive adjuvant ADT). Differences in overall survival were just below and above significance level, depending on whether or not correction for confounders was performed [9].

Trastuzumab in combination with taxane based chemotherapy is the best studied combination on HER-2 targeted therapy. Fifty-seven eligible patients with SDC were enrolled in a phase II study. The overall response rate was 70.2% (95% CI, 56.6% to 81.6%), and the clinical benefit rate was 84.2% (95% CI, 72.1% to 92.5%). Median progression-free and overall survival times were 8.9 months (95% CI, 7.8 to 9.9 months) and 39.7 months (95% CI, not reached), respectively [10]. This combination could potentially be amplified with the addition of another agent targeting HER2 (e.g. pertuzumab, lapatinib) or after progressive disease replacing trastuzumab with the antibody-drug conjugate trastuzumab-emtansine. A oral presentation at the American Society of Clinical Oncology (ASCO) in 2019 emphasizes the potential of trastuzumab-emtansine in *HER2*-amplified SGC, as 9 out of 10 patients (0–3 lines of prior treatment, median of 2) responded on this treatment. Presumably most of these patients were SDC patients. Median PFS was not reached after a median follow-up of 12 months [11]. In analogy with the positive results achieved in HER2 positive breast cancer by adding pertuzumab to docetaxel/trastuzumab and the cases reported on this combination in SDC, this triple combination deserves a continuation in clinical studies in SDC.

Targeting HER2 in SDC patients with HER2 overexpression is thus promising. In patients co-expressing AR and HER2 it is yet unclear whether therapy targeting AR or HER2 is the best approach. However, in case of extensive or rapidly progressive disease, HER2 targeting therapy in combination with taxane-based chemotherapy is the preferable choice over ADT.

Besides AR and HER2, a wide spectrum of mutations, is observed in lower frequencies in SDC, which altogether forms a genetic landscape highly similar to apocrine breast cancer. This includes mutations in *TP53* (53–68%), *PIK3CA* (18–26%), *HRAS* (16–23%), *BRAF* (4%) and *AKT1* (1.5%). Reports on the use of drugs aiming at these targets in clinical practice are scarce [12].

In summary, SDC has many targets amenable for systemic therapy. Elaborate mapping of tumor characteristics regarding receptor expression, genomics and pathway alterations are key to alter the dismal prognosis of patients with locally advanced or metastatic SDC.

NTRK Inhibition

Secretory carcinoma (SC), previously named mammary analogue secretory carcinoma (MASC), is a relatively new entity that was first described in the salivary glands in 2010 [13]. In retrospect, most cases of what is now called SC were initially classified as acinic cell carcinoma and also as polymorphous adenocarcinoma or adenocarcinoma NOS. SC is rare, is most often found in the parotid gland (58–68%) and behaves relatively indolent with a good prognosis. R/M disease is rare (estimated 5 and 10 years survival 95%). The genetic hallmark of SC is a

ETV6-NTRK3 gene fusion as a result of a t(12;15) (p13;q25) translocation, although other gene fusions with *ETV6* have been described (for instance *ETV6-MET* and *ETV6-RET*). *NTRK* gene fusions are known oncogenic drivers and have been described in other tumor types. This *ETV6-NTRK3* gene fusion therefore provides a promising target for systemic therapy, and the body of evidence for efficacy of TRK-inhibitors (e.g. larotrectinib, entrectinib, repotrectinib, LOXO-195) in patients with *NTRK* gene fusions is expanding. A recent phase II trial evaluating the efficacy of larotrectinib in *NTRK* fusion positive patients included 12 patients with recurrent or metastatic (MA)SC and reported a response rate of 75% and the median progression free survival was not reached after median follow-up of 9.9 months [14]. Responses in patients with (MA)SC have also been observed for entrectinib and repotrectinib. A phase I/II trial evaluating LOXO-195 in second line is currently recruiting and is open for inclusion of *NTRK* fusion positive SGC patients previously treated with a TRK-inhibitor (NCT03215511).

Whether *NTRK* gene fusions are present in other subtypes of SGC is currently unknown, but treatment with TRK-inhibitors is a very promising treatment option, an option that should be investigated in all SGC patients with advanced disease.

Future

Patients with rare cancers deserve a different approach. Only then we can make progress to improve care and cure in rare cancers.

In the rare cancer agenda 2030 written by the Joint action on Rare Cancers (JARC) ten recommendations are made [1]. In rare cancers networking is crucial; this means that networking with patients, in health care systems, but also in medical education is extremely important. Furthermore, regulation on rare cancers should tolerate a higher degree of uncertainty, which means that the 'rules' we made for registration of new medications in common cancers (mostly based on phase III evidence) cannot be the same for rare cancers.

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Part V
Nasopharynx Cancer

Chapter 21
Epidemiological Aspects in Nasopharyngeal Cancer

Gemma Gatta

Introduction

Nasopharyngeal cancer (NPC) is a rare cancer in the majority of countries, however NPC is endemic in certain regions of southern China, Southeast Asia and Africa. This paper provides descriptive epidemiology of the epithelial malignant nasopharyngeal tumours, showing incidence and survival variation by sex, age, geographic region/population and time trend. The source of data are the major website as given by the International Agency for Research on Cancer (IARC) Global Cancer Observatory [1], and the RARECAREnet European project [2], for Europe.

The differences in incidence and survival will be interpreted according to the literature.

Incidence

In 2018, 129,000 new cases of NPC were diagnosed worldwide with 85% of cases in the Asiatic population. Figure 21.1 shows the estimated number of new cases and the age-standardized incidence rates for the 10 countries in which NPC is diagnosed most common. Actually, in males the annual crude rate of incidence (per 100,000) dramatically varied between 8 in South-Eastern Asia and <1 in the European regions (Table 21.1).

In Europe (EU28) from European population-based cancer registries, 2600 new diagnoses per year (incidence) were made (1999–2007) and 18,200 people were living, in 2008, with a diagnosis of NPC (prevalence) [2]. Tables 21.2 and 21.3 show

G. Gatta (✉)
Fondazione IRCCS 'Istituto Nazionale dei Tumori', Milan, Italy
e-mail: gemma.gatta@istitutotumori.mi.it

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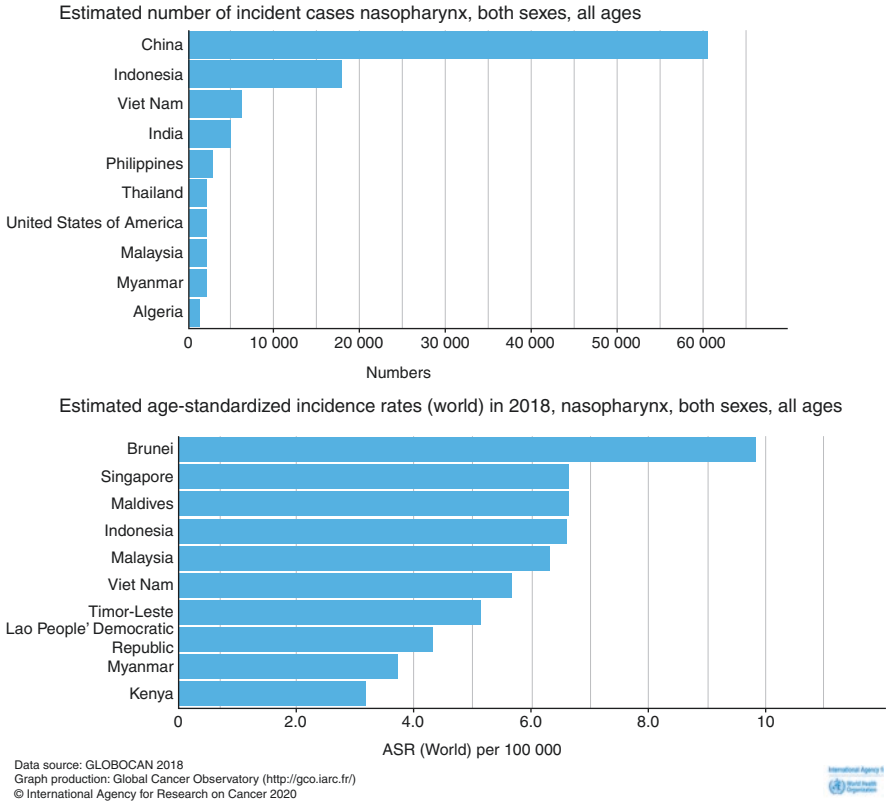

Fig. 21.1 Nasopharyngeal cancer in the ten countries with the highest incidence in 2018. From Global Cancer Observatory [1]

Table 21.1 Estimated number of new cases in 2018, nasopharyngeal cancer, males, all ages

Population	Number	Uncertainty interval	Crude rate[a]	ASR (World)[a]
Eastern Asia	46,783	[44,415.0–49,277.3]	5.5	3.9
South-eastern Asia	25,895	[22,705.8–29,532.2]	7.9	7.8
South-Central Asia	5394	[4542.2–6405.5]	0.53	0.59
Northern Africa	2331	[1726.7–3146.8]	2	2.2
Eastern Africa	2112	[1204.9–3702.1]	0.98	1.7
North America	1709	[1577.8–1851.2]	0.95	0.66
Western Asia	1642	[1321.3–2040.6]	1.2	1.3
Central and Eastern Europe	1345	[1177.2–1536.7]	0.98	0.69
South America	1273	[882.5–1836.2]	0.6	0.55
Western Africa	1170	[637.6–2146.9]	0.61	0.86
Southern Europe	1148	[920.6–1431.5]	1.5	0.97
Western Europe	903	[763.5–1067.9]	0.94	0.59
Middle Africa	515	[223.3–1187.8]	0.61	1
Caribbean	319	[204.7–497.2]	1.5	1.2

Table 21.1 (continued)

Population	Number	Uncertainty interval	Crude rate[a]	ASR (World)[a]
Northern Europe	287	[240.8–342.1]	0.56	0.37
Central America	266	[191.4–369.6]	0.3	0.32
Australia and New Zealand	145	[119.1–176.5]	0.99	0.72
Southern Africa	136	[79.3–233.3]	0.42	0.49
Melanesia	19	[5.7–63.3]	0.36	0.48
Micronesia	18	[12.2–26.6]	6.7	6.3
Polynesia	6	[3.3–10.9]	1.7	1.7

[a]Crude and age-standardized rates per 100,000
From the Global Cancer Observatory [1]

Table 21.2 Nasopharyngeal cancer in Europe, number of observed cases (obs.) and age-adjusted incidence rate (adj. rate) with (95% CI) by sex and age

	Obs.	Adj. rate
All	7439	0.429 (0.419–0.439)
Sex		
Males	5313	0.648 (0.631–0.666)
Females	2126	0.229 (0.219–0.240)
Age		
0–14 years	70	0.027 (0.021–0.034)
15–24 years	260	0.129 (0.114–0.146)
25–64 years	4862	0.571 (0.555–0.587)
65+ years	2247	0.897 (0.861–0.935)

Table 21.3 Nasopharyngeal cancer in Europe, number of observed cases (obs.) and age-adjusted incidence rate (adj. rate) with (95% CI) by time period and region

	Obs.	Adj. rate
European region		
Northern Europe	219	0.242 (0.211–0.277)
Ireland and UK	1823	0.331 (0.316–0.347)
Central Europe	1797	0.360 (0.343–0.377)
Southern Europe	2055	0.702 (0.672–0.734)
Eastern Europe	1545	0.513 (0.487–0.539)
Time period		
1995–1998	2510	0.413 (0.396–0.429)
1999–2002	2591	0.411 (0.395–0.427)
2003–2007	3227	0.400 (0.386–0.414)

From http://rarecarenet.istitutotumori.mi.it/rarecarenet/

incidence (numbers and rates) by sex, age, time period and European region. Incidence is higher in men than women with a ratio 3:1 (Table 21.2). The disease is more frequent in the elderly (65 and more years of age): the incidence rate (per 100,000/year) increases with age at diagnosis from <0.1 to 0.9 (Table 21.2).

In Europe there is an incidence gradient across countries, with the highest rates in the Southern which are 2/3 times higher than in the Northern countries. The occurrence of NPC remains constant during the period 1995–2007.

Survival

Based on about 7300 cases, survival of European patients with NPC were 76%, 57%, and 49% at 1, 3 and 5 years after diagnosis, respectively (Table 21.4). Prognosis (5-year survival) was better in younger patients, aged 15–24 years, at 73%, and dramatically reduced in the elderly, 65 years and more, at 31%. Females had a significantly better prognosis, 5-year survival, 54% versus 47% (Table 21.4).

Five-year survival was between 51% and 55% in all the European regions, except the Eastern of European countries with 36% (Table 21.5).

During the study period (1995–2007), 5-year survival slightly, but not significantly, improved.

Survival in population based studies was analyzed in terms of relative survival, which is an analogous of cause specific survival usually considered in clinical studies. Relative survival is the ratio between the observed survival of the cohort of patients belonging to a specific population (for example Belgium or Estonia) and the survival of the general population of the same country and with the same age distribution.

Table 21.4 Nasopharyngeal cancer in Europe, number of (No.) and 1, 3 and 5-year Relative Survival (RS%) with (95% CI) by sex and age

	No.	1-year RS	3-year RS	5-year RS
All	7276	76 (75–77)	57 (56–59)	49 (48–50)
Sex				
Males	5205	76 (74–77)	56 (54–57)	47 (45–49)
Females	2071	78 (76–80)	61 (59–63)	54 (51–56)
Age				
0–14 years	69	88 (81–96)	83 (75–93)	84 (75–93)
15–24 years	259	95 (92–98)	78 (73–84)	73 (67–79)
25–64 years	4791	82 (81–83)	63 (61–64)	55 (53–56)
65+ years	2157	61 (59–63)	41 (39–43)	31 (29–34)

Table 21.5 Nasopharyngeal cancer in Europe, number of (No.) and 1, 3 and 5-year Relative Survival (RS%) with (95% CI) by time period and European region

Time period	No.	1-year RS	3-year RS	5-year RS
1995–1998	1893	76 (74–78)	56 (54–59)	48 (46–51)
1999–2002	1915	73 (71–75)	54 (52–56)	47 (44–49)
2003–2007	1886	77 (75–79)	59 (56–61)	50 (48–52)
European region				
Northern Europe	218	79 (73–84)	62 (55–70)	55 (47–63)
Ireland and UK	1793	74 (72–76)	58 (56–61)	51 (48–54)
Central Europe	1764	80 (78–82)	63 (61–66)	55 (52–58)
Southern Europe	2030	80 (78–82)	60 (57–62)	51 (48–53)
Eastern Europe	1471	69 (66–71)	45 (42–48)	36 (33–39)

From http://rarecarenet.istitutotumori.mi.it/rarecarenet/

Discussion

Incidence of cancers, given by population-based cancer registries, provides the annual number of new cases of a specific cancer in a defined population/region. NPC is a rare cancer, in Europe not more than 3% of all H&N cancers [2]. Incidence together with prevalence are important for public health planning, to organize centralization of the cure and planning of clinical trials. In Europe, the number of annual cases across country ranged between 2 or less (Malta and Iceland) and 454 (Germany) [2]. The geographical variation of the incidence rates gives insight into possible factors or causes of the disease, again relevant to public health to reduce the new number of cases. The incidence variability around the world of NPC is very high and this variation has been explained by diet. According to the World Cancer Research Fund (WCRF) updated review [3], the largest review on diet, nutrition and physical activity, the major risk factors explaining the difference in incidence in populations are the consuming Cantonese style salted fish, meat and preserved non-starchy vegetable; other established causes include smoking, occupational exposure and infectious agents. There is a strong evidence of risk for NPC consuming Cantonese style salted fish and some evidence consuming red meat and processed meat; while the consumption of a greater intake of non-starchy vegetables decreases the risk of NPC. Cantonese-style salted fish contains nitrosamines and nitrosamine precursors which have been shown to induce the development of cancer. Smoking is attributable to 23% of NPC cases [3] and dust and formaldehyde are the major occupational factors associated to NPC [4]. Epstein-Barr virus (EBV) infection is an important player in this disease, but it needs other factors in addition, as only a fraction of the infected population develops NPC [3].

A recent paper, conducted in the Taiwanese population (≈160,000 participants and 115 NPC cases), showed the effect of air pollution as a risk of developing NPC [5]. The study reported a clear dose response relationship: NPC increased with the increase in nitrogen dioxide (NO_2) from 1.4 to 2.3 compared to lowest concentration levels. The same was with the fine particulate ($PM_{2.5}$) with a double risk.

There is a global reducing incidence of NPC as reported by the review by Tang et al. for the period 1970–2007 [6]. The occurrence of NPC significantly decreased in southern and eastern Asia, north America and Nordic countries with average annual percent changes (AAPCs) of −1% to −5%. Decreasing trends in NPC incidence are due to tobacco control, changes in diets and economic development. The ecological study by Lau et al. [7] investigated in some European and Asiatic countries and in the US the relationship between the NPC incidence with the consumption of salted fish, vegetables and tobacco cigarettes, from the Food and Agriculture Organization (FAO) and Census Statistics. They found markedly decreasing trends of NPC in Hong Kong which was correlated with corresponding secular changes in salted fish consumption per capita, tobacco and vegetable consumption per capita. In many countries the tobacco smoking, which is more connected to the keratinizing squamous cell carcinoma and prevalent in the non epidemic area, is reducing [6].

In Europe, NPC 5-year survival was poorer in males, in the elderly and in the Eastern countries. No progress has been observed in the first years of this century. Interestingly, from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database (1973–2013) [8], Asians showed a disease specific survival advantage over Caucasians, African Americans and Hispanics, when adjusted for sex, age at diagnosis, grade, TNM staging and treatment strategy. Asians showed a less aggressive disease characterized by non keratinizing lesions, smaller size at diagnosis, well differentiated grading and an earlier TNM stage. However, taking into account these prognostic factors in a multivariate analysis, the advantage persisted suggesting that genetic predispositions, viral agents, occupational exposures, and dietary exposures to chemical carcinogens can be responsible of the aggressiveness of the diseases. However, the African Americans had a higher rate of metastasis at the time of diagnosis and the highest proportion of no treatment with the common therapy of NPC (surgery or radiation). These results may be connected to the fact that certain minorities in the US have less access to or make use of medical care in terms of clinic visits, preventative care and diagnostic testing.

NPC is a rare cancer, therefore the correct and fast diagnosis and treatment can be obtained in high volume hospitals with a good expertise. Diagnosis and treatment in reference centres are expected to be more accurate because they benefit from large numbers of cases, which are often discussed in a multidisciplinary setting involving expert professionals. Within the RARECAREnet project [9] centralization of rare cancer patients was studied in 7 European countries, and for the head and neck group of rare cancers 75% of patients were centralised in two top hospitals in Slovenia (2 million population, 266 treatments per hospital per year), and 12 top hospitals in the Netherlands (17 million population, 201 treatments per hospital per year). The level of centralisation was lower in the other countries such as Finland, Ireland, Bulgaria, Navarra and Belgium. However, the period of study was 1999–2007 and the situation will for sure improve in some countries over time. The European Joint Action on Rare Cancers [10] and the institution of the European Reference Network for rare diseases [11] will continue to play a role in this.

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Chapter 22
New Developments in the Management of Nasopharyngeal Carcinoma

Xiaoshuang Niu and Yungan Tao

Unique Characteristics of Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is an epithelial carcinoma with a specific geographic distribution. It affected an estimated 129,000 patients worldwide in 2018, with the highest incidences are in regions in Southeast Asia (especially in South China), and North Africa [1].

NPC is categorized into three pathological subtypes on the basis of WHO criteria. Differentiated tumours with surface keratin are defined as type I, whereas types II and III refer to non-keratinising differentiated and undifferentiated tumours, respectively. Types II and III were combined into a single category of non-keratinising carcinoma which constitutes most cases in endemic areas (>95%) [2]. EBV infection, host genetics, environmental factors and other factors are contributors in the occurrence and development of NPC. Among them, EBV infection may be the most common cause of NPC [3]. The nasopharynx is anatomically concealed, and it is adjacent to important organs such as brainstem, optic nerve and optic chiasm. NPC is prone to early retropharyngeal and cervical lymph node metastasis and local skull base invasion. More than 70% of NPC receive a diagnosis of locoregionally advanced (LA) disease at presentation. Distant metastasis predominates as the pattern of disease relapse, which accounts for approximately 70% of patients with cancer-specific mortality [4]. Radiotherapy (RT) is the cornerstone of initial treatment due to its radiosensitive characteristic and deep-seated location. Given the

X. Niu
Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai, China

Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China

Y. Tao (✉)
Department of Radiation Oncology, Institut Gustave Roussy, Villejuif, France
e-mail: yungan.tao@gustaveroussy.fr

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depth and complex location of the nasopharynx, surgery is especially used as a rescue method for regional lymph node failure, while with limited use for local recurrence.

Radiotherapy

Radiotherapy is the main treatment for non-metastatic NPC. The technology of photon-based RT has developed from two-dimensional radiotherapy (2D-RT), three-dimensional conformal radiotherapy (3D-CRT) to Intensity modulated radiotherapy (IMRT). IMRT is the standard RT technique compared with 2D/3D RT and could reduce late toxicities such as xerostomia. Overall survival (OS) and tumor control could potentially be enhanced by the improved dosimetric properties. In a monocenter randomized trial [5], the 5-year OS rate was 79.6% for the IMRT group and 67.1% for the 2D-RT group ($p = 0.001$). Patients in IMRT group had significantly lower radiation-induced toxicities than those in 2D-RT group. Pow et al. [6] compared directly the effect of IMRT vs. 2D-RT on salivary flow in patients with early-stage NPC. Fifty-one patients with T2N0/N1 NPC were enrolled in a randomized controlled clinical study and received IMRT or 2D-RT. The result showed that IMRT was significantly better than 3D-CRT in terms of parotid sparing for early-stage disease. Results from a similar phase 3 trial of the Groupe D'Oncologie Radiothérapie Tête et Cou (GORTEC trial 2004–01) have been reported in ESMO 2018 by Tao et al. [7], in which IMRT improved significantly xerostomia compared with conformal radiotherapy in locoregionally advanced head and neck squamous cell carcinoma (LA-HNSCC).

Along with the RT technique improvements, precisely defining the target volume and adjacent organs at risk (OARs) has become crucial for a good treatment outcome. Target delineation in NPC often proves challenging because of the notoriously narrow therapeutic margin. High doses are needed to achieve optimal levels of tumor control, despite the apparent radio-sensitivity of the tumor in many patients [8]. We established the international guideline for the delineation of the Clinical Target Volume (CTV) of NPC. This set of consensus guidelines has been developed to provide a practical reference for appropriate contouring to ensure optimal target coverage [8]. Even in the contemporary era of IMRT with extensive use of concurrent chemotherapy, the dosimetric inadequacy enforced by dose constraints on OARs remains one of the most important independent factors affecting treatment outcome. It is often difficult to achieve the optimal balance and trade-off between risks of local recurrence owing to inadequate tumor coverage versus potential serious late complications [9]. A guideline was developed to provide a practical reference for setting dose prioritization and acceptance criteria for tumor volumes and OARs [9]. Both of these two guidelines provided useful references for NPC radiation management. The final decision on the treatment volumes and treatment prescription should be based on the individual clinical situation and the patient's acceptance of optimal balance of risk [8, 9].

Chen and colleagues [10] reported the role of RT in addition to systemic therapy for initially diagnosed metastatic NPC in ESMO 2019. Between April 2014, and August 2018, 173 patients were assessed for eligibility, of whom 126 patients with a complete response (CR) or partial response (PR) after 3 cycles of cisplatin and fluorouracil were randomised (63 patients in the chemo-radiotherapy group and 63 in the chemotherapy group). The median follow-up was 26.7 months. The researchers found that the addition of RT to chemotherapy alone significantly improved OS (hazard ratio [HR] 0.42, 95% CI 0.23–0.77, $p = 0.004$) and progression free survival (PFS) (hazard ratio [HR] 0.36, 95% CI 0.23–0.57, $p < 0.001$) for metastatic NPC patients who obtained objective response after chemotherapy.

There are still several remaining questions: Firstly, in order to reduce the late toxicities, we still need to define the best dose/volume after induction chemotherapy because NPC patients are often young and with long-term survival. Secondly, for stage II NPC patients, whether the IMRT alone could be used instead of the combination of chemo-radiotherapy especially for those patients with T1-T2N0 or N1 with only a single small neck lymph node. Finally, although IMRT is currently the preferred method, there is great interest in using proton or carbon ion RT to further improve the treatment rate of NPC. Compared with IMRT, intensity-modulated proton therapy (IMPT) and intensity-modulated carbon ion therapy (IMCT) have a dosimetric advantage in NPC and better protection for normal tissues [11]. Studies with proton therapy (NCT00592501) and carbon ion therapy (NCT02569788) are under way to provide more information about the application of IMPT and IMCT in NPC.

Chemotherapy and Radiotherapy Combinations

The combination of RT and chemotherapy is the key development in the treatment of LA diseases. A large number of trials have shown that concurrent chemoradiotherapy (CCRT) had a survival advantage compared with RT alone for NPC [4, 12, 13]. The meta-analysis MAC-NPC (Meta-Analysis of Chemotherapy in Nasopharynx Carcinoma) including eight trials with 1753 patients demonstrated an absolute survival benefit of 6% at 5 years from the addition of chemotherapy (from 56% to 62%). The most significant benefit of chemotherapy on OS was CCRT [14]. In the recent actualization of MAC-NPC meta-analysis including 19 trials and 4806 patients, we confirmed that the addition of chemotherapy to RT significantly improved OS (hazard ratio [HR] 0.79, 95% CI 0.73–0.86, $p < 0.0001$; absolute benefit at 5 years 6.3%, 95% CI 3.5–9.1). The most significant benefits of chemotherapy on OS were seen with either CCRT plus adjuvant chemotherapy (HR 0.65 [95% CI 0.56–0.76]) or CCRT alone (0.80 [0.70–0.93]) [15].

However, it is still controversial whether adjuvant chemotherapy after CCRT can bring more survival benefits. A phase 3 multicenter randomized controlled trial in 508 patients with stage III-IV NPC did not show a significant improvement in failure-free survival when the combination of cisplatin and 5-FU (PF regimen) was

given after CCRT (with weekly cisplatin 40 mg/m^2) [16]. Long-term follow-up data confirmed these findings [17]. More recently, the network meta-analysis based on the MAC-NPC data has shown that the addition of adjuvant chemotherapy to CCRT achieved the highest survival benefit and consistent improvement for all end points. However, the addition of induction chemotherapy to concurrent chemo-radiotherapy achieved the highest effect on distant control [18]. These results should be considered with caution because the comparisons in the network meta-analysis were indirect.

Compared with adjuvant chemotherapy, induction chemotherapy may be a promising treatment strategy for NPC due to better tolerance and a stronger effect on micro-metastasis. Several phase 3 trials have shown benefit of induction chemotherapy when added to cisplatin-based CCRT. An early-closed French multicenter phase 3 trial (GORTEC 2006–02) including 86 French/Tunisian patients with stage II–IV NPC showed that 3 cycles of induction chemotherapy with docetaxel-cisplatin-5-FU (the TPF schedule) significantly improved 3-year PFS (hazard ratio (HR) = 0.44; 95% confidence interval (CI): 0.20–0.97, P = 0.042) compared to CCRT (with weekly cisplatin 40 mg/m^2) alone. Similarly, the 3 years OS rate was 86.3% in the TPF arm versus 68.9% in the reference arm (HR = 0.40; 95% CI: 0.15–1.04, P = 0.05). The tolerance of TPF schedule was quite good with 95% of patients who completed 3 cycles [19]. A large-scale Chinese multicenter phase 3 trial has been reported, that confirmed these data. In that study, comprising 480 patients with stages III-IVB NPC (except T3-4N0), they made use of modified TPF dose schedule and used high-dose cisplatin (100 mg/m^2 q 3 weeks) during CCRT. Induction with TPF significantly improved 5-year OS and failure-free survival [20]. More recently, Zhang et al. compared gemcitabine and cisplatin (the so-called GP schedule) as induction chemotherapy plus CCRT with CCRT alone in 480 Chinese patients with stage III to IVb NPC (N1–3). The 3-year recurrence-free survival was 85.3% in the induction chemotherapy group and 76.5% in the standard-therapy group (stratified hazard ratio for recurrence or death, 0.51; 95% confidence interval [CI], 0.34 to 0.77; P = 0.001). OS at 3 years was 94.6% and 90.3%, respectively (stratified hazard ratio for death, 0.43; 95% CI, 0.24 to 0.77) [21]. The induction chemotherapy with the GP schedule was better tolerated in Chinese patients (96.7% of patients with 3 cycles) than the dose-reduced TPF schedule in the previous study (88% tolerated 3 cycles) and therefore could potentially be used more widely (Table 22.1).

Several other phase 3 trials of chemoradiotherapy combinations in LA-NPC are in progress to answer several unresolved issues in NPC. What is the best strategy in combination with platinum-based CCRT, induction chemotherapy or adjuvant chemotherapy? Can we delete the chemotherapy during RT by using induction first, followed by RT and then adjuvant chemotherapy? What are the less toxic drugs combined with radiotherapy? The gemcitabine/cisplatin regimen as an induction regimen plus CCRT is being tested with RT plus gemcitabine and cisplatin as adjuvant chemotherapy (NCT03366415). Replacing cisplatin with nedaplatin, or fluorouracil with capecitabine during induction and concurrent phases may reduce toxicities and improve quality of life (NCT03503136).

Table 22.1 Randomised trials evaluating induction chemotherapy plus concurrent chemoradiotherapy vs. concurrent chemoradiotherapy alone

	Experimental chemotherapy	Control chemotherapy	Sample size	Overall survival	Progression-free survival
Frikha et al. [19]	Induction: Docetaxel 75 mg/m^2 d1; cisplatin 75 mg/m^2 d1; fluorouracil 750 mg/m^2 d1–5; q3weeks × 3; concurrent: Cisplatin 40 mg/m^2 d1; q1week × 7	Concurrent: Cisplatin 40 mg/m^2 d1; q1week × 7	83	86% vs. 69% (3 year results) p = 0.059	74% vs. 57% (3 year results) p = 0.042
Sun et al. [20]	Induction: Docetaxel 60 mg/m^2 d1; cisplatin 60 mg/m^2 d1; fluorouracil 600 mg/m^2 d1–5; q3weeks × 3; concurrent: Cisplatin 100 mg/m^2 d1; q3week × 3	Concurrent: Cisplatin 100 mg/m^2 d1; q3weeks × 3	480	86% vs. 78% (5 year results) p = 0.042	77% vs. 66% (5 year results) p = 0.019
Zhang et al. [21]	Induction: Gemcitabine 1 g/m^2 d1,8; cisplatin 80 mg/m^2 d1; q3weeks × 3; concurrent: Cisplatin 100 mg/m^2 d1; q3week × 3	Concurrent: Cisplatin 100 mg/m^2 d1; q3weeks × 3	480	94.6% *vs* 90.3% (3 year results)	85.3% vs. 76.5% (3 year results) p = 0.002

EBV-DNA

Plasma Epstein-Barr virus (EBV) DNA is an independent prognostic biomarker for NPC [22, 23]. Quantification of plasma EBV DNA is useful for monitoring patients with NPC and predicting the outcome of treatment [24]. Chan and colleagues compared adjuvant chemotherapy against clinical observation for patients with high risk of recurrence based on EBV DNA after completing RT or chemoradiotherapy in a phase 3 trial. In 789 enrolled patients at the completion of chemoradiotherapy, 216 patients with detectable EBV DNA after radiotherapy, 104 patients were randomized to adjuvant GP schedule or observation. They found that the level of post-RT plasma EBV DNA correlated significantly with the hazards of loco-regional failure, distant metastasis, and death. However, no significant difference was found in 5-year relapse-free survival rate between the two arms (49.3% vs. 54.7%; P = 0.75; hazard ratio for relapse or death, 1.09; 95% CI, 0.63 to 1.89) [25]. The ongoing NRG-HN001 trial also uses plasma EBV DNA to identify patients with NPC at a higher risk of relapse for adjuvant chemotherapy. The purpose was to establish whether adjuvant gemcitabine and paclitaxel is better than cisplatin and fluorouracil for patients with detectable EBV DNA, and whether adjuvant cisplatin and fluorouracil can be omitted in patients with undetectable plasma EBV DNA. Lv et al. quantified circulating EBV DNA copy number in 673 NPC patients undergoing radical induction chemotherapy and chemo-radiotherapy. The patients divided into four prognostic phenotypes (early responders, intermediate responders, late responders, and treatment resistant) that were correlated with efficacy of

chemotherapy intensity. Based on the exploratory observations, it was proposed a risk stratified treatment adaptation design that is based on the phenotypic clusters and longitudinal surveillance of cell free EBV DNA. Real-time monitoring of EBV DNA response added prognostic information and had the potential utility for risk-adapted treatment de-intensification/intensification in NPC [26].

Immunotherapy and Chemoradiotherapy

Recently, immune checkpoint blocking therapy has made a breakthrough in cancer treatment. In NPC clinical histological samples, it is characterised by high PD-L1 expression (up to 90% of tumour cells) and abundant infiltration of non-malignant lymphocytes [27, 28]. This nature makes NPC patients potentially suitable for immunotherapy treatment [29]. Fang and colleagues reported the results of two single-arm, phase 1 trials. Camrelizumab (an anti–PD-1 monoclonal antibody) was used as (1) monotherapy in 93 patients with pre-treated recurrent or metastatic disease, and (2) in combination with gemcitabine plus cisplatin in 23 first-line patients. Overall response was 34% in the monotherapy trial and 91% in the combination trial. One year PFS was 27% and 61% in the two arms, respectively. The combination of camrelizumab plus gemcitabine and cisplatin has promising preliminary anti-tumour activity for treatment-naive loco-regionally recurrent or metastatic disease [30]. Several randomized phase 3 trials in NPC patients are ongoing due to promising anti-tumour activity and predictable safety profile of anti-PD-1/PD-L1 therapy. One phase 2 trial (NCT03925090) is assessing neoadjuvant and adjuvant toripalimab (anti PD-1) combined with CCRT in NPC. Two phase 3 trials (NCT03700476 and NCT03427827) are investigating induction and concurrent sintilimab or adjuvant camrelizumab in LA-NPC. These studies will be evaluating the value of adding anti-PD-1 therapy to standard treatment (CCRT or GP-CCRT) in locally advanced NPC.

Conclusions

Nasopharyngeal carcinomas have unique characteristics with a specific geographic distribution and IMRT is the standard radiotherapy technique. Concurrent chemoradiotherapy is a standard of care in NPC (especially N0–1) and induction chemotherapy plus chemo-radiotherapy in stage III/IV N1–3. The benefit of RT in addition to systemic therapy has been shown for the initially diagnosed metastatic NPC. Furthermore, ongoing studies will identify adjuvant chemotherapy according to EBV DNA and the role of immunotherapy in association with chemoradiotherapy.

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Chapter 23
New Drugs for Recurrent or Metastatic Nasopharyngeal Cancer

Olubukola Ayodele and Lillian L. Siu

Introduction

Nasopharyngeal cancer (NPC) is a rare subset of head and neck cancers with geographical diversity, whereby the incidence is approximately 20–30 cases per 100,000 in Eastern versus 1 per 100,000 in Western countries. Early stage and localized NPC generally carry a good prognosis; however systemic dissemination develops in approximately 20% of patients with locoregionally advanced disease [1, 2]. Additionally, about 15% of patients present with distant metastases at primary diagnosis [3]. For these patients with recurrent or metastatic (RM) NPC, treatment options are largely limited to palliative systemic therapies leading to poor outcomes. Although NPC is a chemosensitive disease with some studies reporting response rates of over 80% with platinum-based chemotherapy regimens in the first-line recurrent or metastatic setting [4, 5], resistance invariably develops; therefore better treatment options are needed. Platinum-containing doublet chemotherapy is generally regarded as the standard first-line treatment for patients with RMNPC. The randomized phase III trial of gemcitabine and cisplatin (GC) versus 5-fluorouracil and cisplatin (PF) in RMNPC established GC as the standard of care first-line regimen. The overall response rate (ORR) in the GC arm was 64% compared to the PF arm of 42% [6]. Despite the superior outcome of the GC arm that reported a median survival of 29.1 months, RMNPC patients ultimately succumb to their advanced malignancy.

O. Ayodele
Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, Canada
e-mail: Olubukola.ayodele@uhn.ca

L. L. Siu (✉)
Princess Margaret Cancer Centre, Toronto, ON, Canada
e-mail: lillian.siu@uhn.ca

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The pathogenesis of NPC involves genetic, lifestyle, and viral infection factors [7, 8]. NPC is an Epstein-Barr virus (EBV)-associated cancer in which programmed cell death ligand 1 (PD-L1) expression is upregulated upon EBV activation. This feature is predominately among the WHO class II and III subtypes [9]. Expression of viral proteins in NPC cells can elicit a virus-specific immune response in patients with NPC [9]. Latent membrane protein 1 (LMP-1) expression and interferon-gamma activation can synergistically induce PD-L1 in NPC cells [10]. In fact, PD-L1 expression is reported to occur in 89% to 95% of NPC tumors [10–12]. This increased PD-L1 expression may be associated with better efficacy to immune checkpoint blockade using anti-PD1/L1 antibodies.

Development of new systemic therapies for RMNPC has stagnated in the past 20 years despite the conduct of numerous clinical trials. The exploration of molecular targeted therapy has been hindered by the absence of a definite genetic driver or actionable alterations in this malignancy. There are currently no approved targeted agents for RMNPC and no standard treatment options beyond the first-line setting for patients with platinum-refractory RMNPC.

In this review, we highlight potential new therapeutic options such as immunotherapy and molecularly targeted agents in patients with RMNPC.

Immuno-Oncology in RMNPC

Histologically, NPC is characterized by heavy infiltration of immune cells within its stroma. These cells consist mostly of CD3+, CD8+, regulatory T cells (Treg), natural killer cells, neutrophils, dendritic cells and mast cells [13]. The co-presence of active cancer cells together with abundant immune infiltrates reflects the underlying functional immune suppression within the NPC tumor microenvironment [14]. These dense immune infiltrates were postulated to be involved in the growth and invasive properties of NPC [15]. Studies have demonstrated an inverse relationship between survival and the density of tumor infiltrating CD8+, neutrophils and mast cells [13]. The immune system plays a critical role in the surveillance, prevention and development of cancer. Evasion of the immune system has been established as a hallmark of cancer [16]. It is therefore highly attractive to manipulate the immune system in such a way as to induce an antitumor response. The various immunotherapeutic strategies that have been employed in the management of RMNPC include immune checkpoint inhibitors, adoptive cell therapies, EBV directed vaccines, personalized cancer vaccines and oncolytic viruses.

Immune Checkpoint Inhibitors (ICI)

Due to the overexpression of PD-L1 in RMNPC, several single arm phase I/II studies of anti-PD1 antibodies have been conducted in this malignancy. KEYNOTE-028,

a phase Ib non randomized clinical trial of pembrolizumab (humanized IgG4) was the first single arm study to assess a PD-1 inhibitor in PD-L1 positive RMNPC. The NPC cohort enrolled 27 patients with a mixed background of treatment-naïve or pretreated squamous and non-squamous NPC. The PD-L1 status had to be positive (≥1 combined positive score [CPS] using 22C3 antibody) for trial enrollment. Partial response and stable disease were observed in seven and 14 patients, respectively, for an ORR of 25.9% and a 1-year overall survival (OS) of 63% [17]. A similar study by Ma et al. (NCI-9742) investigated the use of another PD-1 inhibitor nivolumab (fully human IgG4) and demonstrated an ORR of 20.5% and a 1-year OS of 59%. This was a phase II clinical trial of patients who had progressed after first-line chemotherapy containing platinum with no PD-L1 cut off point included (Table 23.1). A subgroup analysis showed response was higher among patients with PD-L1 positive tumors, defined as ≥1 membrane staining in tumor and immune cells using 22C3 antibody [18] (33% vs. 13% for PD-L1 positive versus negative patients respectively).

In another single arm study, Fang et al. [19] reported the findings of a newer PD-1 inhibitor camrelizumab (humanized IgG4) among 93 Chinese patients with RMNPC. This phase I clinical trial had no PD-L1 cut off and demonstrated an ORR of 34% and 1-year progression free survival (PFS) of 27.1%. In the same report, a separate small cohort of 23 patients who received a combination of GC-based chemotherapy with camrelizumab was included. This combination produced a staggering ORR of 91%, 6-month PFS and 12-month PFS of 86% and 61%, respectively. Other PD-1 inhibitors investigated in single arm phase I/II trials in China include toripalimab (JS001) and tislelizumab (BGB-A317). Their results were presented at the American Society of Clinical Oncology (ASCO) annual meeting 2019. Toripalimab, a humanized IgG4 anti-PD1 antibody was used in a phase II open label trial to treat RMNPC who had progressed on at least 2 lines of systemic treatment. One hundred and ninety patients were enrolled with no PD-L1 cut off, reporting an ORR of 25.5% [20]. Tislelizumab was investigated in a phase I/II single arm indication-expansion study among 21 Chinese patients with RMNPC. All patients had received at least one line of systemic treatment. An ORR of 43% was observed which is the highest response rate observed in single arm studies of PD-1 inhibitors [21]. This may be due to tislelizumab being engineered to minimize binding to FcγR on macrophages in order to override antibody-dependent phagocytosis which is a potential resistance mechanism to anti PD-1 therapy. However, inter-study comparisons are fraught with limitations due to heterogeneity in patient populations and other confounding factors.

There have been two randomized phase II studies in the platinum-pretreated RMNPC setting (Table 23.2). The first among them compared the anti-PD-1 antibody spartalizumab (PDR001) monotherapy (n = 82 patients) with chemotherapy (n = 40 patients), the latter can be monotherapy or doublet/triplet combinations. Patients who progressed on chemotherapy were allowed to crossover to the spartalizumab arm (n = 25). This trial did not meet its primary PFS endpoint when spartalizumab was compared to chemotherapy (1.9 vs. 6.6 months, HR 1.36, 95% CI = 0.87–2.12). The ORR (95% CI) in the spartalizumab arm versus chemotherapy

Table 23.1 Immunotherapy in recurrent or metastatic nasopharyngeal carcinoma (RMNPC) (single arm studies)

Author	Year	Country/ Region	Agent(s)	Phase	Sample size (n)	ORR (%)	PD-L1	mF/U (months)	mPFS (months)/1 year PFS (%)	mOS (months)/1 year OS
Hsu et al. KEYNOTE-028	2017	Multiregional	Pembrolizumab	IB	27	25.9	≥1%	20	6.5/34%	16.5/63%
Ma et al. (NCI-9742)	2018	Multiregional	Nivolumab	II	44	20.5	All comers	12.5	2.8/19.3%	17.1/59%
Fang et al	2018	China	Camrelizumab (monotherapy)	I	93	34	All comers	9.9	5.6/27.1%	NA
			Camrelizumab + GC	I	23	91		10.2	NR/61.4%	NA
Wang F et al	2019	China	Toripalimab	II	190	25.2	All comers	NA	NA	NA
Wang S et al	2019	China	Tislelizumab	I/II	21	43	All comers	11.7	10.4	NM

NA not available, *NM* not met, *ORR* overall response rate, *mPFS* median progression free survival, *mOS* median overall survival, *GC* gemcitabine +cisplatin

Table 23.2 Immunotherapy in RMNPC (randomized second line studies)

Author	Year	Country/ Region	Agent(s)	Phase	Sample size (n)	ORR (%)	PD-L1	mF/U (months)	mPFS (months)	mOR/1 year OS
Chan et al. KEYNOTE-122	2016	Multiregional	Pembrolizumab vs. CT	II	NA	NA	NS	NA	NA	NA
Lim et al.	2019	Multiregional	Spartalizumab vs. CT	II	122	17.1 vs. 35	NS	12	1.9 vs. 6.6	25.2 vs. 15.5

NA not available, *NS* not specified, *CT* chemotherapy

arm was 17.1% (9.7–27.0) versus 35% (20.6–51.7) respectively. The ORR for monotherapy chemotherapy was 26.9% and for doublet/triplet chemotherapy was 58.3%. In the crossover arm from chemotherapy at disease progression to spartalizumab, ORR was 8.0% (1.0–26.0). However, of interest, the median duration of response was higher in the spartalizumab arm than in the chemotherapy arm (10.2 vs. 5.7 months). Median OS was also numerically longer in spartalizumab treated patients (25.2 vs. 15.5 months) but this study was not powered for this endpoint [22]. Results from the second randomized trial, KEYNOTE-122 (NCT02611960), are yet to be published.

The high response rate observed in camrelizumab in combination with chemotherapy suggests that combination of immunotherapy with chemotherapy might be the best way to elucidate response in RMNPC. Three randomized phase III trials investigating first-line anti-PD1 antibody with GC combinations are currently recruiting RMNPC patients (NCT03707509, NCT03581786, and NCT03924986) in China (Table 23.3). In addition, an international randomized study comparing nivolumab in combination with GC versus GC alone is actively being planned by the NRG cooperative group (HN007 now activated, NCT04458909).

Adoptive Cell Therapy

Adoptive cell therapy is a new therapeutic strategy based on the modulation, manipulation, and selection of autologous T cells in vitro to overcome the tolerance of the immune system to tumor cells. The T cells can be harvested from tumor infiltrating lymphocytes (TIL) and reinfused into the donor patient after population expansion is ensured. Lymphocyte T cells can also be harvested from peripheral blood, with those that recognize tumor antigens being selectively expanded. Alternatively, lymphocyte T cells can be genetically engineered either by modifying a T cell receptor for a cancer antigen ("transgenic T cell receptor" or TCR T cell) or by adding a chimeric antigen receptor that recognizes a specific cancer antigen (CAR T cell). Endemic NPC is associated with EBV, therefore targeting EBV antigens expressed

Table 23.3 Randomized First line immunotherapy combinations in RMNPC

Clinical trial identifier	Investigative product	Phase	Estimated sample size	Endpoints	Country/ region	Status
NCT03707509	Camrelizumab+GC vs. GC	III	250	PFS, ORR, DCR, OS	China	Active
NCT03581786	Toripalimab+GC vs. GC	III	280	PFS, ORR, DCR, OS	China, Singapore, Taiwan	Active
NCT03924986	Tislelizumab+GC vs. GC	III	256	PFS, OS, ORR, DOR	China	Active

GC gemcitabine+cisplatin, *DCR* duration of continued response

in non-keratinizing and undifferentiated NPC is an attractive approach to improve outcomes for patients with advanced disease.

Adoptive transfer of EBV-specific cytotoxic T lymphocytes (EBV-CTL) as a single agent therapy has shown some benefit in phase I and II NPC studies [23–27]. Chia et al. conducted a phase II trial exploring the role of cytoreductive chemotherapy followed by autologous CTL in previously untreated patients with advanced EBV associated NPC. The patients received four cycles of carboplatin and gemcitabine followed by six doses of EBV-CTL. This combination was well tolerated and resulted in an encouraging response rate of 71.4% with 3 complete and 22 partial responses [28]. Based on these promising results, a multicenter phase III randomized controlled trial using this protocol is underway (NCT02578641). There is strong evidence of antitumor activity for EBV-CTL in patients with NPC, however response rates vary between the reported clinical trials. Contributing factors may include different technical approaches used for the generation of the EBV-CTL; variable patient populations with different stages of disease, genetic predispositions, comorbidities and the impact of prior therapy. A novel approach in adoptive cell therapy is the use of allogeneic EBV-specific TIL, tabelecleucel, in combination with pembrolizumab in platinum-pretreated EBV positive RMNPC (NCT03769467). This is a multicenter, open label single-arm phase Ib/II study. Tabelecleucel will be selected for each subject from a bank of available tabelecleucel cell products based on the matching of ≥2 human leucocyte antigen (HLA) alleles, at least one of which is a restricting HLA allele shared between the tabelecleucel donor and the subject's EBV+ NPC.

The use of CAR T and TCR T cell therapy is relatively new in solid tumors. These represent a promising strategy that has demonstrated effective and durable responses in hematological malignancies. In a preclinical study, Tang et al. demonstrated reduced tumor growth in EBV associated NPC treated with CAR T cells [29]. Several clinical trials including phase I and II treating RMNPC with CAR T and TCR T cell therapy are ongoing (Table 23.4).

The toxic effects attributable to the activation of the host immune system have always been a major concern for adoptive cell therapy. Cytokine release syndrome (CRS) is a common toxicity observed with adoptive cell therapy. It has a heterogeneous presentation but usually involves fever, hypotension, tachycardia and respiratory insufficiency and it can be potentially fatal. The severity of CRS is correlated

Table 23.4 Ongoing CAR-T and TCR-T cell therapy trials

Treatment class	Trial	Clinical trial Identifier
CAR-T	Phase I trial of EpCAM CAR-T	NCT02915445
CAR-T	Phase I/II trial of LMP1-CAR-T	NCT02980315
CAR-T	Phase I trial of NKG2DL-CAR-γδ-T	NCT04107142
TCR-T	Phase II trial of EBV-TCR-T (YT-E001)	NCT03648697
TCR-T	Phase I trial of LMP2-specific TCR-T	NCT03925896

EpCAM epithelial cell adhesion molecule, *CAR-T* chimeric antigen receptor T cell, *LMP1* latent membrane protein 1, *NKG2DL* natural killer group 2D ligand, *EBV* epstein Barr virus, *LMP2* latent membrane protein 2

with tumor burden [30]. Despite the toxicity profile of cell therapy approaches, the rationale of using them in EBV-directed cancer such as NPC can be justified given the presence of viral antigens that can be the target of such EBV-directed therapies.

Therapeutic Vaccines

Cancer therapeutic vaccines are designed to boost the adaptive immune response of patients by delivering different forms of tumor associated antigens into the body. Peptide based and dendritic cell (DC) vaccines have been investigated in EBV associated NPC.

A recombinant vaccinia Ankara vaccine (MVA-EL) is a peptide based vaccine that encodes inactive proteins such as full length LMP2 and C-terminal of EBNA1. This vaccine was investigated in a phase I trial in patients from Hong Kong and United Kingdom [31, 32]. The combined analysis of 27 RMNPC patients, demonstrated detectable immunologic T cell response to at least one vaccine coded antigen in 20 patients (74%). A phase II study is underway for formal efficacy evaluation in RMNPC (NCT01094405).

Dendritic cells (DC) play a vital role in the activation of CD4+ and CD8+ T cells, triggering robust T cell immune response to the tumor antigens. Enhanced CD8+ T cell response was observed in 9 of 16 NPC patients who had been vaccinated with LMP2 peptide epitope pulsed autologous DC in a phase I trial [33]. Partial remission was observed in 2 of 16 patients. Another DC vaccine called CD137L-DC-EBV-VAX is being investigated in a phase I study that is currently recruiting patients with locally advanced or RMNPC (NCT03282617).

Personalized Cancer Vaccines (PCV)

PCV are designed based on cancer specific peptides, or neoantigens, expressed by each patient's tumor tissue which harbor genomic alterations such as mutations. To create an individualized cancer vaccine, neoantigens must be identified, and then a cell-, protein- or nucleic acid based platform is used to deliver these neoantigens to patients to prime the immune system to attack the tumor. Antigen presenting cells such as DC internalize the cancer specific peptides selected for a PCV and display them on their surface with the help of major histocompatibility complex (MHC) proteins. This triggers T cells with receptors that bind these neoantigens to differentiate into effector, or killer T-cells that mobilize an immune reaction against cancer cells. Next generation sequencing data from tumor and normal DNA are aligned and compared to each other to identify tumor specific alterations. Neoantigens are then assessed and prioritized in order to select the ones most likely drive a robust immune response against the tumor. The selected sequences are evaluated by computer models and algorithms that predict the binding of the neoantigens to the MHC proteins

Table 23.5 Ongoing clinical trials investigating personalized cancer vaccines (PCV)

Clinical trial identifier	Phase	Cancer type	Vaccine	Other agents	Target accrual	Status
NCT03313778	I	Unresectable solid tumor	mRNA-4157	Pembrolizumab	90	Active
NCT03289962	I	Advanced solid tumor	RO7198457	Atezolizumab	770	Active
NCT03662815	I	Advanced malignant tumor	iNeo-Vac-P01	GM-CSF	30	Active
NCT03568058	I	Advanced solid tumor	PCV (not specified)	Pembrolizumab	30	Active
NCT03671720	I	Advanced solid tumor (High tumor mutation burden)	PCV (not specified)	Cyclophosphamide	10	Active
NCT02721043	I	Advanced solid tumor	PGV001	Poly-ICLC	20	Active

GM-CSF granulocyte-macrophage colony stimulating factor, *mRNA* messenger ribonucleic acid

that would present them on the surface of cells. These PCV can be either DNA or RNA based. The promising results of early preclinical and clinical work on neoantigen vaccines have led to a number of clinical studies of personalized neoantigen vaccine based immunotherapy. Table 23.5 illustrates ongoing studies investigating PCV in solid tumors in which patients with NPC can be included.

Oncolytic Viruses (OV)

Oncolytic viruses have the ability to kill cancer cells directly as well as induce the secretion of various cytokines and chemokines to facilitate tumor antigen expression and presentation, thereby recruiting immune cells into tumors [34]. They selectively replicate in and kill cancer cells and they spread within the tumor while not harming normal tissue. They have been genetically modified to improve their safety and efficacy. OV encompass a broad diversity of DNA and RNA viruses that are naturally cancer selective. The activity of OV is very much a reflection of the underlying biology of the viruses from which they are derived and the host-virus interactions. Many of the hallmarks of cancer provide a permissive environment for OV; these include sustained proliferation, resisting cell death, evading growth suppressors, genome instability, DNA damage stress and avoiding immune destruction. In addition, insertion of foreign sequences can endow further selectivity for cancer cells and safety. G47Δ, a third generation herpes simplex virus 1 demonstrated some antitumor effect in EBV associated NPC [35]. There are over 10 different oncolytic viruses that have been used in antitumor research [34].

Molecularly Targeted Agents

Several molecularly targeted agents have been considered as second line systemic agents for RMNPC patients with a good performance status who become refractory to platinum-based regimens. Vascular endothelial growth factor receptor inhibitors and epithelial growth factor receptor inhibitors have particularly been studied in RMNPC.

Vascular Endothelial Growth Factor Receptor (VEGFR) Inhibitors

The VEGF-VEGFR interaction activates a signaling cascade that promotes angiogenesis, tumor growth and metastasis [36, 37]. It has been shown that NPC is characterized by high expression of VEGFR-2, which in turn is adversely correlated with poor survival [38]. This mechanism has driven the development of therapies geared towards molecular targeting of VEGF-VEGFR in the management of patients with RMNPC. Axitinib, sorafenib, pazopanib, famitinib and sunitinib are multi-targeted tyrosine kinase inhibitors (TKI) of VEGFR that have demonstrated promising clinical activity in RMNPC. The efficacy of this class of agents has been demonstrated in several clinical trials of single agent VEGFR inhibitors or in combination with chemotherapy. In a comprehensive literature review by Almobarak et al. [39], the highest observed ORR of 77.8% with a median OS of 11.8 months was reported in a trial combining sorafenib with 5FU and cisplatin [40]. Axitinib demonstrated the highest ORR of 30.4% with median OS of 10.4 months as a single agent [41]. However, given the mechanisms of action, bleeding especially tumor-associated hemorrhage is a relevant concern with VEGFR inhibitors in RMNPC, especially in cases with prior high dose radiotherapy to the head and neck and in those with direct vascular invasion by tumor [42].

Newer agents such as apatinib and anlotinib have recently been explored in RMNPC. Results from a phase II trial presented at ASCO 2019 by Jiang et al. [43] demonstrated clinical activity with apatinib. Apatinib, a novel small molecule highly selective inhibitor of VEGFR-2 was given at the dose of 500 mg daily to 33 patients across three centres in China after failure of first-line chemotherapy. This trial yielded an ORR of 36.3%, with disease control rate (DCR) of 54.5% after a median follow up time of 14 months. Median PFS of 5.0 months was observed with 1-year OS rate of 83.1%. In a first-line phase III clinical trial in progress presented by Yang et al. [44] at ASCO 2019, anlotinib which is a novel multitarget TKI that targets VEGFR 1–3, fibroblast growth factor receptor 1–4 and platelet derived growth factor receptor α and β was given in combination with GC compared with placebo plus GC. Fifty eight patients had been recruited out of 336 as at the time of poster presentation in ASCO. Results of this trial should be available sometime in 2021 (NCT03601975).

Epidermal Growth Factor Receptor (EGFR) Inhibitors

The EGF-EGFR interaction activates the Ras-Raf-MEK-ERK signaling pathway, which plays various important biological roles, such as apoptosis, cell growth, cellular differentiation and malignant transformation. Non-keratinizing NPC is characterized by high expression of EGFR, as well as *EGFR* gene amplification in pre-clinical NPC models and patients' tumor samples. EGFR expression in NPC is associated with poor clinical and survival outcomes. Thus, molecular targeting of EGFR is a plausible therapeutic aim in recurrent and metastatic NPC. Cetuximab, gefitinib and erlotinib are EGFR inhibitors that have been studied in RMNPC [39]. Unfortunately, they have not been shown to offer meaningful clinical and survival benefits to patients with RMNPC [39].

Overall, the role of molecularly targeted agents in RMNPC has fallen short of expectations due to shortcomings that include absence of validated predictive biomarkers, small study sample sizes, lack of phase III trials and short duration of follow-up of the studies reporting molecular targeted therapy in patients with recurrent and metastatic NPC. Thus far, molecular targeted therapy in RMNPC has not been able to identify and interrogate the most important and actionable drivers in this malignancy. Furthermore, the absence of evaluation of quality of life before and after administration of molecular targeted therapy is an additional shortcoming. All these caveats, collectively, contribute to a limitation in drawing concrete conclusions. Thus, as it stands now, the role of molecular targeted therapy in patients with RMNPC remains to be further investigated.

Epigenetic Therapy in RMNPC

NPC is associated with genetic alterations on particular chromosomal regions and genes, harboring of specific cancer-associating single nucleotide polymorphisms (SNP), and familial aggregation. Interestingly, recent studies confirm that epigenetic alterations, including the promoter hypermethylation, are also one of the crucial factors that are highly associated with NPC [45].

Two genome-wide methylome studies consistently identified a few important signaling pathways and functions often deregulated by DNA methylation in NPC, including the Wnt, MAPK, Hedgehog, and TGF-β signalling pathways and focal adhesion [45, 46]. In the Wnt signalling pathway, a number of Wnt inhibitors including *DKK1*, *WIF1*, *SFRP1*, *SFRP2*, *SFRP4*, and *SFRP5* are silenced by promoter methylation in NPC. Inactivation of these inhibitors may lead to the aberrant activation of Wnt signaling and transcription of its downstream targets. The enrichment of the genes with homeobox domain in the aberrantly methylated genes in NPC indicates these aberrantly methylated genes are often polycomb complex targets. Many studies have demonstrated that the polycomb repressive complex genes encoding epigenetic gene-silencing proteins contribute to the oncogenesis of various cancers.

Overexpression of the polycomb complex genes *BIM1* and *EZH2* was demonstrated in NPC tumor [47, 48].

MAK683 is an inhibitor of embryonic ectoderm development protein (EED) and allosteric inhibitor of polycomb repressive complex 2 (PRC2) with potential antineoplastic activity. There is a phase I/II clinical trial of MAK683 currently recruiting where patients with RMNPC are eligible (NCT02900651).

Notably, co-deletion of the gene encoding the methionine salvage pathway enzyme methylthioadenosine phosphorylase (MTAP), which is adjacent to the CDKN2A/p16 locus on 9p21.3 is common in NPC [49–51]. Recent studies have uncovered the pharmacologic vulnerability of MTAP-deficient tumors through drugging of the MAT2A/PRMT5/RIOK1 axis [49–51]. In MTAP/CDKN2A-deleted tumors, loss of MTAP leads to disordered methionine metabolism with impaired cleavage and accumulation of metabolite methylthioadenosine, thereby inhibiting protein arginine N-methyltransferase 5 (PRMT5) activity. Knockdown of PRMT5 and its downstream enzyme MAT2A by siRNA has shown selective growth inhibition in MTAP-deleted tumor cells [49–51]. This high frequency of MTAP loss in NPC makes PRMT5 a relevant target. There are several PRMT5 inhibitors currently being tested in clinical trials in which patients with RMNPC are a subset (Table 23.6).

Precision medicine approach on an array of druggable targets in NPC can be helpful for related subsets of patients bearing specific genomic aberrations. Once supported by more preclinical data of gene-based precision therapies for NPC, basket trials would be helpful to determine the clinical benefits of precision therapies for NPC.

Conclusion and Future Approaches

Systemic therapy in NPC represents an unmet clinical need in locally advanced and RMNPC. NPC is a complex disease that involves host genetics, viral infection, and environmental factors. It is characterized by a comparatively low mutation rate, extensive hypermethylation, as well as frequent chromosomal abnormalities and copy number alterations. Overall, EBV plays a critical role in driving the development of NPC, but it may also provide distinctive targets and opportunities for immune therapies, which warrants integrated approaches from basic science and

Table 23.6 Ongoing clinical trials investigating PRMT5

Clinical trial identifier	Phase	Cancer type	Agents	Target accrual	Status
NCT03573310	I	Advanced solid tumor	JNJ-64619178	120	Active
NCT02783300	I	Advanced solid tumor	GSK3326595, pembrolizumab	416	Active
NCT04089449	I	Advanced malignant tumor	PRT811	125	Active

clinical perspectives. In addition, with the rapid development of precision medicine, we can optimize the combination of immunotherapy according to the different genomic characteristics and immune status of individual patients and customize therapy to obtain the maximum clinical effect.

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Part VI
Keynote Lecture

Chapter 24
Innovation and Advances in Precision Medicine in Head and Neck Cancer

Geoffrey Alan Watson, Kirsty Taylor, and Lillian L. Siu

Introduction

The past decade has marked the emergence of precision cancer medicine, a diagnostic and therapeutic approach that aims to comprehensively characterize the clinical, molecular and immunologic aspects of a patient's tumor in order to tailor management [1]. Upon reflection, this approach has encountered a mix of successes with demonstration of clinical utility and failures that have led to disappointments. For the proponents of precision medicine, the glass has been half full and the complete potential of this framework has just begun to be realized. For instance, the genotype-drug matching strategy has potently inhibited oncogenic addiction in some malignancies, yielding spectacular objective responses and sustained clinical benefit. Some examples are disease-specific such as the use of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC) harboring *EGFR* mutations, whereas other indications are histology-agnostic such as neurotrophic tyrosine receptor kinase (NTRK) inhibitors for tumors with *NTRK* gene rearrangements. Furthermore, large scale next generation sequencing (NGS) initiatives to profile cancers have substantively increased knowledge in cancer biology, and provided insights into clonal evolution and mechanisms of therapeutic resistance in oncology. The sharing of clinical and genomic results among institutions worldwide, in efforts such as the American Society of Clinical Oncology (ASCO)'s CANCERLINQ and the American Association for Cancer Research (AACR)'s Project Genomics Evidence Neoplasia Information Exchange (GENIE), has enabled big data learning [2, 3]. Conversely, for the opponents of precision medicine, the proportion of patients who have undergone NGS and ultimately

G. A. Watson · K. Taylor · L. L. Siu (✉)
Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, Canada
e-mail: Geoffrey.watson@uhn.ca; ktaylor13@qub.ac.uk; lillian.siu@uhn.ca

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benefitted from genotype-target matching has been consistently small, raising concerns on the low cost to benefit ratio of this strategy [4].

The Current Landscape of Large Scale Genomics Based Data Research in HNSCC

Squamous cell carcinoma of the head and neck (HNSCC) represents the sixth most common cancer worldwide. Risk factors include smoking, alcohol and infection with high risk types of human papillomavirus (HPV) [5]. The main treatment modalities include surgery, radiation and chemotherapy, although survival benefit is modest in the advanced setting. Until recently therapeutic options for recurrent or metastatic, platinum resistant HNSCC have been limited, however the emergence of immuno-oncology in this setting has been a welcome addition to the treatment armamentarium for these patients [6, 7]. This has been accompanied by an epidemiological shift, with reduced smoking rates resulting in decreased rates of HPV negative (−) cancers in some countries, whereas others are reporting increasing rates of the biologically distinct, more prognostically favorable HPV-associated (+) HNSCC [8–10]. Despite these seemingly advantageous epidemiological and management shifts, survival rates of high risk locoregionally advanced disease, as well as recurrent or metastatic disease, remain poor. As such it is imperative to further elucidate the molecular pathogenesis of these malignancies, which may facilitate attempts in developing a more tailored, patient specific treatment approach to improve outcomes in patients with advanced HNSCC.

It has become increasingly recognised that HNSCCs are comprised of distinct molecular subtypes [11]. While the development of targeted therapies has been met with success in various malignancies, the diversity of genetic aberrations, the heterogeneous mutational spectrum, and the lack of actionability of the majority of genomic-based alterations observed in HNSCC make a precision-medicine based approach particularly challenging. In an effort to identify further actionable targets, concentrated efforts have been made to provide comprehensive multi-platform, genome wide profiling studies to annotate molecular aberrations in a wide variety of malignancies including HNSCC.

Initial attempts at exploring and curating the etiology and landscape of mutations in human cancer resulted in the development of the Catalogue Of Somatic Mutations In Cancer (COSMIC) (https://cancer.sanger.ac.uk) in 2004. COSMIC includes all the genetic mechanisms by which somatic mutations promote cancer, including coding and non-coding mutations, gene fusions, copy-number variants and drug-resistance mutations [12]. More recently scientific innovation has enabled big data analytics; whole exome capture and massive parallel sequencing of cancer genomes have further augmented our understanding of the mutational landscape of HNSCC. The first reports of whole exome sequencing of HNSCC were published in 2011, which provided a glimpse into the extensive network of molecular changes

underlying HNSCC [13, 14]. These studies demonstrated a mutation rate consistent with that seen in other smoking-related malignancies, and identified the six most frequently mutated genes that may potentially encode key signaling molecules for HNSCC tumorigenesis: *TP53*, *NOTCH1*, *CDKN2A*, *PIK3CA*, *HRAS*, and *PTEN* genes. The true significance of these early studies however was in the validation of large-scale sequencing in exposing fundamental tumorigenic mechanisms.

In 2015 The Cancer Genome Atlas (TCGA) then became the catalyst for systematic characterization of diverse genomic alterations underlying human malignancies, which now represents the most comprehensive integrative genomic analysis of HNSCC. TCGA has yielded numerous novel biological insights, and has had a profound impact on how cancer genomics is now conducted. It utilises a collaborative approach to harmonize data and standardize analyses with the ultimate aim of enhancing our knowledge of cancer biology and pathogenesis. TCGA has profiled 500 HNSCC tumors, and has aided in further characterizing the groups of genes implicated in its pathogenesis, such as genes important for cell survival and proliferation (*TP53, HRAS, EGFR*, and *PIK3CA*), cell-cycle control (*CDKN2A* and *CCND1*), cellular differentiation (*NOTCH1*), adhesion and invasion signaling (*FAT1*) [13–15].

Analyses from the first 279 patients reported copy number alterations (CNAs) including losses of 3p and 8p, and gains of 3q, 5p and 8q chromosomal regions resembling squamous cell carcinomas of the lung [16]. The amplification of 3q26/28 region containing squamous lineage transcription factors, *TP63* and *SOX2*; and *PIK3CA* oncogene is seen in both HPV subtypes, but more frequently in the HPV(+) subtype [17, 18]. HPV(+) tumors were distinguished by novel recurrent deletions and truncating mutations of TNF receptor-associated factor 3 (*TRAF3*), the loss of which promotes aberrant NF-κB signaling [19]. In addition, focal amplification of *E2F1* and an intact 9p21.3 region containing the *CDKN2A* gene were seen. This latter region is commonly deleted in HPV(−) tumors, which also feature co-amplifications of regions containing genes implicated in cell death/NF-κB and Hippo pathways such as 11q13, containing *CCND1*, *FADD* and *CTTN*, and 11q22 containing *BIRC2* and *YAP1*. Recurrent focal amplifications in receptor tyrosine kinases (*EGFR*, *ERBB2* and *FGFR1*) also predominate in HPV(−) tumors. However, a potential limitation in the TCGA data is that most of the sequenced tumors were acquired from early-stage surgical samples, while samples of recurrent/metastatic disease were underrepresented. The latter would likely reveal distinct genetic profiles due to various phenomena including clonal evolution and treatment selection pressures, thus TCGA data may not entirely inform the biological drivers of recurrent and metastatic HNSCC in which most novel targeted agents are currently being tested. Moreover most studies also included only a small number of HPV(+) cases, and many were conducted in heterogeneous patient populations without detailed clinical annotation; as such they may lack the power to determine prognostic and predictive value of genetic alterations identified [18].

An emerging knowledgebase in the current genomic era is the coordinated acquisition and examination of data derived from real world NGS initiatives. The AACR's Project GENIE is another collaborative, international effort aimed at integrating

large scale cancer genomic data and clinical outcomes obtained from participating institutions in the real world setting [3]. To date, the AACR GENIE dataset includes nearly 80,000 de-identified genomic records collected from patients treated at each of the consortium's participating institutions, which are then made available to the global scientific community. The combined dataset now includes data for 80 major cancer types including samples from approximately 1300 patients with HNSCC, and almost 40% represent those collected in the metastatic disease setting. The relative frequencies of the most common somatic mutations in each of the aforementioned databases are quite similar. Some of the frequently mutated genes have matching targeted therapies that may be used to treat HNSCC cases with specific aberrations, generally under the auspice of clinical trials (Fig. 24.1) [3, 20–22].

Biomarker-Based Treatment Strategies

The above mentioned data-sharing platforms have profoundly promoted translational and clinical discovery, providing the impetus for the development of novel therapeutic targets, design of new biomarker-driven clinical trials, and offering a deeper understanding of patient response to therapy. As an increasing number of genetic alterations are identified, one pivotal challenge has been the difficulty matching effective drugs to genomic profiles. Potential targets include driver oncogenes such as *PIK3CA*, of which genomic alterations are associated with both HPV(+) (56%) and HPV(−) (34%) HNSCC cases [23, 24]. Several trials exploring agents that target the PI3K pathway in patients with HNSCC have been largely disappointing, however notable exceptions include combination studies of apelisib (BYL719), a PI3K class I α isoform inhibitor, co-administered with cetuximab; and

Fig. 24.1 The list of common mutations identified in head and neck squamous cell carcinoma in The Cancer Genome Atlas and the frequency of each mutation to date in samples catalogued in the AACR GENIE (American Association for Cancer Research-Genomics Evidence Neoplasia Information Exchange) database. Courtesy of AACR GENIE [3] via cBioPortal [21, 22]

buparlisib, a pan-PI3K inhibitor, co-administered with paclitaxel, where some signals of activity have been observed in early studies [23, 25–27].

The value of DNA-based biomarkers has already demonstrated clinical utility in cancer therapeutics, with many key examples such as anti-HER2 therapies for *HER2* amplified breast cancer and EGFR inhibitors for *EGFR* mutated NSCLC [28, 29]. To date there have been few biomarker-driven trials dedicated to HNSCC. Beyond *PIK3CA*, actionable mutations in other oncogenic driver genes in HNSCC such as *ERBB*, *FGFR*, and *MET* are relatively rare, making it challenging to conduct biomarker directed clinical trials. The EORTC 1559 study (NCT03088059) sought to address this, and is the first international umbrella biomarker-driven study implemented for patients with recurrent and/or metastatic HNSCC [30]. EORTC 1559 (UPSTREAM) attempts to better ascertain upfront the patients who will benefit from a specific treatment, by investigating the activity of immunotherapy or targeted agents in tumors harboring a pre-defined biomarker(s). NGS is carried out to identify somatic mutations and copy number alterations with a custom panel that included 13 oncogenes and tumor suppressor genes (*EGFR, HER2, TP53, PIK3CA, CCND1, NRAS, KRAS, HRAS, PTEN, FGFR1, FGFR2, FGFR3*, and *cMET)*. The analysis also includes p16 and PTEN expression by immunohistochemistry [31]. Based on the molecular aberrations identified and a pre-defined algorithm, patients were allocated to different treatment cohorts including afatinib, palbociclib, niraparib and entrectinib. Patients not eligible for these biomarker-driven cohorts were included in one of the immunotherapy cohorts (monalizumab monotherapy or monalizumab plus durvalumab) [30]. The UPSTREAM study design is dynamic and allows new treatment arms that target other important genetic aberrations, such as *PIK3CA* and *HRAS,* to be added through protocol amendments. Of note recent phase II data evaluating the efficacy of the farnesyl transferase inhibitor tipifarnib in patients with recurrent and metastatic *HRAS*-mutant HNSCC reported objective responses, and thus further investigation in this malignancy is warranted (NCT02383927) [32].

Innovative Clinical Trial Designs

Despite the development and implementation of innovative, precision medicine clinical trial design strategies such as the EORTC 1559 trial described above, to date these trials have largely been centred on molecular matching strategies with predetermined monotherapies [33–40]. Limitations of this approach include low matching rates, possibly due to limited gene panels, restrictive matching algorithms, non-targeting of co-existing resistance aberrations and lack of drug availability [41]. As such combination strategies have begun to be explored in this setting. Traditionally combination strategies have often been employed to induce a synergistic effect and enhance the anti-tumor activity of therapeutic agents, and impede the development of resistance. This approach has been met with some success already, using the aforementioned PI3K inhibitors in combination with both paclitaxel and cetuximab.

Another example is the combination of palbociclib, a cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor that is associated with objective responses in HPV (−) HNSCC patients when combined with cetuximab [42]. To further explore the customization and personalization of multidrug combination regimens, the I-PREDICT study (NCT02534675) was designed for patients with refractory malignancies [43]. This multi-institutional prospective study utilised tumor DNA sequencing and relied on timely recommendations from a molecular tumor board to provide personalized treatment decisions with combination therapies. The feasibility of this approach was demonstrated with 49% of consented patients receiving individualized combination treatment. Strategies to design clinical trials that test personalized combination regimens in HNSCC are needed.

While the evolution of NGS has augmented the identification of potentially actionable molecular variants, it has become increasingly recognised that these patients may be treated with drugs outside of their approved label indications, and outcomes after employing these targeted therapies may not be systematically collated and shared. The Drug Rediscovery Protocol (DRUP) was implemented to address this shortcoming, with the goal of identifying signals of response in patients with defined tumor types and molecular variants, who are being treated with anticancer drugs outside of their approved label [44]. The study reported an overall rate of clinical benefit (defined as complete or partial response, or as stable disease beyond 16 weeks) of 34% in 215 treated patients, comprising 136 patients who received targeted therapies and 79 patients who received immunotherapy. The overall median duration of clinical benefit was 9 months (95% confidence interval of 8–11 months), including 26 patients who were experiencing ongoing clinical benefit at data cut-off [44]. This trial again demonstrated feasibility of multidrug precision oncology trials, and facilitated the defined use of approved drugs beyond their labels in rare subgroups of cancer.

Similarly, the Targeted Agent and Profiling Utilization Registry (TAPUR) (NCT02693535) study, led by ASCO, was also designed to describe efficacy and toxicity of commercially available, targeted anti-cancer drugs prescribed for treatment of patients whose tumors have a genomic variant known to be a drug target, or to predict sensitivity to a drug [45, 46]. Patients were matched into multiple parallel cohorts defined by tumor type, genomic alteration, and drug. Examples of drug targets and respective treatment arm include *MET* (Crizotinib), *CDKN2A* (2 arms – palbociclib and abemaciclib) and *ERBB2* (trastuzumab and pertuzumab). The Canadian Profiling and Targeted Agent Utilization Trial (CAPTUR) (NCT03297606) is a Canadian Cancer Trials Group led study that leverages existing clinical genomic profiling platforms, and also aims to test the activity of commercially available targeted agents in patients with advanced cancers with 'druggable' mutations [47]. Cohorts are again defined by tumor type, genomic alteration and matched drug treatment. Examples of those with potential relevance to HNSCC include *MET* (crizotinib), *EGFR* (erlotinib), *CDKN2A/CDK4* (palbociclib), *FGFR* (sunitinib), *PIK3CA* (temsirolimus) and *ERBB2* (trastuzumab and pertuzumab).

An innovative development in the pursuit to identify druggable targets involves functional testing, such as small interfering RNA (SiRNA) and drug libraries on

patient derived cell cultures [48]. siRNAs may be used as tools to study single gene function both in vivo and in vitro and represent an attractive new class of therapeutics, particularly against undruggable targets. Xu et al. recently performed comprehensive genomic analyses together with genome-scale siRNA using low-passage tumor cells derived from a patient with treatment-resistant HPV (−) HNSCC. While genomic analysis revealed a heterogeneous mutational profile typical for HPV (−) HNSCC, no drug targets were identified. In contrast, siRNA profiling identified 391 candidate target genes, 35 of which were preferentially lethal to cancer cells. Further studies are warranted but functional profiling may potentially become a useful adjunct to DNA sequencing to guide the therapeutic decision making process for precision oncology.

Adapting to the Evolution of Cancer

For precision medicine to be truly efficacious, it is necessary to recognize and adapt to the evolution of cancer. As discussed this has become an attainable goal due to advances in our ability to comprehensively examine tumor derived material, coupled with the development of increasingly sensitive assays and massive parallel sequencing technologies to detect and analyse cancer specific analytes and their alterations. This has paved the way for the introduction of liquid biopsies, a minimally invasive method designed to assess circulating tumor (ct) DNA, which has received considerable attention as a potential biomarker and surrogate for tissue biopsy [49, 50]. The evaluation of ctDNA is a powerful tool that can be used to longitudinally inform on the real time presence or absence of cancer, compared to a tissue biopsy which only gives a single, static snapshot in space and time. There exists several potential applications for ctDNA, for example monitoring for molecular residual disease (MRD), which describes the detection of cancer-derived molecular biomarkers when the cancer may be radiologically occult (Fig. 24.2). Other examples include early

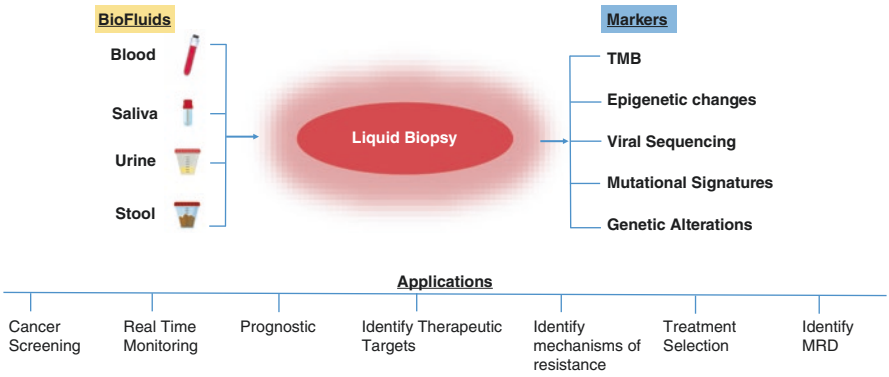

Fig. 24.2 Liquid biopsy sources, markers and applications

assessment of treatment response and further informing on the mechanisms of response or resistance to personalize treatment strategies [50, 51].

It is important to recognize however, that many factors have the potential to influence the abundance and detectability of ctDNA in cancer patients. At diagnosis, anywhere from >90% to <0.1% of plasma DNA is tumor-derived [52]. Tumor type and location influence ctDNA levels, as do prior treatments; other potential confounders such as demographic, comorbidity and environmental factors are less well characterized [51]. Furthermore, ctDNA has a short half-life (of around 1 h) and its kinetics can be complex, thus the timing of blood collection is also significant in order to ensure accurate interpretation of results.

Monitoring in Minimal Residual Disease (MRD)

One of the most appealing clinical applications of ctDNA is to detect cancer recurrence in the MRD setting after definitive local or locoregional therapy, as it offers the opportunity to initiate salvage therapy early (if available), eradicate micrometastatic disease and maximize cure. Observational studies correlating the presence of ctDNA or specific genomic aberrations with disease outcome have shown a prognostic role across multiple tumor types, with positive ctDNA status typically preceding the occurrence of clinical relapse by a few months [53]. In addition to somatic alterations, other cancer-specific biomarkers that may potentially be evaluated by ctDNA include mutational signatures, tumor mutational burden, tumor associated epigenetic changes and methylation patterns, and viral sequencing (Fig. 24.2) [50]. This has been coupled with the development and maturation of technologies and their associated platforms designed to facilitate this evaluation, such as NGS, Digital-PCR, Real-time PCR and mass spectrometry. Wang et al. previously demonstrated feasibility of this approach in HNSCC patients, detecting tumor DNA in postsurgical patients months before the onset of clinical recurrence [54]. More recently ct HPV DNA was longitudinally monitored in patients with HPV associated oropharyngeal cancer post treatment with curative intent to explore its role as a potential biomarker in detecting recurrence, and demonstrated high positive and negative predictive values as a post treatment surveillance strategy [55].

Selecting Patients for Personalized Treatment

In addition to the above applications, ctDNA offers insight into genomic changes in the tumor that may guide therapeutic decisions. ctDNA data generated using high-throughput NGS panels can provide value by directly identifying known or new actionable mutations for genotype–drug matching. For example, ctDNA has been incorporated into standard of care as a less invasive alternative to tissue biopsy for detecting the *T790 M* mutation in *EGFR* mutant NSCLC patients who are

progressing on first-generation tyrosine kinase inhibitors [56]. The B-FAST trial is a phase 2/3 multicentre multi-cohort study evaluating the safety and efficacy of targeted therapies or immunotherapy as single agents, or in combination, in participants with unresectable, advanced or metastatic NSCLC (NCT03178552). Patients were enrolled into four specific molecularly defined treatment cohorts based on identification of genetic alterations using only blood-based NGS [57]. Studies similar to the B-FAST design can be extrapolated to HNSCC to enable precision medicine evaluation using ctDNA as a minimally invasive tool.

Prediction of Treatment Outcome

Early changes in ctDNA dynamics after treatment can inform on therapeutic efficacy, as demonstrated in a retrospective analysis of samples from the phase III PALOMA-3 trial in advanced estrogen-receptor-positive breast cancer. A decline in PIK3CA ctDNA levels compared to baseline after 15 days of treatment with palbociclib and fulvestrant was predictive of progression-free survival [58].

The incorporation of ctDNA into clinical trials of immune checkpoint blockade enables the evaluation of its role as a predictive biomarker. The INSPIRE trial (NCT02644369) is a pan-cancer study which collected tumor and ctDNA samples to correlate with clinical outcome in patients treated with pembrolizumab [59]. A bespoke ctDNA assay was used, whereby 16 patient-specific somatic variants were identified based on paired pre-treatment normal-tumor whole exome sequencing. Change in ctDNA, collected at about 6–7 weeks post initiation of pembrolizumab, compared to baseline, was strongly associated with clinical efficacy parameters including objective response, progression-free survival and overall survival in this study [60]. The dynamics of ctDNA may be leveraged to select out patients, including those with HNSCC, who are most likely to benefit from immune checkpoint blockade.

Moving Beyond Genomics in HNSCC

Over the last several years, the increasing recognition of the complexity and molecular diversity of HNSCC has been coupled with the development and expansion of additional high throughput ‘omics’ technologies, such as epigenomics, transcriptomics, proteomics, metabolomics and shotgun metagenomics. These single level omics approaches may individually shed further light on epigenetic alterations, or molecular subtyping of HNSCC tumors based on protein expression, however they are limited in their ability to fully portray the relationship between molecular signatures and the phenotypic manifestation of the hallmarks of cancer [61–64]. Ultimately, by integrating these biomedical frameworks and developing *multi-omics* approaches there exists an opportunity to further expose the intricate molecular

mechanisms underlying HNSCC phenotypic manifestations, and may potentially offer predictive and prognostic value.

Transcriptomics

Transcriptomics is perhaps the most advanced novel omics approach beyond genomics, with techniques such as RNA sequencing (RNA-seq) developed to detect and quantify all RNA transcripts including messenger RNA (mRNA), long noncoding transcripts (LncRNAs) and microRNAs. This has enabled careful scrutinization of their expression profiles and assessment of the impact of their alterations, which may aid in disease classification and progression. In contrast to the static genome, the transcriptome exhibits dynamic changes depending on cellular, environmental, extracellular, and developmental stimuli [64]. The increasing interest to perform transcriptomic profiling to further delineate therapeutic targets is exemplified by the WINTHER trial (NCT01856296) [65]. This was a collaborative international precision medicine study involving investigators from five countries that prospectively matched patients to therapy according to either DNA-guided NGS or transcriptional analysis, specifically comparing tumor to matched normal tissue. This study successfully guided 35% of patients (n = 107) (69 patients DNA guided (64.5%) and 38 patients RNA guided (35.5%)) with refractory cancers to a therapeutic agent and demonstrated the utility of transcriptomics in exposing otherwise unspecified avenues of therapy. Overall efficacy between transcriptome-matched drugs and genotype-matched drugs was similar with response rates ranging between 20 and 30%.

Epigenomics

Epigenomics can be defined by the genome-wide identification of chemical modifications such as methylation and acetylation of DNA and/or DNA-binding histone proteins. Alterations in epigenetic mechanisms have been implicated in numerous malignancies including HNSCC, and represent an active area of research [66, 67]. Epigenetic changes have been recognised as fundamental mechanisms for carcinogenesis, and may have a role in early detection, treatment, and prognostic assessment for the cancer patients [66–72]. DNA methylation has become an increasingly attractive diagnostic biomarker that can be measured and evaluated with ctDNA.

Metabolomics

The field of metabolomics has garnered increasing attention in recent years, and there has been renewed interest in its role as a potential modulator of cancer

metabolism, which may further inform on phenotype [73]. Metabolomics is centred on the study of a metabolite within a system, and the levels of various metabolites can reveal an exclusive 'fingerprint' specific to that individual, providing information on the effect of gene/post-transcriptional regulation and altered pathway interactions [74]. Several studies have reported the role of tumor metabolism in cancer development and therapeutic response and resistance, and recently the role of glycolysis has come to the forefront [75–77]. Jiang et al. recently reported glycolytic activity was likely correlated with active immune signatures in various cancers, and highly glycolytic tumors presented an immune-stimulatory tumor microenvironment [78]. They found that glycolytic activity enhances PD-L1 expression on tumor cells and promotes anti-PD-1/PD-L1 immunotherapy response, suggesting a role as a potential predictive biomarker. Further, Cascone et al. identified tumor glycolysis as a pathway associated with immune resistance in melanoma [75]. In addition, new efforts have focused on identifying tumor-specific metabolite profiles including in HNSCC using different biological sample types and a variety of novel metabolomic platforms and technologies [79]. For example, the salivary metabolite profile has recently been shaped by the emerging knowledge of oral host–microbiome interactions.

Microbiome

The human body, particularly the oral cavity and gut, is host to rich and taxonomically diverse multi-species microbial communities. The microbiota typically exists in a symbiotic relationship with the host, regulating immune function and providing protection from pathogens. Disturbances in this intricate relationship, referred to as dysbiosis, often as a result of poor oral health or antibiotic use, may alter the community composition and induce inflammatory reactions, DNA damage and apoptosis. This results in altered metabolism and has subsequently been implicated in the pathogenesis of various malignancies including HNSCC [79–83]. In these patients chemoradiotherapy has recently been implicated in dysbiosis, where increases of potentially pathogenic species were found in patients with locally advanced oropharyngeal cancer [84]. Retrospective cohort studies have demonstrated varying microbiota composition in the saliva of HNSCC patients compared with healthy controls, while the presence of specific strains of bacteria has been associated with reduced risk of developing HNSCC [83, 85–88]. In the immuno-oncology setting differences in species population have been reported in both responders and non-responders. For example in melanoma patients whose baseline microbiota was enriched with *Faecalibacterium* genus and other Firmicutes showed a longer PFS and OS than those whose baseline microbiota was enriched with *Bacteroides* upon ipilimumab treatment [89]. Recent studies have also suggested that the immune microbiome plays a role in the development of toxicity [89–92]. Taken together the presence of specific bacterial strains may have the ability to modulate cancer progression and impact therapeutics [93]. As such metagenomic profiling and whole

genome shotgun sequencing of these microbial communities have become yet another increasingly attractive area of cancer research and precision medicine. Attempts to manipulate the gut microbiota to modulate the host immune response and further elucidate the mechanisms of response and toxicity are ongoing (NCT03686202, NCT03838601).

Artificial Intelligence/Radiomics

The field of artificial intelligence (AI) is also evolving and being incorporated into the clinical arena, particularly pertaining to the increasing use of immunotherapeutic agents and in the context of radiation therapy. Machine learning (ML) is an AI tool that can process enormous amounts of imported data, enabling classification with predictive capabilities, uncovering patterns that can predict outcomes with a high degree of accuracy. It has potential roles in cancer screening, diagnostics and prognostication; with a recent report demonstrating its ability to predict genotypes associated with poor prognosis in patients with lung cancer [94]. AI is also becoming an important decision support tool in the management of radiation oncology complications. Recently computational modelling has been shown to accurately predict two of the most challenging side effects associated with radiation therapy for head and neck cancer patients; weight loss and the need for feeding tube placement [95]. This AI precision oncology approach may thus have the potential to better identify patients who might benefit from early supportive interventions.

In HNSCC, radiomic efforts are currently concentrated on pathological classification and risk stratification of disease, aiming to prognosticate survival and predict response to treatment [96]. Several studies have demonstrated the potential in identifying clinically relevant molecular phenotypes such as HPV status, and the ability to determine histological diagnosis and stage of disease [96–99]. Models combining radiomic and clinical features have shown better accuracy in determining locoregional control and lymph node failure than either parameter independently, in both CT and MRI based studies [100–102]. In a study by Aerts et al., radiomic analysis of independent data sets from 1019 head and neck and lung cancer patients revealed a prognostic radiomic signature that was associated with intratumoral heterogeneity. This non-invasive, low-cost technique provides an opportunity for prognostic stratification of patients that may help guide treatment choice [103]. Quantitative analyses of available CT images of head and neck cancer patients have revealed a pattern of radiomic signatures that could be used to predict patterns of response and resistance to immune checkpoint inhibitors [104]. A retrospective radiomic response evaluation of recurrent/metastatic HNSCC patients treated with pembrolizumab within the KEYNOTE-012 study is ongoing, with tumor and peritumoral features of target lesions at baseline aiming to predict lesional level and overall response [105]. Successful modelling would allow for improved patient selection, increasing likelihood of response and reducing unnecessary toxicity and cost.

Although very much in its infancy, radiomics is a non-invasive 'omic' area that complements the advancement towards personalized cancer medicine. The limitations at this stage include heterogeneity in study methodology and statistical modelling, leading to challenges in comparison, reproducibility and validation of results [106]. As such the role in precision oncology remains uncertain and will require significant safeguards in place to reduce biases and allow meaningful translation into the clinic [107].

Conclusion

It is evident there has been tremendous advances in precision oncology in head and neck cancer in recent years. While this has largely been led by the field of cancer genomics, the increasing design and incorporation of innovative methodology and technology will continue to broaden the therapeutic scope for these patients. Increased understanding of the tumor microenvironment and host immunity will also advance precision immuno-oncology and the development of rational combination strategies. Despite these advances, sustained scientific collaboration remains paramount to realise the goal of precision medicine in HNSCC patients.

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