Critical Issues in Head and Neck Oncology

Key Concepts from the Eighth THNO Meeting

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Editors

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Preface

The eighth Trends in Head and Neck Oncology (THNO-8) took place at the Novotel Amsterdam City in Amsterdam, The Netherlands, November 11–13, 2021. It was organized by the same organizing team as on the last three occasions with support from Pharma (Merck, MSD, PCI Biotech) and practical logistical support from Congress Care. The conference was also endorsed by the European Head and Neck Society (EHNS), the European Organization for Research and Treatment of Cancer (EORTC) and the European Society for Radiotherapy and Oncology (ESTRO). As on previous occasions, the setup was educational, with a multidisciplinary focus. Case presentations, organized by some members of the coordinating team, stimulated lively interaction between faculty and audience and stressed the importance of individualized patient care underpinned by the best available evidence. 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 them for their efforts in realizing this important goal. Special thanks goes to Dr. Petr Szturz, who helped in the review process for the different manuscripts.

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Chapter 1
The Bi-Directional Communication Between Tumour Cells and Other Components of the Tumour Microenvironment

Philip Sloan

Introduction

The tumour microenvironment may be a suitable site for biomarker expression that relies on signalled changes from neoplastic cells. Complex bi-directional interactions occur and involve cell-intrinsic and cell-extrinsic mechanisms. Most attention has been focussed on the reciprocal signalling between cancer stem cells and tumour infiltrating immune cells, but other components of the tumour microenvironment play important roles in tumour initiation and progression. These include endothelial and pericytic cells, stromal fibroblasts, extracellular matrix macromolecules and dendritic cells [1]. Signalling molecules released from neoplastic cells can induce changes in adjacent tissue compartments, resulting in tissue changes than may be exploited for developing novel biomarkers.

Biomarkers

Biomarkers are now an essential part of clinical practice. In pathology, molecular testing is increasingly used routinely alongside immunohistochemistry for diagnosis [2]. Such testing ranges from single molecular markers detected by fluorescent or chromogenic in situ hybridisation through to whole genome sequencing, which currently can be completed within a two-week turnaround in the United Kingdom. In addition to using biomarkers for diagnosis, predictive biomarkers for drug response

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form a substantial part of the pathologist's workload in a cancer centre. Both diagnostic and predictive biomarkers must be validated and ideally accredited before they can be used outside a clinical trial setting.

Prognostic biomarkers are also an expanding field and levels of evidence for their use vary. Many prognostic biomarkers are available commercially that are supported by published evidence but which have not been adopted into clinical guidelines for routine use. Often the reason for this is that prognostic biomarkers are typically developed in the laboratory using retrospective samples from clinical trials or biobanks. Testing in a prospective setting is normally required by regulators and health economic modelling must also be undertaken to demonstrate benefit by change of clinical practice. For example, if a biomarker can be used to 'rule out' disease progression in a cancer, then unnecessary follow up and expensive investigations can be avoided, with significant health cost savings. Even more importantly perhaps, patients can be relieved of anxiety and there are wider societal impacts as well as reduction in time off work, which may be impossible to include in health economic modelling. Developers of prognostic biomarkers can face the challenge that expensive prospective clinical trial data is expected by clinicians, regulators and the expert groups that formulate guidelines [3]. Real world data cannot be obtained until the biomarker is introduced and used for a considerable period. For these reasons, some prognostic biomarkers are offered commercially for years before achieving accreditation for routine clinical use.

The need for reliable prognostic biomarkers is exemplified by cutaneous melanoma, where in the American Joint Committee on Cancer (AJCC) stage I and II disease, only 20% of patients will undergo progression but all patients must be managed and followed up in the same way. In a recent Delphic survey of melanoma experts, thresholds for clinical follow up, cross sectional imaging and adjuvant therapy were determined, and are an early step towards individualising management recommendations based on risk [4]. Validated prognostic biomarkers have the potential to offer personalised management based on better knowledge of actual risk.

There is good evidence that patients are increasingly seeking information about their cancers and are keen to find prognostic tests that can contribute to a fuller understanding of their individual risk [5]. In a recent survey presented by Miley L-B et al., at the 2022 Fall Clinical PA & NP Conference, over 90% of patients with cutaneous melanoma surveyed wanted prognostic information about their melanoma at the time of diagnosis. Over 75% wanted to increase their knowledge and over 45% wanted a test that could inform treatment decisions. Provision of good quality information and guidance by the clinical team is key as patients may access unreliable sources by web based searching. It is important that the possible benefits and harms of taking a personalised risk test using a prognostic biomarker are explained and reference to the source scientific data may be required to provide a balanced view.

Several prognostic markers based on gene expression signature (GES) for cutaneous melanoma are commercially available [3]. The signatures include genes from the melanoma and its microenvironment. The biological mechanisms that underpin GES tests are often not known. It is likely that the different levels of gene expression detected rely on cross-talk between the melanoma cells, immune cells and stromal
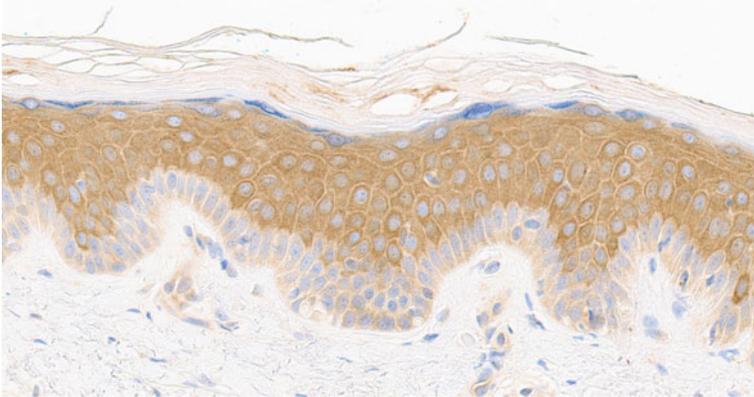
Fig. 1.1 Immunohistochemistry expression of AMBRA1 in normal epidermis. The protein is expressed in the cytoplasm only and shows a gradient with weakest staining in the basal layer increasing in intensity to the stratum corneum where staining ends abruptly

cells. An emerging prognostic biomarker for cutaneous melanoma (AMBLor) that relies on detecting changes in the microenvironment is described below. The test is based on finding changes in autophagy regulation expression in the epidermis overlying cutaneous melanoma.

Autophagy

Autophagy is a fundamental cellular process that eliminates molecules and subcellular elements, including nucleic acids, proteins, lipids and organelles, through lysosome-mediated degradation to promote cellular homeostasis, differentiation, development and survival. The discovery of selective autophagy receptors demonstrated that autophagy is a highly selective cellular clearance pathway regulated by bi-directional cellular cross talk. AMBRA1, (autophagy/Beclin-1 regulator 1), is a key activating molecule in Beclin-1-regulated autophagy. It is a highly-conserved adapter protein that plays multiple roles in the autophagy signalling network [6].

In the normal epidermis, immunohistochemistry has shown that AMBRA-1 is expressed in the cytoplasm of the keratinocytes. Expression is weakest in the basal layer and there is a gradient of increasing intensity through the prickle cell layer to the granular layer, where expression ends abruptly (Fig. 1.1). As the epidermis renews, daughter basal stem cells undergo amplification and then enter on a pathway of terminal differentiation. Autophagy appears to be essential to normal epidermal differentiation and as the terminally differentiating keratinocytes move away from the nutritional supply of the dermal stroma, recycling of their cytoplasmic components presumably becomes essential to normal maturation.

Development of AMBRA-1 as a Prognostic Biomarker

In a study of early stage cutaneous melanoma, it was found that AMBRA1 expression detected by immunohistochemistry in melanoma cells did not correlate with clinical outcomes. However, when the overlying epidermis in early stage melanomas was considered, it was observed that loss or reduction of AMBRA1 expression was frequently present. Retention of AMBRA-1 correlated with lack of disease progression, providing a potential biomarker of prognosis based on signalled changes occurring in the tumour microenvironment. Combining AMBRA1 with a second immunohistochemical marker, Loricrin (Fig. 1.2) to examine epidermis in AJCC Stage I melanomas resulted in an effective prognostic biomarker test [7]. In order to develop these observations into a prognostic biomarker for clinical use, a further multicentre study has recently been undertaken in a mixed cohort of 334 AJCC Stage I and 77 Stage II cutaneous melanomas from Roswell Park Cancer Centre, Buffalo USA (n = 241) and the Peter McCallum Cancer Centre, Melbourne, Australia (n = 170). Clinical follow up ranged from 60 to 287 months in these retrospective cohorts. Each cohort was powered to represent rates of metastasis of 10% for AJCC Stage I or up to 20% for Stage II disease. Results showed that a positive combined AMBRA1 and Loricrin test (AMBLor) with maintenance of either or both proteins, was associated with significantly increased disease-free survival of 97% compared to 87% for patients in which expression of both was lost (P = 0.01, 95% CI 0.9–0.42), and a negative predictive value of 97.14% (Fig. 1.3). The analysis was performed using newly created and validated humanised antibodies to AMBRA1 and Loricrin to ensure that consistent and quality controlled reagents would be available for future use. The antibodies can be used on the Ventana and Bond platforms which are the most widely used in pathology laboratories worldwide.
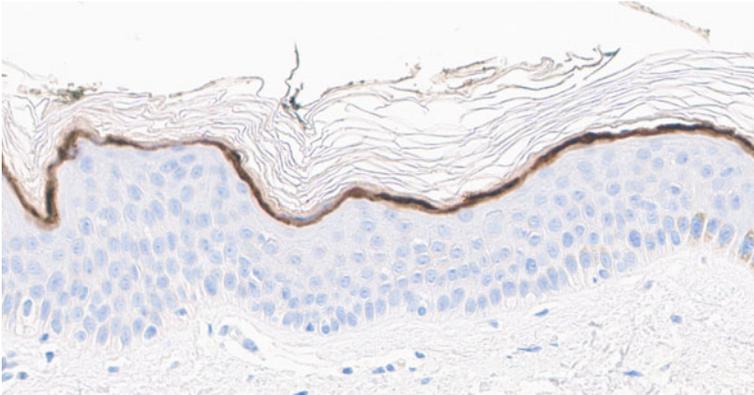
Fig. 1.2 Loricrin is expressed in the stratum corneum as a continuous band in normal skin. Fine granular cytoplasmic is seen and nuclei can be labelled. Single cell gaps may be present
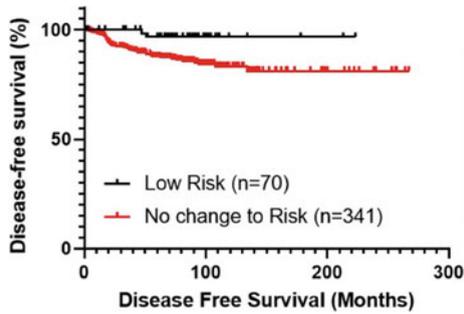
Fig. 1.3 Interim analysis of AJCC stage I and II non-ulcerated cutaneous melanomas (n = 411) in cohorts from Melbourne and Buffalo. There were 70 cases in the low risk group and 341 cases lost expression of both AMBRA1 and Loricrin, leaving their AJCC risk unchanged. Only two patients progressed in the low risk group after a minimum of five years of follow up. A negative predictive value of 97.14% for progression prediction was found. In the group maintaining one or both markers, 44 cases progressed out of 341 studied. Analysis of additional cohorts using a blinded prospective-retrospective study design is ongoing

The AMBLor test can only be performed on non-ulcerated Stage I and II cutaneous melanomas, that have been removed with a small margin of normal surrounding skin. The marginal skin serves as an excellent positive control and the pathologist interprets the test by comparison of the protein expression in the epidermis overlying the melanoma with that in the marginal skin. Additional negative and positive normal skin batch controls are also used. A training programme has been developed for pathologists and an interpretation guide is available.

Further larger validation cohorts using a 'prospective-retrospective' study design in which the biomedical scientists and pathologists are blinded to the clinical outcomes are currently being evaluated. If successful, UKCA and CE marking for the antibody test will be sought.

AMBLor has successfully passed the second stage of the National Institute for Clinical Excellence (NICE) accreditation process in the United Kingdom through a MedTech Innovation Briefing [8]. A prospective clinical trial to ascertain whether having a low risk AMBLor result would change clinical decision making and what the impact of a positive or negative test result would be on patient anxiety is about to be launched.

Sentinel Node Biopsy in Melanoma

The use of sentinel lymph node biopsy (SLNB) in melanoma is controversial. Current guidelines such as the National Comprehensive Cancer Network (NCCN) recommendations do not recommend SLNB for patients where the risk of metastatic disease is less than 5%, unless there is significant uncertainty about the local staging [9]. In a recent addition to these guidelines, gene expression profiling (GEP) is included

but should not guide clinical decision making in this group. In higher risk groups a GEP prognostic test may be used to inform the likelihood of a positive SLNB on an individual basis. The ability of AMBLor to predict SNLB status is not known but will be tested in a prospective clinical trial. Such a biomarker could help to inform clinical decision making. It should be remembered that SLNB has a morbidity and identification of cases where the procedure is inappropriate would benefit a subset of patients.

Cross-talk Mechanism in Melanoma

Some insight into the mechanisms by which melanoma is able to deregulate autophagy in the epidermis has been gained by the discovery of a paracrine mechanism mediated through the Transforming Growth Factor beta (TGFβ) pathway [10]. Using semi-quantitative immunohistochemistry, it was demonstrated that increased TGFβ2 in the melanoma cells was associated with loss or significant reduction of AMBRA1 in the epidermis overlying the melanoma and with ulceration. Further, TGFβ2 treatment of keratinocytes in culture resulted in downregulation of AMBRA1, which is followed by downregulation of loricrin and claudin-1. It can be speculated that the TGFβ2 paracrine mechanism is the cause of spontaneous ulceration in melanoma, which is known to be an adverse prognostic feature, as reflected in AJCC staging. Loss or reduction of AMBRA1 expression in the epidermis overlying melanoma is interpreted in the AMBLor test by comparison with the normal epidermis at the margin (Fig. 1.4).

Autophagy and Squamous Cell Carcinoma of the Head and Neck

Oropharyngeal Squamous Carcinoma

Although oropharyngeal squamous cell carcinomas (OPSCC) that actively transcribe high-risk human papillomavirus (HPV) have a more favourable prognosis that their HPV-negative counterparts, the mechanism remains undefined. In vitro studies have shown that HPV-positive OPSCC cells exhibit reduced macroautophagy/autophagy activity, mediated by the ability of HPV-E7 to interact with AMBRA1, to compete with its binding to BECN1 and to trigger its calpain-dependent degradation [11]. Further, pharmacological inhibition of autophagy and downregulation of AMBRA1 have been shown to sensitize HPV-negative OPSCC cells to the cytotoxic effects of cisplatin. Immunohistochemical analysis using a tissue microarray (TMA) showed that AMBRA1 expression appears reduced in HPV-positive compared to HPV-negative OPSCCs [11]. The data suggest that AMBRA1 may be a key target of HPV
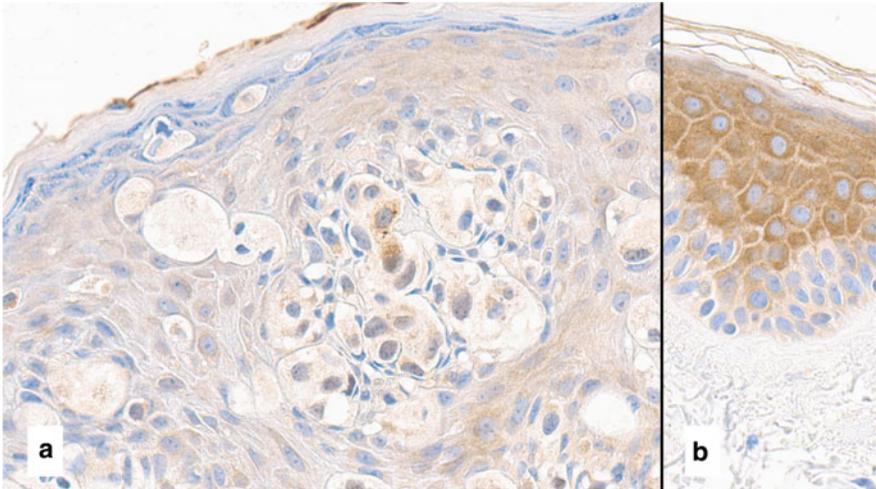
Fig. 1.4 Immunohistochemistry for AMBRA1 showing cellular interactions in melanoma. The keratinocytes adjacent to melanoma cells show reduced expression of AMBRA1 **a** compared to expression in the normal skin in the surrounding margin **b** where cytoplasmic expression is intense. This is mediated through the TGFβ2 pathway

resulting in impairment of autophagy. This leads to the proposition that targeting of autophagy could be a possible therapeutic strategy for improving the response of HPV-negative OPSCC to chemotherapy [11].

Whole genome sequencing (WGS) of both the viral and somatic genomes in HPV positive OPSCC has revealed a complex picture, in which HPV may play different roles in different tumours. Although WGS appears to reveal subgroups within HPV positive OPSCCs, the patterns are currently too complex to translate WGS into a biomarker for clinical use. The finding that AMBRA-1 expression is downregulated in HPV positive OPSCC, raises the possibility that it may be a useful prognostic marker. In our larger (unpublished) cohort study based on whole sections of OPSCC evaluated by p16 immunohistochemistry and HPV in situ hybridisation, no clear prognostic pattern emerged, however. Currently, re-evaluation of the cohort for changes in stromal and endothelial AMBRA1 expression using AI is underway.

Cutaneous Squamous Cell Carcinoma

Currently there are no validated prognostic biomarkers in routine clinical use for prediction of metastatic behaviour of cutaneous squamous cell carcinoma (cSCC). Staging systems are of limited clinical utility with regard to identification of primary squamous cell carcinomas that have metastatic potential. The staging system proposed by the Brigham and Women's has great utility [12] but biomarkers that

could identify low risk primary cSCC with a very high negative predictive value could relieve patient anxiety and save considerable health service resource.

The evidence for a role in autophagy in cSCC is mostly from laboratory studies and the data suggest that autophagy may have a protective effect, allowing malignant keratinocytes to escape apoptosis [13]. Our initial studies examining AMBRA1 in a cohort of cSCC by semi-quantitative immunohistochemistry have shown a trend towards expression in the neoplastic keratinocytes associating with adverse outcome, but statistical significance has not been achieved (data unpublished).

A promising approach is to utilise Artificial Intelligence (AI) in combination with AMBRA1 expression and this has shown improved prognostic power in our preliminary study. In a recent study of whole slide images, AI has shown the ability to distinguish between rapidly metastatic and non-metastatic primary cSCC with an area under receiver operator curve (AUROC) of 0.747 [14]. Combining the AI with known adverse factors in a risk model increased the AUROC to 0.917. The risk factor model with AI predicted high 5-year disease specific survival (DSS) for patients with cSCC with 0 or 1 RFs (100 and 95.7%) and poor DSS for patients with cSCCs with 2 or 3 RFs (41.7 and 40.0%). Perhaps the most intriguing finding is that the AI system appears to recognise morphological features in routine sections of primary cSCC that are associated with metastasis that pathologists do not identify. Whether these features are in the neoplastic keratinocytes or the tumour micro-environment is unknown.

Conclusion

The biological behaviour of neoplasms depends not only on the properties of the neoplastic cells but on their interactions with cells in the microenvironment. The development of the AMBLor test is an example of the way that detecting changes in the tumour microenvironment can be exploited to predict prognosis in a cancer. In the future, it seems likely that biomarker tests will be incorporated into risk models that combine AI and clinical features to provide personalised management plans.

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Chapter 2
Immune Checkpoint Inhibition and Radiotherapy in Head and Neck Squamous Cell Carcinoma: Synergisms and Resistance Mechanisms

Nikko Brix and Kirsten Lauber

Introduction

In 1895, Wilhelm Konrad Roentgen described a novel radiation quality which he termed "X-rays" [1]. The importance of this discovery was immediately recognized by the scientific community and was spread rapidly across the globe. It reached Émil Grubbé in Chicago: a 21-year old student who was attending Hahnemann Medical School at that time. He was probably the first who used the novel radiation quality in a therapeutic setting in order to treat cancer–not even one year after Roentgen's discovery [2, 3]. This was the beginning of radiotherapy. In 1908, the first case report on what today would be classified as an "abscopal effect" of radiotherapy was published: A case of head and neck cancer described by H.D. McCulloch who also presented his hypothesis on how "immunity" contributed to spontaneous tumor regression upon irradiation of the "lymphatic glands" [4]. Since then, radiotherapy has gone through a series of impressive technical improvements and physical refinements and has evolved to a central treatment modality for various types of solid cancers, including head and neck squamous cell carcinoma (HNSCC) [5].

Radiotherapy and Immunotherapy in HNSCC Treatment

For locally advanced HNSCC, radiotherapy is implemented in definitive or adjuvant settings. State-of-the art techniques include intensity-modulated and volume-modulated arc treatment protocols in daily fractions of 1.8–2.0 Gy, alone or combined with concomitant chemotherapy [6, 7]. In recurrent or metastatic disease stages,

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immune checkpoint inhibition has emerged as a central part of the standard-of-care [8], together with the EXTREME chemotherapy protocol involving 5-fluorouracil, cisplatin/carboplatin, and cetuximab [9], and/or stereotactic body radiotherapy with high single doses and steep dose gradients as a palliative option [10].

Clinical responses upon immune checkpoint inhibition are impressive but remain limited to a minority of patients [11, 12]. Primary resistance of never-responders is considered to derive from host- and tumor-specific characteristics, the latter comprising immune checkpoint activity, tumor immune contexture, tumor mutational burden, (neo-)antigen load, and others (Fig. 2.1). Secondary resistance of initially responding patients in addition, appears to be driven predominantly by irreversible T-cell exhaustion and therapy-induced selection of tumor cell clones with mutations in critical genes involved in the immune checkpoint response [13, 14].
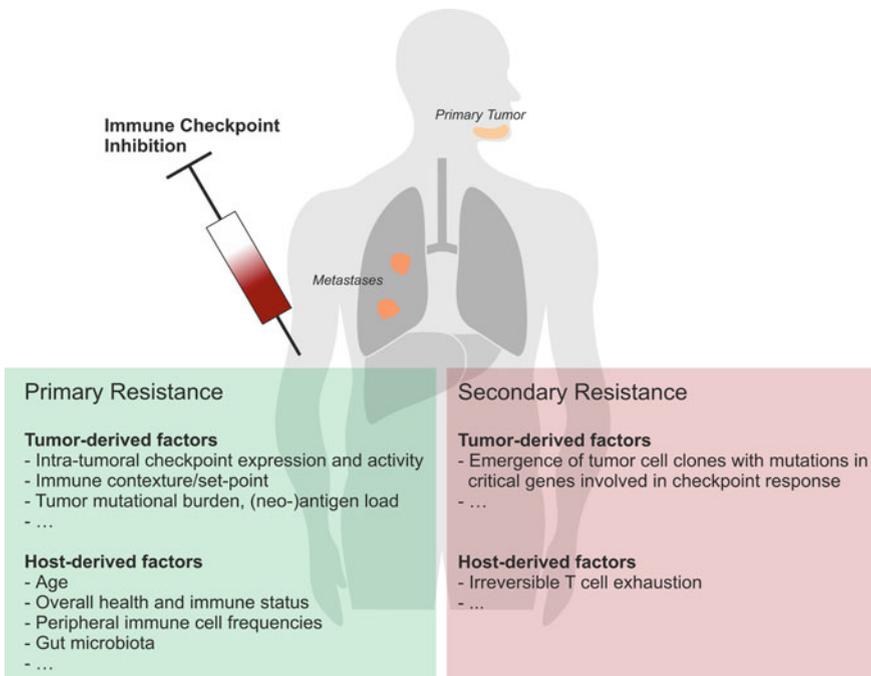
Fig. 2.1 Mechanisms of primary and secondary resistance against immune checkpoint inhibition

Immunological Effects of Radiotherapy

With particular focus on primary resistance against immune checkpoint inhibition, scientific interest of preclinical and clinical researchers currently aims at the development and evaluation of combined modality treatment approaches, for instance in combination with radiotherapy: Can the immune contexture be altered in order to convert immunologically cold tumors into hot ones? And can tumor-antigenicity be increased, for instance by enforcing the presentation of neo-antigens? In this regard, the immunological implications of radiotherapy and the anecdotally reported abscopal effects are of interest. It took nearly 50 years until a term was coined for H.D. McCulloch's initial observation, and it was Robert Mole who defined it: Abscopal effects are radiation effects at a distance from the irradiated volume but within the same organism [15]. Meanwhile, this rather general definition is being nearly exclusively used in (oligo-)metastatic tumor settings describing the phenomenon of tumor regression at out-of-field locations distant from the primary site of local (radio-) therapy. In-depth preclinical analyses have shown that the underlying driving force of this phenomenon is a (re-)activation of systemic anti-tumor immune mechanisms and the cancer immunity cycle [16]. Irradiated tumor and normal tissue cells stimulate the recruitment and activation of antigen-presenting cells (APCs) which capture tumor antigen, migrate to the draining lymph nodes, and prime tumor-specific T-cell responses, particularly CD8+T-cell responses, which finally contribute to local and distant lesion regression. The basic idea of this concept and pioneering preclinical data for its validation were provided by Sandra Demaria and Silvia Formenti. From their and others' experiments with mouse tumor models in which irradiation of the primary tumor stimulated regression of a secondary out-of-field tumor in a T-cell-dependent manner, they concluded that radiation can generate an in situ cancer vaccine [17–21].

An effective cancer vaccine consists of tumor-specific antigens, i.e. tumor-associated antigens or neo-antigens, and immune cell activating adjuvants. Accumulating evidence suggests that radiotherapy can affect both (Fig. 2.2).

The irradiated state of tumor cells bears interesting analogy to the anti-viral state [22]. Fragments of nuclear and mitochondrial DNA that are released into the cytosol can stimulate cytosolic nucleic acid sensors to mount an intra-tumoral type I interferon response which is essential to (re-)activate the cancer immunity cycle and (re-)invigorate systemic anti-tumor T-cell responses [23, 24]. The optimal irradiation dose and regimen to trigger these mechanisms are still under debate and seem to reveal non-linear dose–response behavior. Both, super-hypofractionated protocols (i.e. 3 × 8 Gy) as well as low dose radiotherapy with single fractions of around 2 Gy were reported to be effective in this regard [25–27].

Apart from the intra-tumoral type I interferon response which essentially contributes to the maturation and activation of APCs in the irradiated lesion, cytosolic DNA fragments and persisting DNA damage also determine the overall cell fate in response to radiotherapy. This strongly shapes the adjuvanticity of irradiated cells as well. Whereas non-malignant cells with functional cell cycle checkpoints commonly
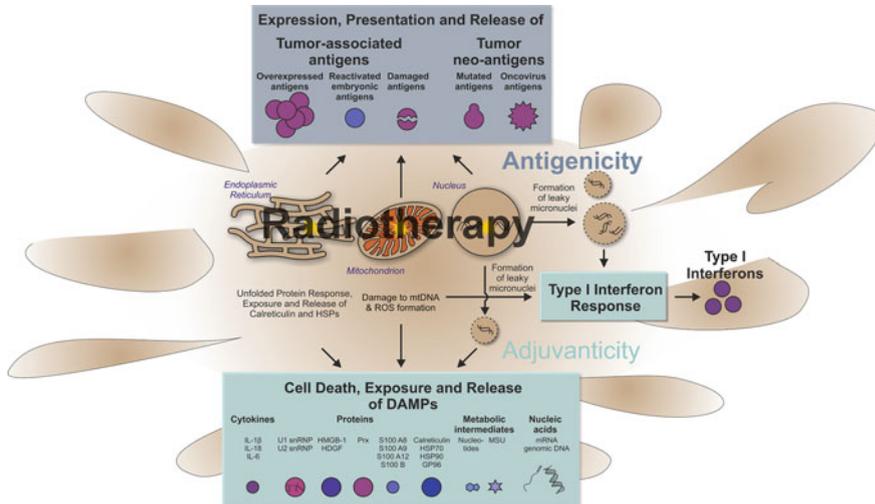
Fig. 2.2 Determinants of tumor antigenicity and adjuvanticity and their modulation by radiotherapy

undergo cellular senescence upon irradiation, tumor cells often fail to properly arrest in cell cycle until the damage is repaired. In consequence, they experience several rounds of aberrant mitosis and finally commit to cell death of different morphotypes [28–30]. Depending on a spectrum of physical and biological parameters, including radiation quality and dose, origin and genetic repertoire of the irradiated cells, and the functionality of cell cycle checkpoints, regulated forms of apoptotic or necrotic morphology can be observed. In tumors of epithelial origin, such as HNSCC, the regulatory machinery of apoptotic cell death is frequently perturbed, and different forms of regulated necrosis appear to be dominating in response to irradiation, including but not limited to necroptosis, ferroptosis, pyroptosis, and parthanatos [31, 32] which–although regulated via different signaling cascades–all share in common that the plasma membrane disintegrates and cellular contents are released [30, 33]. Danger signals and/or damage-associated molecular patterns (DAMPs) leaking out of the dying cells activate pattern recognition receptors on neighboring cells, endothelial cells, and immune cells, and trigger an immunological reprogramming of the tumor microenvironment [29, 30].

If cell cycle checkpoint function is operational, tumor cells can commit to irradiation-induced cellular senescence. Similar to irradiated non-malignant cells, they arrest in cell cycle, increase in size, and reshape their intercellular connections and stress fibers. They produce a wide spectrum of cytokines, chemokines, and growth factors, the so-called senescence-associated secretory phenotype or SASP, which exerts multiple effects in the tumor microenvironment [34, 35]. SASP factors can contribute to vascular remodeling and immune cell recruitment [26, 36]. On the contrary, they can also support cancer cell stemness, therapy resistance, tumor repopulation, and invasion [37, 38]. So, radiotherapy-induced senescence and the

corresponding secretome present as double-edged swords which can precondition the tumor microenvironment for immune checkpoint inhibition and at the same time can drive (radio-)therapy resistance and tumor progression. Accordingly, the current discussion about the implementation of broad-range senolytic and/or senomorphic drugs in the context of multi-modal cancer therapy should also include selective targeting of distinct SASP cytokines [39, 40].

Apart from elevated tumor adjuvanticity, several reports have described increased tumor antigenicity upon radiotherapy originating from radiation-induced expansion of the major histocompatibility class I/II ligandome and the exposure of neo-antigens [41–44]. Collectively, radiotherapy thus may serve as a means of personalized in situ cancer vaccination which can synergize with immune checkpoint inhibition and may help to undermine primary resistance against immune checkpoint inhibition.

Clinical Experiences with a Combination of Radiotherapy and Immune Checkpoint Inhibition in HNSCC

Given that the mechanisms described above are operational, the (re-)activation of systemic anti-tumor immunity by a combination of radiotherapy and immune checkpoint inhibition in preclinical model systems can be reportedly achieved on a reliable and regular basis [21]. However, clinical experiences are different. Here, the description of abscopal tumor regression remains limited to scattered case reports and few retrospective analyses. Nevertheless, the first cases were described at the very beginning of the therapeutic application of ionizing irradiation, and their numbers appear to be increasing–particularly since the advent of immune checkpoint inhibition [4, 45]. The most prominent case of abscopal lesion regression upon radiotherapy with ongoing immune checkpoint inhibition was reported by Michael A. Postow and colleagues. It was a case of metastatic melanoma, in which upon progression during anti-CTLA4 treatment stereotactic irradiation at 3×9.5 Gy was applied to a paraspinal lesion. The irradiated lesion showed a good response, and interestingly also the non-irradiated splenic lesions did regress [46]. Similar case reports can be found predominantly for melanoma, lymphoma, and lung cancer [21]. However, corroborating these case reports by higher level evidence in a randomized phase II trial has failed so far–at least for HNSCC. Sean McBride and colleagues compared inhibition of programmed cell death protein 1 (PD-1) versus PD-1 inhibition plus concomitant stereotactic body radiotherapy in unselected patients with metastatic HNSCC, and the rate of abscopal effects was the primary endpoint (i.e. objective response rate of non-irradiated lesions) (NCT02684253). No evidence of abscopal effects and no improvement in response rates were observed [47].

Encouraged by the success of palliative immune checkpoint inhibition in relapsed and/or metastatic HNSCC, its concomitant addition to curative-intent radiochemotherapy for locally advanced HNSCC is currently being investigated [48]. Despite good tolerability, efficacy data reported so far are rather disappointing. As

such, JAVELIN Head and Neck 100, the first randomized, placebo-controlled phase III trial adding concomitant PD-L1 inhibition to definitive radiochemotherapy in locally advanced HNSCC led by Nancy Lee did not meet its primary objective of prolonging progression-free survival [49]. Further randomized phase II and III trials evaluating the concomitant addition of PD-1/PD-L1 blockade to radiochemotherapy and/or radiobiotherapy (e.g. KEYNOTE-412 (NCT03040999) or GORTEC 2017–01 "REACH" (NCT02999087)) are still ongoing. Yet, reported interim analyses prognosticate that at least the latter may not change the current standard-of-care for locally advanced HNSCC.

Considering the successful implementation of adjuvant immune checkpoint inhibition in other cancer entities–yet with clearly different treatment schedules–these results are rather disappointing. In the randomized phase III PACIFIC trial (NCT02125461), Scott J. Antonia and colleagues compared inhibition of programmed cell death ligand 1 (anti-PD-L1) as maintenance therapy after radiochemotherapy versus placebo in patients with stage III non-resectable non-small cell lung cancer. For both co-primary endpoints of progression-free and overall survival, the immune checkpoint inhibition arm was clearly superior [50]. Similarly, adjuvant PD-1 inhibition was also successful in patients with esophageal or gastroesophageal junction cancer as reported in CheckMate 577 (NCT02743494), a randomized, placebo-controlled phase III trial led by Ronan J. Kelly [51].

The reasons underlying these discrepant trial results need to be investigated in order to refine and optimize treatment concepts and to develop radiochemoimmunotherapy protocols with improved outcomes for patients with locally advanced HNSCC. Obviously, different strategies of immune checkpoint inhibition (anti-PD-1 or anti-PD-L1 blockade) and different immunoglobulin G (IgG) classes with different epitopes were used. These reported disparities have an impact on efficacy and safety profiles [52]. Of note, JAVELIN Head and Neck 100, KEYNOTE-412, and GORTEC 2017–01 "REACH" all rely on targeting the ligand PD-L1 and not the receptor PD-1. This may have implications for tumor-cell-intrinsic, retrograde signaling of PD-L1 which has recently been reported to support tumor cell growth, stemness, as well as DNA damage repair, and thus may drive resistance against the concomitantly administered radiochemotherapy [53, 54]. Furthermore, the treatment sequences need consideration. Immune checkpoint inhibition with concomitant radiochemotherapy provided negative results in JAVELIN Head and Neck 100, whereas adjuvant immune checkpoint inhibition after completion of radiochemotherapy was used in the successful PACIFIC and CheckMate 577 trials. Preclinical studies in diverse cancer models had shown that simultaneous immune checkpoint inhibition (with or without a loading dose prior to the start of radiotherapy) was superior to the adjuvant treatment sequence [55], and thus guided the trial designs of JAVELIN Head and Neck 100, KEYNOTE-412, and GORTEC 2017–01 "REACH". In this regard, the lymphotoxic effects of concomitant radiochemotherapy during immune checkpoint inhibition may need to be considered. Chemoradiation of the circulating blood pool as well as of the tumor draining lymph nodes may interfere with the successful release of immune checkpoints and may be of minor importance in the context of adjuvant or neoadjuvant immune checkpoint inhibition [56].

Accordingly, the question arises if sparing of the lymph nodes–at least in the early phase of immune checkpoint inhibition–or alternative treatment sequences could be beneficial. Combinations of radiochemotherapy with adjuvant or neoadjuvant immune checkpoint inhibition for locally advanced HNSCC are currently underway [48]. Another relevant parameter is the fractionation regimen of radiotherapy. As described above, preclinical data suggest that the synergism between radiotherapy and immunotherapy reveals a non-linear dose–response relationship, and the optimal fractionation protocol for the (re-)activation of systemic anti-tumor immune mechanisms is still under debate. Presumably, there is no "one-fits-all" regimen, and entity-specific characteristics may need to be considered [57]. Along these lines, unique and so far disregarded aspects of HNSCC biology and/or immunology may render the implementation of immune checkpoint inhibition into the standard-of-care with curative intent for patients with locally advanced HNSCC so difficult.

Conclusions

– Immune checkpoint inhibition has emerged as an integral part of the standard-of-care for recurrent and/or metastatic HNSCC, but response rates remain limited to a minority of patients.
– Mechanisms of primary and secondary resistance comprise tumor- and host-derived factors, including immune checkpoint activity, immune contexture, tumor mutational burden, neo-antigen load, and others.
– Preclinical studies and clinical case reports have shown that radiotherapy can function as a means of in situ cancer vaccination to (re-)activate systemic anti-tumor immunity, to synergize with immune checkpoint inhibition, and to break primary resistance against immune checkpoint inhibition.
– Evaluation in randomized clinical trials has provided heterogeneous results, particularly for HNSCC.
– Scheduling and dosing of combined modality treatment regimens appear to be challenging.
– Unique aspects of HNSCC biology and/or immunology may be responsible that the combination of radiotherapy and immune checkpoint inhibition is so difficult.

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Chapter 3
DNA Repair Mechanisms as a New Target in Head and Neck Cancer

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Introduction

Radiotherapy is one of the most effective and frequently used treatments for a variety of cancers. Approximately half of cancer patients receive radiotherapy at some point in their treatment [1], whether in the curative or palliative settings. Radiotherapy causes cell death or senescence via DNA damage. In general terms, necrotic or apoptotic cell death occurs depending on cell type, radiotherapy dose and fractionation schedule [2]. Cancer cells that evade apoptosis and continue to divide with accumulated DNA damage may die via mitotic catastrophe. Classically, the outcome of fractionated radiotherapy is governed by the principles of the 5 Rs of radiobiology, one of which is repair of DNA damage [3]. Therefore, combining radiation with agents that can target, and inhibit, DNA damage repair pathways represents an important new avenue towards enhanced therapeutic outcomes.

In addition to its direct anti-cancer cytotoxicity, ionising radiation can promote anti-tumour immune responses by triggering pro-inflammatory signals, DNA damage-induced immunogenic cell death (ICD) and innate immune activation. Anti-tumour innate immunity can arise from recruitment and stimulation of directly active natural killer (NK) cells. In addition, dendritic cells (DCs) can be recruited and activated with subsequent tumour-specific adaptive T-cell priming and immunostimulatory cell infiltration. The reverse effect can also occur through radiotherapy-induced immunosuppression and generation of anti-inflammatory mediators that can confer radioresistance. Approaches that target the DNA damage response (DDR) concomitantly with radiotherapy are attractive strategies for circumventing radioresistance, by enhancing the radiosensitivity of tumour relative to normal tissues, but also by reprogramming the tumour microenvironment to create an immunostimulatory milieu.

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This doubly-targeted approach seeks to exploit tumour-intrinsic genomic instability as a means of preventing immune evasion.

Here, we review targeting of ataxia telangiectasia and Rad3-related kinase (ATR) and the potential this brings for interactions with druggable immunomodulatory signalling pathways, including nucleic acid-sensing mechanisms (Toll-like receptors (TLR); cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) and retinoic acid-inducible gene-I (RIG-I)-like receptors), and immune checkpoint inhibitors (ICPI). Central to these discussions are considerations of how these approaches might be exploited to enhance the effects of radiation therapy.

Immunostimulatory Effects Mediated by Radiotherapy

The innate immune system uses pattern-recognition receptors (PRRs) to detect microbial pathogenic molecules known as pathogen-associated molecular patterns (PAMPs). However, these pathways are not exclusively limited to foreign molecules and immune activation can also occur without microbial infection. In such cases, it may be triggered by inflammatory signals released from stressed or dying cells, collectively known as damage-associated molecular patterns (DAMPs) [4]. Radiotherapy-induced cellular stress and ICD can stimulate immune responses through the generation of DAMPs [5], which can be detected by their cognate PRRs [6]. ICD has been defined as the chronic expression of DAMPs in the tumour microenvironment (TME) and this can induce innate and adaptive anti-tumour immune responses in the host [7].

Classically, ICD-related DAMPs include: adenosine triphosphate (ATP) secretion; high-mobility group box-1 (HMGB1) protein release; and calreticulin expression on the cell surface. Extracellular ATP functions as a "find-me" chemoattractant signal [7] and promotes recruitment and activation of dendritic cells [8, 9]. HMGB1 protein, released from the nucleus during ICD, binds to TLR-4 and is critical for activating DCs and facilitating antigen-processing and presentation to T-cells [10]. Calreticulin exposure on the external surface of dying cells provides an "eat-me" signal to antigen-presenting cells (APCs) and results in their phagocytosing target cells [11]. ICD leads to release of tumour-associated antigens (TAA) and, subsequently, their acquisition, processing and presentation by APCs, potentially leading to priming of a cancer-specific adaptive immune response.

Radiotherapy-induced DNA damage can act as a viral mimic through the accumulation of cytosolic DNA or RNA in irradiated cells [12]. Cytosolic DNA and RNA activate cGAS-STING and RIG-I/mitochondrial antiviral-signalling protein (MAVS) pathways, respectively [13]. These pathways activate complex downstream signalling via interferon regulatory factor-3 (IRF-3)/TANK-binding kinase 1 (TBK1) and nuclear factor kappa B (NF-κB) that results in production of Type I interferon (IFN) and other inflammatory cytokines (e.g. interleukin [IL]-1, tumour necrosis factor [TNF]-α) [12]. Detailed consideration of all of these pathways is beyond the scope of this review, but there are a number of active programmes of research

seeking to generate activators of cGAS-STING, RIG-I and TLR pathways to augment anti-tumour immune responses.

There are also data demonstrating that radiation can enhance cancer cell antigenicity through upregulation of genes involved in DNA damage repair and cellular stress responses [12]. Immune cell recruitment is increased via expression of adhesion molecules (e.g. intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and E-selectin) [14] and chemokines (e.g. CXCL16) [15]. Within the appropriate inflammatory environment, APCs take up antigens in peripheral tissues, mature and migrate to draining lymph nodes, where they activate naïve T-cells and promote their differentiation into effector T-cells [16]. Radiotherapy-induced ICD increases TAA presentation that can lead to specific T-cell priming, expansion of tumour reactive CD8+ T-cells and infiltration into the TME [17].

In summary, inflammatory DAMP signalling generates a favourable TME for activated DCs to process and cross-present TAAs from irradiated cells as a "tumour vaccine" to naïve T-cells. These primed and expanded T-cells can sustain a systemic tumour-specific immune response, in effect converting an initial innate to an adaptive anti-tumour response with the potential for durable, systemic activity and the development of long-lasting anti-tumour memory. The T-cell receptor (TCR) repertoire is also known to be shaped following radiotherapy, including when used in conjunction with ICPI [18–20].

Immunosuppressive Mechanisms Triggered by Radiotherapy

Pro-inflammatory signalling, as reviewed above, can trigger beneficial anti-tumour effects, but cancer cells learn to adapt and survive with mechanisms such as hypoxia resistance and unrestricted proliferation that can result in a state of chronic inflammation and evasion of immune surveillance [21–23]. Cancer cells can also adapt to down-regulate or lose TAA expression and interferon signalling pathways. In addition, tumours frequently evolve to use the programmed cell death 1 (PD-1)/ligand 1 (PD-L1) axis as a means of nullifying attack by immune cells. Evasion of immune recognition or immune escape [24] is now enshrined as a hallmark of cancer [8]. This proliferative signalling is mediated by changes in cytokine signalling (TNF-α, IL-1β, IL-6, IL-10 and TGF-β) [25, 26] and recruitment of suppressive immune cells such as tumour-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) [27] and regulatory T-cells (Tregs) [28, 29] into the TME.

Targeting DNA-Damage Response (DDR) Pathways

Ionising radiation induces lesions in DNA, ranging from simple purine and pyrimidine lesions to single-strand (SSB) and double-strand breaks (DSB) in the DNA [30]. DSB are potentially the most lethal DNA lesions induced by radiotherapy and therapies that can prevent their repair/resolution have the potential to be profoundly radiosensitising. There are specific mechanisms to detect and repair radiation-induced abnormalities in DNA structure: DSBs are repaired by non-homologous end-joining (NHEJ) repair during G1 phase of the cell cycle and by high-fidelity homologous recombination (HR) in S and G2 phases; SSBs and base damage are repaired through the base excision repair (BER) pathway [31].

Different types of radiation-induced DNA damage are sensed by mechanisms that activate specific DDR kinases: ataxia telangiectasia-mutated (ATM) and ATR, which phosphorylate the checkpoint kinases, Chk1 and Chk2. In turn, these proteins transfer the signal to different effector molecules that mediate cell cycle arrest, initiate repair functions or trigger cell death – depending on the level of damage sustained by the cell and its capacity to survive and repair that damage. The specific pathways involved are illustrated in simplified form in Fig. 3.1.

ATM and ATR Inhibitors

ATM and ATR are key mediators of the DSB signalling response that induce cell cycle arrest to facilitate DNA repair [32]. Conditions that activate ATM and ATR as part of DDR may also participate in regulating the innate immune system and alert it to potentially 'dangerous' tumour cells [33].

In response to DSB, the MRE11-RAD50-NBS1 (MRN) complex assembles at DSB sites to act as a DNA damage sensor that activates and recruits ATM to DSB sites [34]. Briefly, when a cell triggers the DDR, ATM initiates a massive signalling cascade with the phosphorylation of hundreds of substrates, including p53 and CHK2 kinase. Activated p53 transactivates the expression of p21$^{Cip1/kip1}$, which inhibits cyclin-dependent kinase 2 (CDK2) and CDK4/6 to induce G1/S arrest [30]. Inhibition of the ATM/Chk2 axis can lead to replication stress and accumulation of cytosolic DNA that subsequently activates the cGAS-STING-mediated innate immune response [34].

Inhibition of either ATM or ATR has the potential to improve radiotherapy outcomes since they are both key mediators of the DDR [32]. ATM inhibitors such as caffeine [35], wortmannin [36], CP-466722 [37], KU-55933 [38], KU-60019 [39] and KU-59403 [40] increase cell radiosensitivity [41, 42], particularly in p53 low/deficient and PI3K highly-expressing cells [35, 43]. In a preclinical study in vivo with KU60019 and radiotherapy, in addition to tumour cell sensitisation, combination treatment enhanced TBK1 activity, type I interferon production and antigen presentation and increased tumour-infiltrating CD8+ T-cells; moreover, complete responders
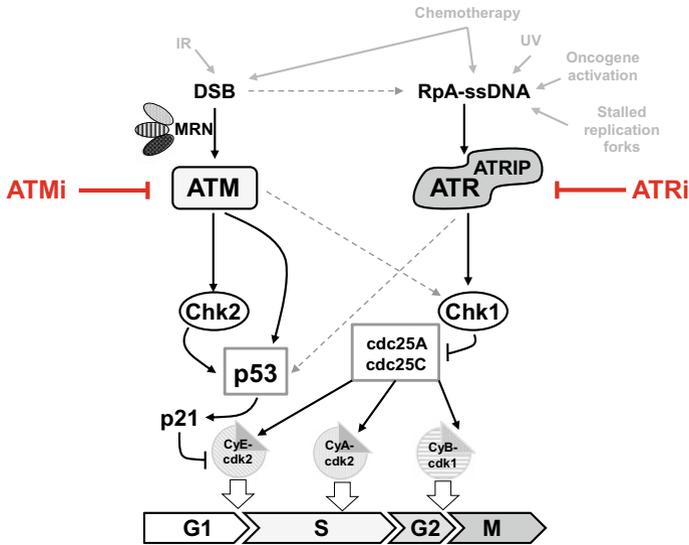
Fig. 3.1 A simplified schematic of the DNA damage response. The ATM pathway is activated by DNA double-strand breaks (DSBs), causing activation of Chk2 and p53 with subsequent G1 cell cycle arrest. Diverse inputs converging on RpA-coated single-stranded DNA(ssDNA) activate the ATR-ATRIP complex, with downstream phosphorylation of Chk1, amongst other targets, resulting in G2/M cell cycle arrest arrest. The ATR pathway also plays an important role in S-phase progression and replication fork stabilisation. There is evidence of substantial crosstalk between these pathways (indicated by the dotted arrows). Both ATM and ATR inhibitors (ATMi, ATRi) are in clinical development. (adapted from Dillon et al. Clin Oncol (R Coll Radiol). 2014;26(5):257-65). **Key:** ATM –ataxia telangiectasia-mutated kinase; ATR –ataxia telangiectasia and Rad3-related kinase; ATRIP –ATR-interacting protein; cdc25A –cell division cycle 25 homolog A; cdc25C –cell division cycle 25 homolog C; cdk1 –cyclin-dependent kinase 1; cdk2 –cyclin-dependent kinase 2; Chk1 –checkpoint kinase 1; Chk2 –checkpoint kinase 2; CyA –cyclin A; CyB –cyclin B; CyE –cyclin E; G1 –gap (or growth) 1 phase of cell cycle; G2 –gap (or growth) 2 phase of cell cycle; IR –ionizing radiation; M –mitosis phase of cell cycle; MRN –Mre11, Rad50 and Nbs1 complex; RpA –replication protein A; S –synthesis phase of cell cycle; UV –ultraviolet light

had established immunological memory with the ability to resist tumour re-challenge [44]. The ATM inhibitor (AZD1390) and radiotherapy is being investigated in a phase I clinical trial in brain cancer (NCT03423628). A dual ATM and DNA-dependent protein kinase (PKc) inhibitor (XRD-0394) is also in clinical development with a phase I trial in combination with radiotherapy recruiting patients (NCT05002140).

ATR is activated by single-stranded DNA (ssDNA) structures that may arise at resected DNA DSBs or stalled replication forks. ATR is recruited via interaction of the regulatory protein ATRIP with ssDNA-bound replication protein A (RPA) [45] (Fig. 3.1). RPA-ssDNA complexes stimulate loading of the RAD9–HUS1–RAD1 (9–1–1) heterotrimer, that recruits TopBP1 which activates ATR [46]. Once ATR is activated, downstream targets, including Chk1, promote DNA repair [47, 48], restart stalled replication forks [49] and intra-S and G2/M cell cycle arrest [50, 51]. In

response to DNA damage, activation of the intra-S-phase cell cycle checkpoint slows progression of DNA replication to allow time for resolution [50, 51]. In addition, the ATR-dependent G2/M cell cycle checkpoint is activated through degradation of Cdc25A [51], and phosphorylation of Cdc25C phosphatase inhibits its ability to activate nuclear Cdc2 and, hence, entry into mitosis [52]. Most cancer cells are defective in DNA damage-induced checkpoints, for example through p53 pathway mutations, and this leads to dependence on the intra-S-phase and G2/M checkpoints for cell survival [32]. Therefore, ATR inhibition will lead to accumulation of DNA damage, premature entry into mitosis, mitotic catastrophe and cell death [32].

ATR inhibitors include schisandrin B [53], NU6027 [54], NVP-BEZ235 [55], VE-821 [56], VE-822 [57], AZ20 [58] and AZD6738 [59]. NVP-BEZ235 has been reported to induce marked radiosensitivity in Ras-overexpressing cancers [60], and NU6027 has been shown to increase sensitivity to DNA-damaging agents in breast and ovarian cell lines [54]. VE-822 results in selective sensitisation of pancreatic tumours to radiation in vivo by increasing persistent DNA damage, decreasing cell cycle checkpoint maintenance and reducing homologous recombination repair [57]. In vitro, ATR inhibition downregulates radiotherapy-induced PD-L1/2 expression to sensitise cancer cells to T-cell killing, in addition to potentiating DNA damage [61].

Immune Effects of ATR Inhibition

Promising preclinical in vivo studies of the ATR inhibitor AZD6738 in combination with radiotherapy have shown an enhanced type I/II interferon response and increased immune cell infiltrate [62], increased RT-stimulated CD8+ T-cell infiltration [63, 64], NK-mediated anti-tumour immunity [65], as well as reversal of the Treg immunosuppressive effect [63, 64]. Dillon et al. [62] reported significant radiosensitization to radiotherapy by ceralasertib alongside a marked increase in immune cell infiltration. Increased numbers of CD3+ and NK cells were identified, but the greatest part of the inflammatory infiltrate was composed of myeloid cells. Ceralasertib plus radiation produced a gene expression signature matching a type I/II interferon response with upregulation of genes involved in nucleic acid sensing. Increased major histocompatibility complex class I (MHC-I) levels were observed on tumour cells, with transcript-level data indicating increased antigen processing and presentation within the tumour. Significant modulation of cytokine gene expression (particularly CCL2, CCL5 and CXCL10) was found in vivo, with in vitro data indicating CCL3, CCL5 and CXCL10 are produced from tumour cells after combined therapy with ATR inhibitors (ATRi) and radiation. All of these data point towards opportunities to modulate immune responses triggered by ATRi and radiotherapy through the use of ICPIs that target key regulatory immune checkpoints.

In further studies, Patin et al. [65] evaluated the addition of ICPI (i.e. anti-PD-1, anti-PD-L1, anti-TIGIT) to the ceralasertib and radiotherapy combination, with a view to evaluating if there was further improved response and long-lasting immunity. They showed that ATR inhibition potentiated radiation-mediated tumour control in

mouse models of head and neck cancer (MOC2, AT84). ATRi enhanced radiotherapy-induced inflammation in the tumour microenvironment, with NK-cells playing a central role in maximising the effect of treatment. Anti-tumour activity of NK-cells could be further boosted with ICPI targeting TIGIT and PD-L1. In addition, NK-cells were shown to be critical for the induction of T-cell-based immune memory response in mice cured by radiotherapy/ATRi/ICPI combination regimens. Interestingly, analyses of clinical samples from patients receiving ceralasertib confirmed the translational potential of the preclinical studies, including evidence of NK and T-cell activation. Further evaluation of clinical trial material should shed more light on the potential value of ATR inhibitors as adjunctive treatments to immunotherapy-based therapy strategies.

There are, to date, three early phase clinical studies investigating ATR inhibition and radiotherapy. PATRIOT, a phase I study of AZD6738 in combination with palliative radiotherapy, has completed recruitment and is awaiting report (NCT02223923) (Fig. 3.2). BAY1895344 in combination with radiotherapy and pembrolizumab in recurrent head and neck squamous cell carcinoma (HNSCC) (NCT04576091) and M6620 with radiotherapy and chemotherapy in solid cancers (NCT03641547) are also ongoing studies. For at least some of these trials, additional analyses of the immune effects of treatment have the potential to provide further insights into the potential integration of DDR-targeted agents alongside radiation and immunotherapy for the treatment of head and neck (and other) cancers.

Conclusions

In this brief review, we have introduced the concept of targeting the DDR pathway, in this case through ATR inhibition, as a means of sensitising to radiation-induced cell death and triggering anti-tumour immunity. There are, however, a number of clinical challenges that need to be overcome in combining radiotherapy with DDR-targeted agents. These include: (i) the need to define optimal radiation dose-fractionation schedules to be used with DDR inhibitors and ICPIs; (ii) the need to understand how to integrate standard chemoradiation-based therapy regimens into a treatment paradigm based on combining radiation, DDR inhibition and ICPI; and (iii) considerations related to the potentially deleterious effects of wide-field irradiation, for example treatment that encompasses large volumes of tissue containing tumour-draining lymph nodes and large blood vessels (and the circulating immune cells within the bloodstream). In addition, careful attention will need to be paid not just to the acute effects of combination regimens potentially involving radiation, chemotherapy, DDR inhibition and immunotherapy, but also the risks of long-term toxicities. Nonetheless, this is an exciting time for the development of novel treatment strategies that have the potential to change outcomes for patients being treated with curative intent for newly diagnosed locally advanced head and neck cancers.
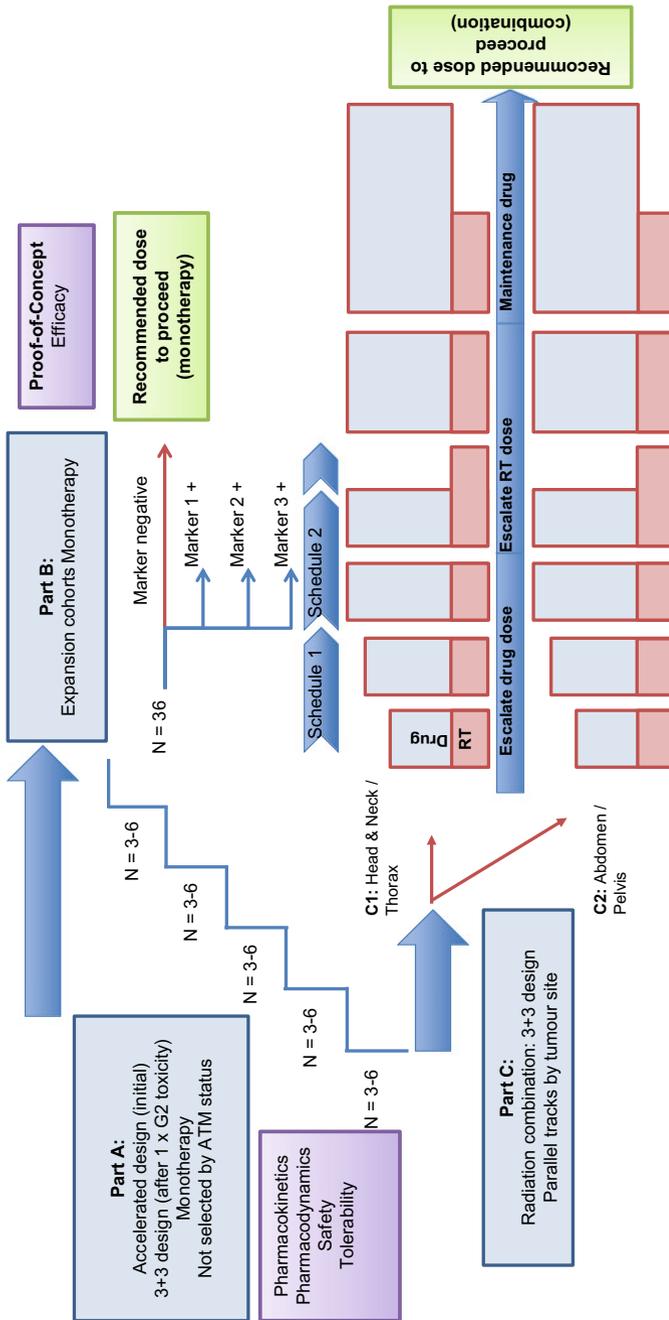
Fig. 3.2 Clinical trial design for the PATRIOT study of the ATR inhibitor, ceralasertib(AZD6738). Part A consists of a dose-escalation study of single-agent ceralasertibto define the recommended phase II dose. Part B consists of expansion cohorts using putative markers of replication stress. Part C is a study of ceralasertib at increasing doses and for increasing treatment durations (as indicated by the height and length of the grey boxes labelled "drug". (adapted from Dillon et al. Clin. Trans. Radiat. Oncol. 2018; 12: 16-20)

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Chapter 4
How to Standardize Molecular Profiling Programs for Routine Patient Care

Ingeborg Tinhofer, Ulrich Keilholz, and Damian Rieke

Introduction

Precision oncology is a rapidly evolving approach of tailoring therapeutic interventions to the individual molecular features of patients and/or their disease that moves beyond the conventional approach of stratifying patients into treatment groups based on tumor stage and phenotypic biomarkers [1]. Central to precision oncology is the ability to characterize precisely the molecular and cellular features of a tumor and its microenvironment, to determine which treatments are likely to confer the greatest benefit. For adequate counseling, patients are presented to Molecular Tumor Boards (MTBs) established at virtually all large cancer centers worldwide within the framework of precision oncology programs. The aim of MTBs is to identify and discuss all potential therapeutic strategies, based on genetic analysis, for patients who are not responding to standard-of-care systemic therapies. The individualized treatment

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recommendations by the MTB should be derived from a multidisciplinary discussion, including not only specific molecular alterations but also features concerning the patient (e.g., performance status, comorbidities).

Major scientific advances, in particular high-throughput sequencing technologies, animal and (increasingly also) *ex vivo* organoid models, play a pivotal role in the translational research that reinforces the current practice of medical oncology. However, the simply stated goal–tailoring oncological treatment to individual characteristics of a cancer patient–hides much complexity, and there are considerable challenges to be addressed. For a patient with cancer, major factors to consider include technical feasibility and validity of biomarkers, inter- and intratumoral heterogeneity, the challenging task of integrating and interpreting the ever-increasing volume of *omics* data, and the changing perspectives on value and cost-effectiveness in personalized cancer medicine.

The analysis of molecular profiles of tumors with the primary goal of detecting targets for molecular therapies relies on the collection of an appropriate biological specimen, optimal sample handling and processing, and accurate data acquisition and analysis (Fig. 4.1). Several efforts have led to technological improvements in terms of biospecimen acquisition and processing, parallel with assay procedure homogenization. Professional bodies have been providing guidance for the validation of clinical next-generation sequencing (NGS) tests [2], and rigorous validation programs [3, 4] have helped to successfully implement and continuously improve the usage of NGS tests in a routine clinical setting [5]. In the following sections, we will discuss relevant aspects to be considered in order to obtain high-quality samples and to standardize molecular analysis. We will review current challenges and potential solutions to maximize the clinical utility of a molecular profiling program.

Fig. 4.1 Overview of the precision oncology workflow. Relevant steps are listed in the left and important considerations in the right boxes
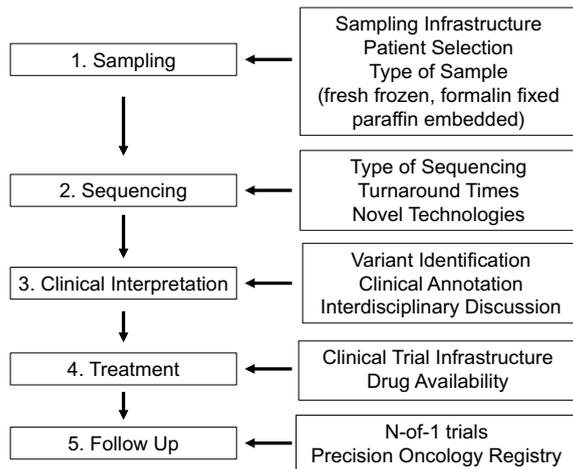
Relevant Aspects for Sample Collection and Processing

Though sample collection and processing can differ depending on the type of molecular analysis, there are general quality aspects to be considered for any type of NGS-based molecular profiling (Table 4.1). Low tumor cellularity and thus insufficient tumor DNA present in a sample is one of the most frequent reasons of molecular testing failure. According to our experience from molecular profiling programs at the Charité Comprehensive Cancer Center, tumor specimens selected for sequencing do not pass quality control in approximately 10% of patients referred to the MTB [6], stressing the importance of pathologic assessment in sample selection. The neoplastic cell, stromal and necrotic content can be highly variable within a sample, and even expert pathologists can judge the sample purity and suitability for testing very differently. Training programs for pathologists [7] that cover the principles and pitfalls of tumor cellularity scoring on sections [8] might help in reducing the reported wide variation in cellularity scoring amongst pathologists [8]. Latest technological advancement in digital pathology, including tissue scanners capable of scanning whole slides at high resolution also opens the possibility to leverage artificial intelligence-based image analysis techniques to further improve scoring accuracy [9].

Table 4.1 Standardized sample collection and processing

	Challenges	Potential solutions
Patient factors	Patients not eligible for tissue biopsy	Consider liquid biopsy (e.g. blood plasma, saliva)
Institutional factors	Tissue collection harmonization	Standardize collection tube, sample volume and time
	Sample selection	Training program for pathologists; automated AI-based image analysis
Tissue factors	Sample quality	Evaluate suitability of fresh biopsy *versus* archival tissue (FFPE)
	Sample fixation	Optimize type, temperature, and timing
	Sample processing	Evaluate availability of tumor samples not subjected to acid decalcification since it severely damages nucleic acids
	Tumor cellularity	Evaluate availability of sections containing >20% of viable tumor; standardize cellularity scoring; microscopic control of the sample to be submitted for molecular analysis
	Intratumoral heterogeneity	Multiregion sampling, consider complementary use of liquid biopsy

Abbreviations: AI, artificial intelligence; FFPE, formalin fixed paraffin embedded

Intratumoral heterogeneity (ITH) has been described as a further potential confounding factor in molecular profiling of tumors [10]. Regionally separated driver mutations, as reported among others for patients with non-small cell lung cancer [11] and renal cancer [12], are likely to be missed by single biopsy-based routine mutational analysis. Considerable ITH has also been reported for head neck squamous cell carcinoma [13–18], further increasing evidence that a single biopsy might not be enough for capturing the entire mutational landscape of individual tumors. A recent systematic review of studies on genetic mutation testing revealed that formal guidelines on how to avoid sampling bias due to ITH are lacking [19]. Of note, only 58% of the 40 genetic / biomarker studies included in this review reported on tumor purity thresholds, widely ranging from 10 to 100% [19]. As potential strategies to reduce sample bias due to ITH, Pongor and colleagues tested the effect of sample size, pooling as well as sequencing depth on the results of multiregional sequencing in ovarian cancer [20]. They observed similar genetic compositions from spatially neighboring regions, with only few private mutations [20]. Pooling samples from multiple distinct regions of the primary tumor did not increase the overall number of identified mutations. They further showed that pooling of multiregional biopsies was especially not suitable for hypermutated tumors since it diluted subclonal private mutations below detection thresholds [20]. In view of the limitations of present technologies, they recommended only one sequencing run per sample combined with high coverage (100–300x) sequencing, regardless of the number of samples taken from the same patient, as affordable and practical approach [20]. Another potential solution in tumor entities with known high ITH might be a complementary approach, in which mutational profiling of a single tumor biopsy is combined with the analysis of cell-free circulating tumor DNA [21], representing the cumulative reservoir of regionally separated mutant variants in the tumor.

Despite increasing numbers of studies focusing on the prevalence of ITH, its impact on clinical management of patients remains largely unknown. Evidence from breast cancer research has shown that molecular profiling of multiple tumor foci rather than the single largest tumor can lead to a change in treatment in 12.5% of cases [22]. In neuroblastoma, high degree of spatial heterogeneity was observed for genetic alterations in the druggable target genes *ALK* and *BRAF* [23]. Temporal ITH reflected by considerable differences in the genetic profiles of primary HNSCC tumors and their local relapses [24] stresses the importance of considering ITH in molecularly guided treatment selection in HNSCC as well. This preliminary evidence strongly supports further studies of ITH in precision oncology.

Clinical Next-Generation Sequencing

Clinical NGS has experienced rapid uptake in recent years, with a large number of academic and commercial certified laboratories offering NGS testing of tumor specimens [25]. Targeted NGS panels have come into widespread use for solid

cancer patients including also patients with head neck cancer [26, 27]. Large-scale sequencing platforms, such as whole-genome and whole-exome sequencing (WGS and WES, respectively), are already frequently used for research purposes, revealing prognostic or predictive profiles that may ultimately guide therapy [28]. In the prospective observational DKTK-MASTER study by the German Cancer Consortium, it was shown that WGS/WES and RNA sequencing enables molecularly informed treatments that lead to clinical benefit in a substantial proportion of patients with advanced rare cancers [28]. Further proof that WGS can meet high-quality diagnostics standards in clinical routine was recently provided by Roepman and colleagues who, after optimizing sample and data processing procedures, reported a technical success rate of 95.6% for WGS analysis of fresh-frozen tumor samples [29]. It is thus expected that in the near-future, WES and WGS will be applied in standard care.

However, since formalin fixation and paraffin embedding (FFPE) will remain the most widely used method for tissue fixation in the diagnostic setting, targeted NGS will likely remain the predominant application in molecular pathology [30]. This is due to the fact that: (1) WES and WGS are not yet ready for clinical FFPE-based NGS; (2) low sequencing coverage remains an issue especially for samples with low tumor cell content; (3) turn-around times are still too long for the routine diagnostic setting; (4) the costs are much too high; and last but not least, (5) the majority of sequence information generated by WES and WGS cannot directly be translated into clinical intervention. By contrast, targeted NGS strategies can overcome most of these disadvantages. Since the first reports showing that it is feasible to use small amounts of genomic DNA derived from FFPE biopsies for NGS-based analyses [31], several protocols have been established that adapt DNA extraction methods as well as target region capture to the specific requirements of FFPE tissue.

Targeted Gene Panel Sequencing

The clinical demand for mutation detection within multiple genes from a single tumor sample requires molecular diagnostic laboratories to develop rapid, highly sensitive, accurate and high-throughput testing within tight budget constraints. To meet this demand, most clinical laboratories including the Molecular Pathology at our institution use NGS panels which interrogate a specific set of clinically relevant cancer-related genes (Table 4.2). The targeted sequences of interest may be enriched by either amplification or hybrid capture. While amplification-based sequencing offers certain advantages including a generally lower input requirement and faster turn-around times, hybrid capture often gives greater library complexity and uniformity [32]. Validated assays incorporating both approaches have demonstrated strong performance characteristics on all major variant classes, including single nucleotide variants, insertion/deletions, copy number variants and structural rearrangements such as gene fusions.

Table 4.2 Current portfolio of certified NGS tests at the Charité university hospital molecular pathology

Certified NGS test	N genes	N regions	Type of alteration
Small gene panels			
Oncomine™ focus assay	52	n.a.	SNVs, indels, CNVs, fusions
nNGM lung cancer panel	19	102	SNVs, indels
Ion AmpliSeq™ cancer hotspot panel v2	50	207	SNVs, indels
Oncomine™ lung cfDNA panel	11	35	SNVs & indels
Oncomine™ breast cfDNA panel	10	26	SNVs & indels
Oncomine™ BRCA1/BRCA2 panel	2	256	whole exonic region
Large gene panels			
Oncomine™ TML assay	409	n.a.	TML, SNVs, indels
Molecular health IVD 600+ panel	624	n.a.	SNVs, indels, TML

Abbreviations: cfDNA, cell-free deoxyribonucleic acid; Indels, insertions / deletions; n.a., not applicable; NGS, next-generation sequencing; nNGM, national Network Genomic Medicine [50]; SNVs, single nucleotide variants; TML, tumor mutational load

The list of gene alterations targeted by clinical NGS panels varies largely. It may be focused on one or a few histologies, such as lung or colon cancer, that have a high prevalence of clinically actionable mutations (e.g. the 52-gene Oncomine focus assay, ThermoFisher Scientific). More typically however, the panel includes several hundred pan-cancer genes, such as the MH IVD 600+ gene test (a customized test from Molecular Health GmbH, Heidelberg, Germany), the 505-gene MSK-IMPACT test [33] or the 324-gene FoundationOne CDx® test [34]. The overlap between these comprehensive gene panels is moderate (Fig. 4.2), with only 208 (22.8%) of the 911 genes captured by all of them. The impact of this heterogeneity in available NGS tests on the clinical benefit rate of molecular profiling programs remains unclear. Also unresolved remains the question whether focused panels targeting only a limited number of molecular alterations are sufficient for routine patient care since they cover all alterations for which molecular drugs are currently available. For a preliminary analysis, we determined the portion of molecular alterations in HNSCC classified as main actionable targets based on the European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets (ESCAT) [35] that would be captured by the small 52-gene Oncomine Focus assay or the more comprehensive 324-gene FoundationOne CDx® test. As shown in Fig. 4.3, alterations in 14 of the 34 (41%) genes could be captured by both tests, and 4 of 34 (12%) genes by none of them. Of note, alterations in 16 of the 34 genes (47%) would be missed by the small NGS test, including genes of the DNA repair (*BRCA2, PALB2, POLE*), oxidative stress (*KEAP1, CUL3, NFE2L2*) and PI3K pathways (*PTEN*). Alterations in these genes mostly classify to the ESCAT tier III category [35] which is defined by a clinical benefit demonstrated in other tumor types or for similar molecular targets.

Fig. 4.2 Venn diagram of unique and common gene targets among three clinical next generation sequencing tests (blue: Molecular Health IVD 600+, red: MSK-IMPACT, green: FoundationOne CDx®)
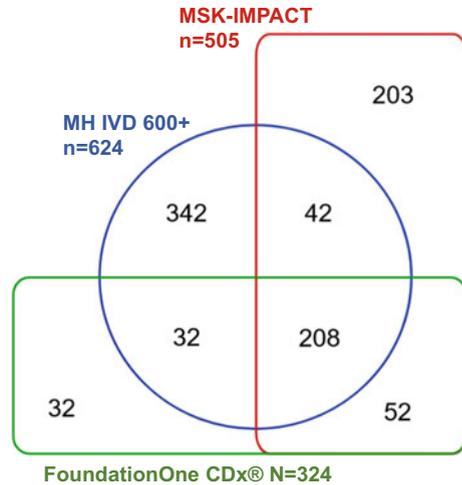
Clinical Interpretation of Molecular Alterations

Personalized treatment requires the identification of predictive biomarkers. In an evidence-based medicine sense, these are biomarkers that are associated with response to a particular treatment, irrespective of the mechanism. In this sense, any molecular alteration that provides information on the probability of response to a therapy is a predictive biomarker [36]. At the same time, the inter- and intra-tumoral heterogeneity of tumors and the number of different alterations make it important to keep biological mechanisms in mind, to adequately interpret molecular alterations that might be similar but not identical to previously described ones [37]. Thus, the identification of molecular alterations and their clinical interpretation requires interdisciplinary analyses by bioinformaticians, biologists and physicians. Identified molecular alterations in a tumor need to be interpreted and annotated in an interdisciplinary setting in a third step to assess their value for guiding personalized therapy (Fig. 4.1). Furthermore, predictive biomarkers are not limited to mutations (e.g. amplification, methylation, gene expression changes) and change rapidly with new data and drugs. These challenges furthermore stress the need for the timeliness of data as well as workflow flexibility.

Usually, these workflows consist of the identification of published data for a given alteration, their annotation with an evidence level, rating the quality and applicability of the data and the interdisciplinary discussion of annotated molecular alterations in an interdisciplinary molecular tumor board [6]. Several databases have been established to allow for up-to-date searches of predictive biomarker data, of which part of the OncoKB database has recently gained FDA-recognition [38, 39]. Due to the wealth of clinical and preclinical data, most databases contain non-overlapping information [40], which has sparked the development of a meta-database [41]. Identified clinical and preclinical studies then need to be evaluated and ranked, for which

a *NSD1 STK11 NTRK KMT2C*

b *TP53 PTEN KMT2D BRCA2 PALB2 CUL3 CDKN2A IGF1R KEAP1 NOTCH1 TERT ARID1A NFE2L2 POLE*

Selected Molecular Alterations in HNSCC

FoundationOne CDx® n=324

4[a]

257   16[b]

37   14[c]

1   0

Oncomine Focus Assay n=52

c *ERBB4 CDK6 FGFR1 MET FGFR2 BRCA1 AKT1 NF1 EGFR CCND1 PIK3CA ERBB3 ERBB2 HRAS FGFR3 BRAF*
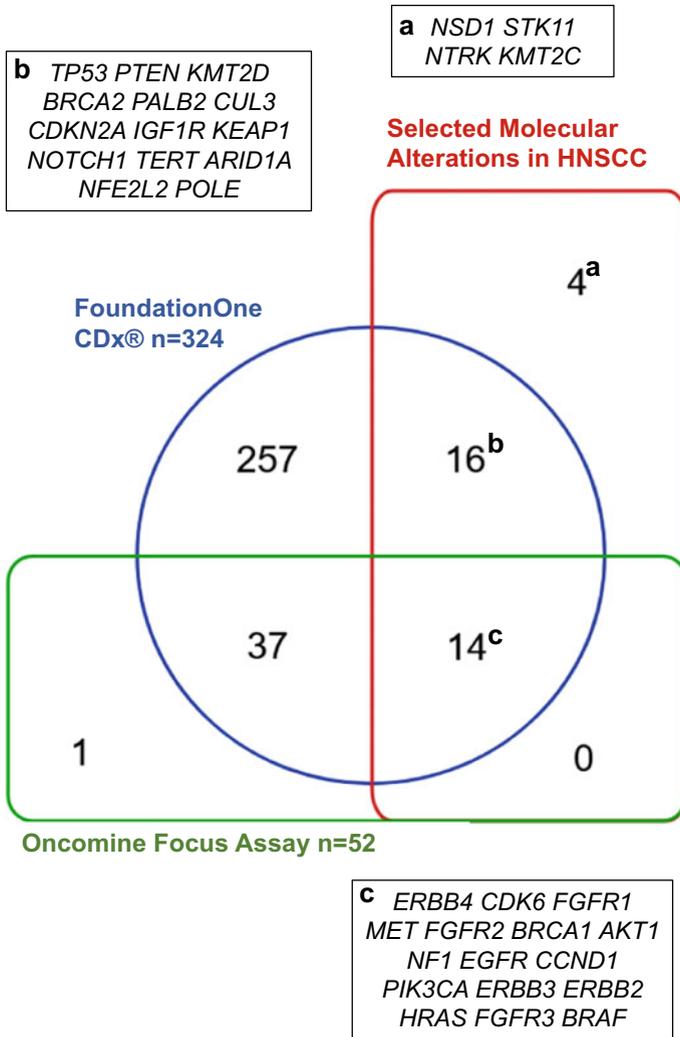
Fig. 4.3 Venn diagram of genes affected by selected potentially targetable molecular alterations in head neck squamous cell carcinoma (red), captured by a large (blue: FoundationOne CDx®) or small next generation sequencing panel (green: Oncomine™ Focus Assay). Genes captured by both tests (n=14), only the large test (n=16) or none of the tests (n=4) are listed in the boxes

several evidence level systems have been created [42, 43]. These evidence levels consider type and quality of underlying clinical trials, tumor histologies or even preclinical data. Negative predictive biomarkers indicating resistance to a specific therapy should also be integrated [6].

The individual annotation of molecular alterations, especially in the context of co-occurring alterations, is largely manual work and has been coined the bottleneck of

personalized oncology [44]. This manual work remains unstandardized and substantial heterogeneity in the interpretation of molecular alterations, especially in patients with complex tumors and not well-described alterations, was shown, whereas fewer better-described alterations led to more concordant treatment recommendations [45, 46]. Following the identification of potential predictive biomarkers, supporting data and the annotation of molecular alterations with evidence levels, an interpreted molecular patient profile is usually presented in an MTB. These MTBs have been established at many institutions but standards, guidelines, or quality requirements for MTBs are currently absent. All are oriented to molecular tumor profiling and its relevance to treatment decisions, and all consist of a multidisciplinary team. Clinicians (mostly medical oncologists) and pathologists form the core of virtually every MTB, and medical biologists and bioinformaticians take part in most MTBs, but other than that, composition can vary widely [6, 45, 47, 48]. The MTB critically appraises molecular alterations, identified predictive biomarkers and patient factors to ultimately identify treatment options.

Treatment and Follow-up

Though individualized treatment recommendations provided by the MTB based on molecular aberrations, relevant patient characteristics, drug and clinical trial availability can guide therapy selection, the final recommendation to the patients remains at the discretion of the treating physician. In the above mentioned DKTK-MASTER study by the German Cancer Consortium in patients with rare cancers, the recommended therapies were administered in 32% of cases [28]. A lack of drug and trial availability, low evidence levels for identified treatment options and deteriorating performance status of patients remain the major causes for this relatively small percentage. Prioritization of recommendations by the MTB was also identified as an important factor in clinical decision-making, as the highest-ranked recommendations could be implemented in 84% of cases [28]. Another important observation of the DKTK-MASTER trial was also that 25% of recommendations were based on ESCAT tier III molecular alterations, for which potential clinical benefit may be predicted because they represent specific alteration (as tiers I and II) but the molecular alteration-drug efficacy relationship was established in a different tumor type.

About one third of patients receive molecularly targeted treatment in unstratified trials [28, 49]. To extend the potential benefit to more patients, overarching precision oncology trials, an access program for drugs and an early integration of precision oncology are required. Available data suggest a greater benefit for patients that receive treatment that is better matched to their individual tumor's molecular profile [49]. Therefore, the integration of novel drugs and customized drug combinations need specific attention. The resulting complexity makes the development of prospective clinical trials to answer specific questions relating to these highly heterogeneous patient cohorts extremely difficult. Precision oncology programs are therefore also required to collect evidence from treated patients through a structured follow-up

program. These data should ultimately be standardized and shared between centers to allow for an optimal use of available evidence.

Conclusions

Molecular profiling programs are highly complex and still largely unstandardized. Many of the technical aspects such as sample acquisition, sequencing and variant reporting as well as the clinical interpretation of the results are already performed in a routine setting at many cancer centers, even though many aspects such as tumor heterogeneity require specific attention. Given that the knowledge of specific biomarker actionability and the armamentarium of molecularly targeted drugs are rapidly evolving, it can be envisioned that the fraction of patients who will benefit from genomically agnostic precision oncology will significantly increase in the next years. Therefore, continuously revisiting the whole MTB workflow from NGS platform selection, data acquisition, annotation and interpretation within molecular profiling programs is warranted to capture the actionable cancer genome and transcriptome as completely as possible.

The translation of findings into clinical care will continue to depend on additional factors like patient performance status and the availability of drugs and clinical trials. The integration of novel drugs and drug combinations as well as increasing evidence from dedicated follow-up programs is expected to improve outcome of precision oncology programs.

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Chapter 5
Novel Immune Oncology Targets Beyond PD-1/PD-L1 in Head and Neck Cancer

Edith Borcoman and Christophe Le Tourneau

Introduction

Tumor cells can induce T-cell immune tolerance via engagement of coinhibitory immune checkpoints molecules like cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or programmed cell death 1 (PD-1), leading to the escape from tumor-specific T-cell response and tumor progression [1, 2]. Therapeutic strategies to enhance cancer-specific T-cell immune response have been developed by inhibiting these specific coinhibitory immune checkpoints and re-activating T cells, such as anti-CTLA-4 or anti-PD-1/PD-L1 fully human monoclonal antibodies (MoAb) [1, 2].

Immune checkpoint inhibitors made a breakthrough in medical oncology in many different types of tumors. Regarding the treatment of recurrent and/or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC) patients, two anti-PD-1 immune checkpoint inhibitors have been approved in the second-line setting for

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patients who have failed platinum-based therapy, i.e. pembrolizumab which received FDA approval in August 2016 in patients with PD-L1 positive tumors (defined by a tumor proportion score [TPS] $\geq$ 50%) and nivolumab in November 2016 regardless of the PD-L1 status [3, 4].

In the first-line R/M-HNSCC setting, it has been more than a decade that no new treatment option showed a survival benefit in comparison to the standard of care (SOC) EXTREME regimen (platinum/fluorouracil plus cetuximab) [5]. Recently, the results from the KEYNOTE-048 study changed the paradigm in that setting [6]. The KEYNOTE-048 study assessed the efficacy of pembrolizumab alone or in combination with platinum/fluorouracil-based chemotherapy versus the SOC EXTREME regimen in previously untreated patients with R/M-HNSCC and showed improvement in overall survival (OS) in both pembrolizumab arms compared to the EXTREME regimen in patients with PD-L1 positive tumors, defined by a combined positive score (CPS) $\geq$ 1 [6].

However, despite these encouraging results and impressive durable responses in a minority of patients, not all patients in the three above mentioned studies derived benefit from anti-PD-1 immune checkpoints inhibitors. In fact, observed overall response rates (ORR) ranged from 13 to 19% in the anti-PD-1 monotherapy arms in these three studies, and OS still remained poor [3, 4, 6, 7] (Table 5.1). Furthermore, although the majority of HNSCC patients have PD-L1 positive tumors (approximately 85%), around 15% of patients present PD-L1 negative tumors for whom SOC EXTREME is still indicated and for whom novel treatment options are urgently needed [6, 7]. This emphasizes the need to improve immunotherapy strategies for HNSCC patients beyond PD-1/PD-L1 inhibitors.

We will further discuss in this review the novel immunotherapy strategies in development beyond PD1/PD-L1 in head and neck cancer.

Targeting Other Immune Checkpoints

Several strategies have been assessed to further improve T-cell priming beyond the PD-1/PD-L1 blockade, either for patients who progressed under anti-PD-1/PD-L1 treatment, or to assess new immunotherapy strategies in immune checkpoint inhibitors naïve patients. Various novel antibodies targeting immune checkpoints have been investigated alone or in combination with anti-PD-1 agents, all showing disappointing results.

Several studies assessed the combination of an anti-CTLA4 MoAb with an anti-PD-1/PD-L1 agent in patients with R/M-HNSCC. In the first-line R/M disease setting, two phase III studies assessed whether the combined use of an anti-PD-1/PD-L1 MoAb with an anti-CTLA-4 MoAb (nivolumab [anti-PD-1] plus ipilimumab in the CheckMate-651 study and durvalumab [anti-PD-L1] plus tremelimumab in the KESTREL study) would be superior over the EXTREME regimen [8] (NCT02551159). However, both had a negative outcome in terms of OS benefit

Table 5.1 Summary of efficacy data from phase III studies of approved anti-PD-1 immune checkpoints in recurrent and/or metastatic HNSCC patients

	CheckMate-141 Nivolumab Monotherapy 2nd line setting [3]	Keynote-040 Pembrolizumab monotherapy 2nd line setting (4)	Keynote-048 Pembrolizumab Monotherapy 1st line setting (6, 7)
ORR, N (%)	32/240 (13.3)	36/247 (14.6)	51/301 (17%)
mOS			
Months (95% CI)	7.5 (5.5–9.1)	8.4 (6.4–9.4)	11.5 (10.3–13.4)
PD-L1 < 1%	N = 73, 5.7 (4.4–12.7)	–	–
PD-L1 ≥ 1%	N = 88, 8.7 (5.7–9.1)	–	–
CPS < 1	–	N = 50, 6.3 (3.9–8.9)	N = 44, 7.9 (4.7–13.6)
CPS ≥ 1	–	N = 196, 8.7 (6.9–11.4)	N = 257, 12.3 (10.8–14.9)
TPS < 50%	–	N = 182, 6.5 (5.6–8.8)	–
TPS ≥ 50%	–	N = 64, 11.6 (8.3–19.5)	–

HNSCC = head and neck squamous cell carcinoma; PD-L1 = programmed death-ligand 1; ORR = overall response rate; mOS = median overall survival; CI = confidence interval; TPS = tumor proportion score

[8](AstraZeneca announcement 5.2.2021). In the second-line R/M-HNSCC setting in platinum-pretreated patients, the EAGLE study, a phase III assessing the combination of durvalumab plus tremelimumab versus treatment at the investigator's choice (cetuximab, a taxane, methotrexate, or a fluoropyrimidine) showed no significant differences in OS [9].

Tiragolumab is a MoAb that targets the coinhibitory receptor T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domain (TIGIT), which is expressed on lymphocytes and suppresses the immune response to cancer by limiting T cells and natural killer (NK) cells proliferation. Tiragolumab is currently assessed in combination with atezolizumab (an anti-PD-L1 MoAb) in a randomized phase II trial in the first line for patients with R/M previously untreated HNSCC, with PD-L1 positive tumors (TPS ≥ 5%) (Table 5.2) [10].

Another coinhibitory checkpoint molecule, lymphocyte-activation gene 3 (LAG-3) is currently targeted by different strategies. Relatlimab is a MoAb that is being investigated either in combination with nivolumab plus ipilimumab or with nivolumab plus an indoleamine 2,3-dioxygenase-1 (IDO1) inhibitor in a phase I/II study in the advanced setting (NCT03459222) (Table 5.2). Eftilagimod alpha is a soluble LAG-3 protein that binds to a subset of major histocompatibility complex (MHC) class II molecules to mediate antigen presenting cell activation and CD8

Table 5.2 Ongoing immunotherapy strategies assessed in recurrent and/or metastatic HNSCC patients

Study	Study drug	Clinical setting
Phase II randomized trial [10] (NCT04665843)	Tiragolumab (anti-TIGIT) + atezolizumab versus placebo + atezolizumab	Recurrent and/or metastatic HNSCC patients in the first line setting with PD-L1 positive tumors (TPS ≥ 5%)
Phase II trial (NCT03459222)	Relatlimab (anti-LAG-3) + nivolumab + ipilimumab or + nivolumab + anti-IDO1	Recurrent and/or metastatic HNSCC patients who have progressed after platinum-based chemotherapy
Phase II trial [11] (NCT03625323)	Eftilagimod alpha (soluble LAG-3 protein) + pembrolizumab	Recurrent and/or metastatic HNSCC patients who have progressed after platinum-based chemotherapy, unselected for PD-L1 tumor expression
Phase Ib trial [13] (NCT04196283)	ABBV-368 OX40 agonist + tilsotolimod ± nab-paclitaxel ± budigalimab	Recurrent and/or metastatic HNSCC patients previously treated with an anti-PD-1 inhibitor, with at least one accessible lesion for intratumoral injection
Phase I trial [14] (NCT02315066)	Ivuxolimab OX40 agonist + 4-1BB agonist	Advanced solid tumors including HNSCC patients
Phase III trial [16] (NCT04590963)	Monalizumab (NKG2A inhibitor) + cetuximab vs. placebo + cetuximab	Recurrent and/or metastatic HNSCC patients who have progressed after platinum-based chemotherapy and anti-PD-1 inhibitors
Phase II basket trial (NCT04357873) [22]	Pembrolizumab + vorinostat (histone deacetylases [HDAC] inhibitor)	Patient with recurrent and/or metastatic HNSCC, lung, cervix, anus, vulva, or penis squamous cell carcinoma

Table 5.2 (continued)

Study	Study drug	Clinical setting
Phase I trial [20] (NCT02517398)	Bintrafusp alfa (bifunctional fusion protein targeting TGF-β and PD-L1)	Recurrent and/or metastatic HNSCC patients who have progressed after platinum-based chemotherapy
Phase I/II trial [21] (NCT04009681)	THOR-707 is a recombinant human IL-2 either plus cetuximab or plus pembrolizumab	Recurrent and/or metastatic HNSCC, either treatment naïve or post PD-1 inhibitor
Phase I/II trial [23] (NCT03162224)	MEDI0457 vaccine + durvalumab	Recurrent and/or metastatic HNSCC with HPV16/18 positive cancer who have progressed after platinum-based chemotherapy

HNSCC = head and neck squamous cell carcinoma; PD-1 = programmed cell death 1; PD-L1 = programmed death-ligand 1; TPS = tumor proportion score; TIGIT = T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domain; LAG-3 = lymphocyte-activation gene 3; IL-2 = interleukin 2; NKG2A = natural killer group 2 member A; TGF-β = transforming growth factor β; HPV = human papillomavirus

T-cell activation, that was studied in combination with pembrolizumab in a phase II trial for HNSCC patients who had progressed on or after a first-line platinum-based therapy (NCT03625323) [11]. First reported results showed an encouraging ORR of 31.4% (95% CI: 16.9–49.3%) in patients unselected for PD-L1 tumor expression and deserves further development [11] (Table 5.2).

Along with immune checkpoint inhibitors, another strategy has been to develop antibodies targeting costimulatory immune checkpoints on T cells, like the inducible T-cell co-stimulator (ICOS), to improve priming of T cells. The feladilimab, was a first-in-man ICOS agonist developed in heavily pre-treated HNSCC patients having an anti-PD-1/L1 treatment-naïve disease that showed encouraging efficacy results in early phase with an ORR of 26% (95% CI: 12.9–44.4) [12]. However, subsequent randomized phase II trials were stopped by a recommendation of the Independent Data Monitoring Committee after obtaining results from a pre-specified futility analysis.

Others agonist antibodies have been developed to target OX40, a potent costimulatory protein in the tumor necrosis factor receptor superfamily (CD134, TNFRSF4), allowing to stimulate effector T cells and inhibit regulatory T cells suppression [13, 14]. OX40 agonists are currently being assessed mainly in combination with other immunotherapies. ABBV-368, an immunoglobulin G1 agonistic anti-OX40 MoAb is evaluated in a phase Ib study in heavily pre-treated HNSCC patients when given together with intratumoral injection of tilsotolimod, a synthetic Toll-like receptor

9 (TLR9) agonist, in combination with nab-paclitaxel and/or budigalimab (ABBV-181), a MoAb targeting PD-1 modified to reduce Fc receptor interactions and limit effector function [13]. In this study, patients had to have failed an anti-PD-1/PD-L1 inhibitor and must have had at least one tumor lesion accessible for intratumoral injection. Other OX40 agonists are also in early-development, like ivuxolimab, a fully human immunoglobulin G2 agonistic MoAb, that unlike immunoglobulin G1-based approaches, does not induce antibody-dependent cellular cytotoxicity and does not deplete OX40-expressing cells (Table 5.2) [14].

NK cells play a critical role in immunosurveillance and control of tumor growth. NK cell activation is negatively regulated by inhibitory killer-cell immunoglobulin-like receptors (KIRs). Blocking KIR function may potentiate an anti-tumor immune response and complement other immuno-oncology strategies that enhance T-cell activity. Lirilumab, a fully human MoAb inhibiting KIRs on NK cells promoting NK cells activation, was studied in combination with nivolumab in a phase I/II trial including patients with advanced solid tumor who have failed at least one prior line of standard treatment (NCT01714739) [15]. In the subgroup of HNSCC patients, the combination of lirilumab plus nivolumab showed an ORR of 24.1% (7/29) and was therefore further investigated in a phase II randomized study versus placebo plus nivolumab. This study, however, showed negative results.

Another immune checkpoint inhibitor, monalizumab is currently being studied in R/M-HNSCC patients previously treated with platinum-based chemotherapy and PD-(L)1 inhibitors [16]. Monalizumab is a first-in-class antibody that inhibits the coinhibitory molecule CD94/NK group 2 member A (NKG2A) expressed by cytotoxic CD8 T cells and NK cells. Results from a phase II study investigating monalizumab in combination with cetuximab in this heavily pretreated population showed an ORR of 20% (95% CI: 11–35%) with a confirmed partial response in eight out of 40 patients included [16]. The phase III study is currently ongoing (NCT04590963) (Table 5.2).

Combination of Anti-immune Checkpoint MoAbs with Other Type of Molecules

In addition to immune checkpoint inhibitors, other molecules have been developed to stimulate the T-cell antitumoral immune response.

The IDO1, an enzyme that catalyzes the degradation of tryptophan, has been described to induce anergy and apoptosis of T cells and can be expressed by tumor cells, dendritic cells and macrophages. Epacadostat, a highly selective oral IDO1 inhibitor, has been assessed in combination with pembrolizumab in a phase III study in the first-line R/M-HNSCC setting [17], but the global development of the molecule was stopped after the negative results of a randomized phase III study published in patients with advanced melanoma [18].

Transforming growth factor β (TGF-β) is implicated in multiple tumorigenic processes and further has a pivotal function within the immune system by maintaining immunotolerance via the regulation of lymphocyte proliferation, differentiation, and survival [19]. Bintrafusp alfa, a first-in-class bifunctional fusion protein targeting TGF-β and PD-L1 was evaluated in a phase I dose-expansion cohort in patients with R/M-HNSCC [20]. The reported ORR was 13% with four partial responses among the 32 included patients, and four patients with disease stabilization. Clinical activity was shown irrespective of tumor PD-L1 expression. A particular adverse event to note related to the treatment was one case of grade 3 keratoacanthoma and one case of grade 3 squamous cell carcinoma of the skin, but these cases were managed with simple excision followed by clinical observation (Table 5.2) [20].

Another interesting strategy is the assessment of interleukin (IL)-2 recombinant cytokines. THOR-707 is a recombinant human IL-2 molecule that includes a polyethylene glycol (PEG) moiety irreversibly bound to a novel amino acid via click chemistry to block the alpha-binding domain (IL-2 receptor [IL-2R], CD25) while retaining near-native affinity for the beta/gamma subunits. This molecule reduced risk of immune toxicities, via blocking CD25 activation on regulatory T cells, and reduce vascular leak syndrome seen with standard human IL-2, along with a maintained selective activation of effector T cells via the beta/gamma subunits binding [21]. The dose escalation phase is ongoing (NCT04009681) in R/M-HNSCC patients, either in combination with cetuximab of pembrolizumab (Table 5.2).

Beside the combination of two immunotherapy agents, or combination with chemotherapy or targeted therapies already approved in the treatment of HNSCC, another strategy is to assess the effect of epidrugs (epigenetic enzyme inhibitors), like vorinostat, a Histone DesACetylases (HDAC) inhibitor in combination with immune checkpoint inhibitors, as preclinical evidence has suggested that modulating the epigenome might modulate antitumor response and improve the efficacy of current immunotherapies (NCT04357873) (Table 5.2) [22].

Immunotherapeutic Vaccines for Human Papillomavirus (HPV)-Positive HNSCC

MEDI0457, a therapeutic DNA vaccine containing plasmids for E6 and E7 oncogenes for HPV16/18 and IL-12 adjuvant, has been shown to be safe and to induce an immune response against the expressed antigens, along with interesting preliminary efficacy results (ORR of 22.2%) when given in combination with durvalumab to HPV16/18 positive R/M-HNSCC patients (Table 5.2) [23].

Tipapkinogene sovacivec (TG4001) is another therapeutic recombinant vaccine based on the non-propagative highly attenuated Modified Vaccinia Ankara (MVA) virus vector that contains inserted transgenes coding for three proteins, the HPV E6 and E7 oncoproteins and IL-2 as an adjuvant. TG4001 has shown promising activity in a phase Ib/II trial including HPV16-related malignancies with an ORR of

23.5%, but limited efficacy on liver metastases (NCT03260023) [24]. However, the further development of TG4001 in a phase II randomized study is now focusing on HPV16-positive anogenital cancer patients only, with limited hepatic involvement.

Immunotherapy in the Early Stage Setting

In parallel with the continued increase of the number of studies evaluating immuno-oncology molecules in the R/M setting, these agents are currently studied in primary disease and are being assessed in the neodjuvant/adjuvant setting of HNSCC, showing promising response rates, without any surgical delays [25–27].

One randomized phase II trial assessed 2 cycles of nivolumab or nivolumab plus ipilimumab in the neoadjuvant setting for the treatment of patients with resectable squamous cell carcinoma of the oral cavity ($\geq$T2, or clinically node positive) [25]. No surgical delays were observed in this study and the pathologic response rate was 54% in the nivolumab arm and 73% in the nivolumab plus ipilimumab arm.

Pembrolizumab alone was investigated in a non-randomized phase II study in patients with resectable HPV-negative, locally advanced HNSCC [26]. In this study, the pathologic response rate after neoadjuvant pembrolizumab was 44% (16/36), without any delayed surgery, and a favorable one-year relapse rate of 16.7% (95%CI: 3.6–41.4%). The KEYNOTE-689 randomized phase III study further evaluates neoadjuvant and adjuvant pembrolizumab in combination with SOC adjuvant chemoradiotherapy in patients with previously untreated, resectable locally advanced HNSCC (NCT03765918).

Bintrafusp alfa has also been investigated in a window-of-opportunity phase II trial in patients with previously untreated, resectable HNSCC (NCT04428047). In this study the bintrafusp alfa was administered for 2 doses before surgery. The study was early terminated after sponsor decision following cases of hyperprogression and early toxicities reported in lung cancer trials.

The combination of lirilumab plus nivolumab was assessed in the neoadjuvant and adjuvant setting in patients with recurrent but resectable HNSCC [27]. In this open-label phase II trial, 28 patients received nivolumab plus lirilumab for one dose between 7 and 21 days prior the planned salvage surgery, then received 6 cycles in the adjuvant setting. Importantly there were no delays to surgery in this study, and pathological response to the combination was observed in 43% (12/28) of patients, with a favorable 1- and 2-year OS of 85.7% (95% CI: 66.3–94.4%) and 71.1% (95% CI: 48–85.3%), respectively.

However, it is not yet known if these preliminary results will translate into significant clinical benefit. Results from larger randomized studies and later survival endpoints are awaited. Furthermore, the definition of pathological responses needs to be standardized, and the establishment or pathological response as a surrogate for survival should be confirmed.

Conclusion

Despite a major craze for the development of immune-oncology drugs beyond PD1/PD-L1 inhibitors, for now, very few have shown significant antitumor activity as single agent in R/M-HNSCC patients, and further results are awaited from ongoing trials. All randomized trials assessing novel immune-oncology drugs in combination with an anti-PD-1/PD-L1 agent have failed in HNSCC patients so far. Many other immune-oncology drugs are in early clinical development and will hopefully improve patient outcomes.

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Chapter 6
Understanding Head and Neck Cancer Evolution to Guide Therapeutic Approaches

Ben O'Leary

Introduction

Cancer evolution is now better understood at both a pan-cancer and tissue-specific level, with the work of international consortia such as The Cancer Genome Atlas (TCGA) [1] and Pan Cancer Analysis of Whole Genomes (PCAWG) [2, 3] providing us with increasingly rich molecular datasets, including for squamous cell cancers of the head and neck (HNSCC). Darwin's theory of evolution was first directly applied to cancer by Peter Nowell, who hypothesized that from a single cell of origin, new cell lineages could evolve through genetic instability, with selection at a population level influenced by factors such as interaction with the immune system, metabolic adaptation to the microenvironment, and anti-cancer treatment [4, 5]. At the time Nowell made his observations, the most granular means of examining the genome was through karyotyping, direct visualisation of metaphase chromosomes within cancer cells allowing semi-quantitative assessment of chromosomal number and structure. Though Sanger sequencing enabled DNA characterisation at a greater degree of scale than had been previously possible, it has been the advent of massively parallel next generation sequencing in the last two and a half decades that made feasible the first sequencing of the human genome [6, 7], a project that remains ongoing [8].

These advances in sequencing technology, combined with complex computational analyses, now allow detailed examination of cancers at a genomic and transcriptomic level, through comparison to the germline DNA which the cancer genomes are derived from. The wealth of available cancer genomic data has allowed new insights into cancer biology, and revealed some of processes that underpin cancer evolution, such as the influence of mutational signatures [9–11], copy number and structural variation [12], and interaction with the immune system [13, 14]. Studies involving high-depth whole genome sequencing, and those with sequencing data from matched

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multi-regional samples can allow the timing of genomic aberrations to be inferred, revealing changes that occur early and late in tumour development [15]. At present, studies like these are extremely resource-intensive and challenging, and although the associated costs have been reducing as technology develops, much work remains to be done to demonstrate their clinical utility [16, 17].

The genomic landscape of HNSCC is now well-described, at least at a whole exome sequencing level [18]. However, there are limited whole genome data available for HNSCC as they are under-represented in the existing pan-cancer datasets [3, 18, 19]. There remains an ongoing paucity of data for metastatic disease. This article will discuss how new analytical approaches allow us to unpick the evolution of HNSCC before and during treatment, providing opportunities for novel therapeutic strategies.

Evolution of Treatment Naïve HNSCC

Genomic Landscape of HNSCC

There are now enough genomic data from a few sizeable clinical cohorts to confidently outline the genomic landscape of HNSCC, a key starting point for understanding the cancer-specific evolutionary processes. An important milestone for this was the initial publication from the TCGA in 2015, which included 279 cancers with whole exome sequencing of tumours and matched germline DNA, a much larger cohort than it had been previously possible to assess [20, 21]. The TCGA cohort has a strong bias towards HPV-negative disease, with 36/279 (12.9%) classified as HPV-positive, as defined by a significant number of mapped reads to *E6* and *E7* in the RNA data. Other biases included the dominance of oral cavity cancers (n = 172/279, 62%) and the heavy smoking history in the cohort, with a mean pack years of 51 [18]. The TCGA cohort has now been increased to 523 patients, largely confirming the previous observations and with a similar split between HPV-negative versus HPV-positive cancers (HPV + n = 72, 13.8%) [22, 23]. Nonetheless, this study has delivered key insights into HNSCC genomics and highlights differences in the biology of HPV-positive and HPV-negative cancer. As such it is an important starting point to understand HNSCC evolution.

Mutations in *TP53* were confirmed to be near ubiquitous among patients with HPV-negative disease (86%), an observation hinted at in smaller cohorts [21, 24, 25], while only a single case of HPV-positive disease had a non-synonymous mutation in *TP53* identified. Other genes noted to be more frequently mutated in HPV-negative disease were *CDKN2A*, the gene encoding the p16 protein, a key mediator of the cell cycle and of the G1/S checkpoint apparatus, and *FAT1*, a gene important in Wnt signalling, but which has also been implicated in resistance to cell cycle inhibitors through activation of CDK6 [26]. These findings confirm a phenotypic convergence in HNSCC towards dysregulation of cell cycle control, in HPV-negative disease through the functional loss of genes vital for cell cycle control but

achieved in HPV-positive disease through expression of viral oncoproteins. Mutated *NOTCH1* (17.1%) and *CASP8* (10.1%) were prevalent in HPV-negative cancers but rarer in HPV-positive disease, *CASP8* in particular being prevalent in oral cavity disease [18]. Compared to HPV-negative cancers, HPV-positive cancers demonstrate a higher prevalence of mutations in *PIK3CA*, the gene encoding the catalytic subunit of phosphoinositide 3-kinase (PI3K), an important intracellular signal transduction protein [19]. *PIK3CA* is remarkable for being the only frequently observed mutated oncogene in HNSCC, a cancer that is otherwise dominated in mutation terms by tumour suppressor genes. Increased prevalence of mutations in *PTEN,* the phosphatase and tensin homolog, upstream of PI3K signalling, highlights the importance of the PTEN/PI3K/mTOR pathway in HPV-positive HNSCC [19]. Other genes that may be more frequently mutated in HPV-positive disease as compared to HPV-negative disease include *ZNF750*, *CASZ1, EP300* and *FGFR3* [19], though much of the functional biology related to these specific alterations within the context of HNSCC is poorly understood at this time.

Combining the TCGA cohort with the whole genome data available from an HPV-positive cohort published by Gillison et al. demonstrates copy number changes that are commonly seen in both HPV-negative and HPV-positive cancers, such as loss of 3p, gain of 3q (seen across all squamous cell carcinomas [1, 27]), gain of chromosome 8 and loss of 9p [18, 19]. In some cases, such as with 9p, the loss is often observed to be relatively focal, specifically including the 9p21 locus that harbours *CDKN2A*, commonly observed to be mutated and providing evidence for phenotypic convergence towards loss of functional p16 resulting in dysregulated cell cycle. Copy number changes observed to be significantly different between HPV-positive and HPV-negative cancers included the 11q region containing *CCND1*, the gene encoding cyclin D1, which was gained in 14% of HPV-negative cases and lost in 17% of HPV-positive cases. A separate study of 108 HPV-negative cancers using a combination of whole exome and whole genome sequencing identified gain of 11q13.3 as being mutually exclusive with truncating mutations of *FAT1*, with concurrent proteomic analysis suggesting this signified convergent evolution towards dysregulated actin dynamics [28]. Losses in 11q, 13q, 14q and 16 were more commonly observed in HPV-positive cancers in the Gillison et al. analysis [19]. Interestingly, within the HPV-positive cohort, deletions in the region of *RB1* were identified in approximately a third of HPV-positive cases (34%), a counterintuitive finding in light of expression of the E7 oncoprotein presumed to abrogate the inhibitory control exerted on the cell cycle by Rb [29], and perhaps an indication that further fitness advantage can be gained through additional attrition on the function of Rb even in the presence of the E7 oncoprotein.

Mutational and Copy Number Signatures

The availability of large pan-cancer sequencing datasets has made possible the development of a number of analytical approaches that shed light on the processes that

drive mutagenesis and genomic variation in cancer genomes [30]. These approaches examine the specific mutations that occur across the genome within their genomic context, that is, the sequence immediately preceding and following the observed mutation [9, 31, 32]. Observing associations between these signatures and long understood clinical associations with various cancers (for example UV light [33, 34], smoking), along with experimental models, has allowed inference of causation and estimates of the relative importance for specific mutagenic processes within specific cancers [9, 11, 31, 35–38]. It is possible to extract mutational signatures from whole exome sequencing, but the power to discriminate between subtle differences is much greater with whole genome sequencing [39].

The mutational signatures of HNSCC have mostly been analyzed in the pan-cancer setting, but the available data do suggest some clear patterns. Some features are common to many cancers, such as the prominence of single base substitution (SBS) 1, one of the mutational signatures associated with ageing [35], and SBS 8, 16, 17 and 18, the cause of which remain unknown. An unsurprising finding is that SBS 4, related to tobacco, is prevalent in HNSCC [10, 11]. A single base substitution signature has not been confidently identified for alcohol, although certain patterns of mutations have been observed in cohorts of esophageal cancers [40, 41], and experimental models have shown that acetaldehyde exposure, an oxidation product of alcohol can lead to a double base substitution pattern [42]. Interestingly, one study examining a possible role for a new *E. coli*-related mutational signature in colorectal cancer also identified the same signature in one case of HNSCC [43].

In addition to these signatures, a further key evolutionary process identified for HNSCC, specifically HPV-positive disease, is the apolipoprotein B mRNA-editing enzyme, catalytic polypeptide (APOBEC), found in a high proportion of HPV-positive HNSCC [11, 19]. This family of cytidine deaminases are hypothesized to have evolved as a mechanism of cellular defence against DNA viruses, such as HPV, by causing mutagenesis in single stranded DNA during viral replication or transcription [44]. The mutational signatures, SBS2 and SBS13 [9, 10], have been ascribed to the APOBEC 3A and APOBEC3B enzymes deaminating cytosines, preferentially those immediately preceded by a thymine, though there may also be a role for DNA secondary structure for bases not following a thymine [45] (Fig. 6.1).

It is hypothesized that HPV infection drives APOBEC activity, potentiating mutagenesis indirectly as well as activating cellular growth and proliferation, this leading to increased genomic diversity and thus adaptability [46]. This is supported by modelling that suggests APOBEC activity could explain the excess of *PIK3CA* E542K and E545K mutations observed in HPV-positive HNSCC, both mutations being consistent with APOBEC activity [47, 48]. Mutations in *PIK3CA* are one of the most commonly identified in all cancers, usually clustered in one of two hotspots in the exon 9 helical domain and the exon 20 kinase domain [49]. In HNSCC compared to other cancers with a high frequency of *PIK3CA* mutations [50] there is a preponderance of the canonical helical domain mutations, E542K and E545K. This is also seen in the predominantly HPV-positive driven cervical carcinomas, favouring a role for associated HPV-related evolutionary processes. A large study of 1001 cell lines
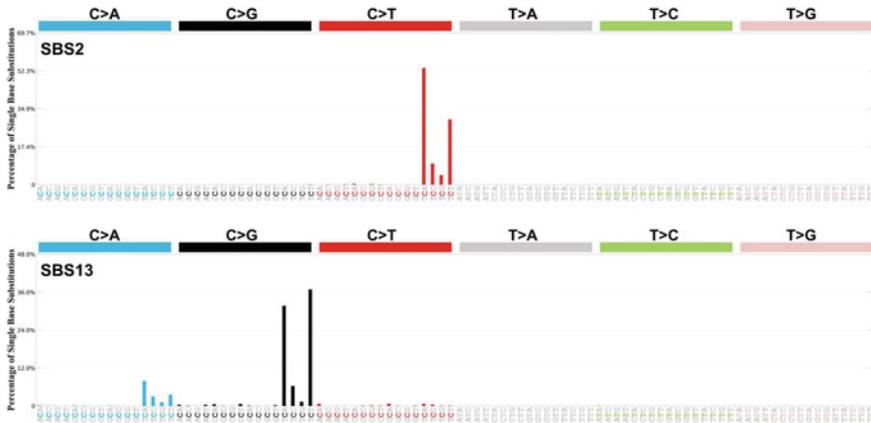
Fig. 6.1 The two APOBEC-related single base substitution signatures, SBS 2 and SBS 13. Frequency of base changes is shown within a specific trinucleotide context. Data accessed from https://cancer.sanger.ac.uk/signatures/

and 577 xenografts exposed to a variety of mutagenic stimuli observed marked variation in the APOBEC mutation rate over time, with multiple events clustered in time, for reasons that are unclear [51], but consistent with the phenomenon of kataegis, a process by which a large number of similar mutations occur in a focused area of the genome [52, 53].

More recently, in addition to unpicking mutational signatures by investigating genomic context, a number of groups have developed analogous techniques for copy number changes [54]. This involves identifying various features of copy number changes, often referred to as 'genomic scars' such as numbers of breakpoints, segment size and copy number aberration distribution across the genome and resolving them into cohesive patterns. This was first attempted in ovarian cancer and revealed considerable complexity in terms of the relationship between features of each signature, with seven different signatures identified in total [12]. The methodology was expanded upon in sarcoma, and later a pan-cancer cohort of approximately 10,000 to develop a total of 21 copy number signatures [55]. How copy number signatures may relate to the evolution of HNSCC remains an area that needs to be explored.

Intra-tumoural Heterogeneity and Timing Evolutionary Events

Though the gold standard for tracking cancer evolution is through analysis of longitudinally collected clinical samples, this is logistically challenging, often limited by the impossibility of obtaining samples from before the time of cancer diagnosis. Efforts to elucidate the critical early evolutionary events in head and neck cancer have been focused on pre-malignant disease, such as leukoplakia in the setting of oral cavity

disease [56–61]. This approach is predicated on the model popularised by Fearon and Vogelstein in colorectal cancer [62] of incremental genomic changes which eventually promote outgrowth of a clonal population. This model is complicated by the observation that many pre-malignant lesions never progress to invasive malignancy for reasons that are poorly understood. The 'field effect' conceptual framework for HNSCC [63, 64], where cancers arise within a wider population of abnormal but not malignant cells, has been recently updated by findings of considerable genomic diversity within normal tissues [65, 66].

Intra-tumour heterogeneity can be inferred from sequencing a single tumour sample through deconvolution of the clonal architecture. At its simplest this can be achieved through analysis of the distribution of observed variant allele frequencies, with the largest peak consistent with clonal variants—those that are present in all cells of the cancer (Fig. 6.2). Peaks at lower frequency describe the presence of subclones. The spread of allele frequencies can be used as a crude metric for heterogeneity within a tumour, and has been shown in an analysis of the HNSCC TCGA to associate with poor clinical outcome [67, 68]. The accuracy of subclonal deconvolution can be improved through the integration of copy number, ploidy and tumour content, all of which can influence the measured allele fraction [31, 69–71]. In one study of whole exome sequencing of HPV-positive oropharyngeal cancers, increased heterogeneity based on a single-sample analysis was associated with a poorer relapse free survival [72].
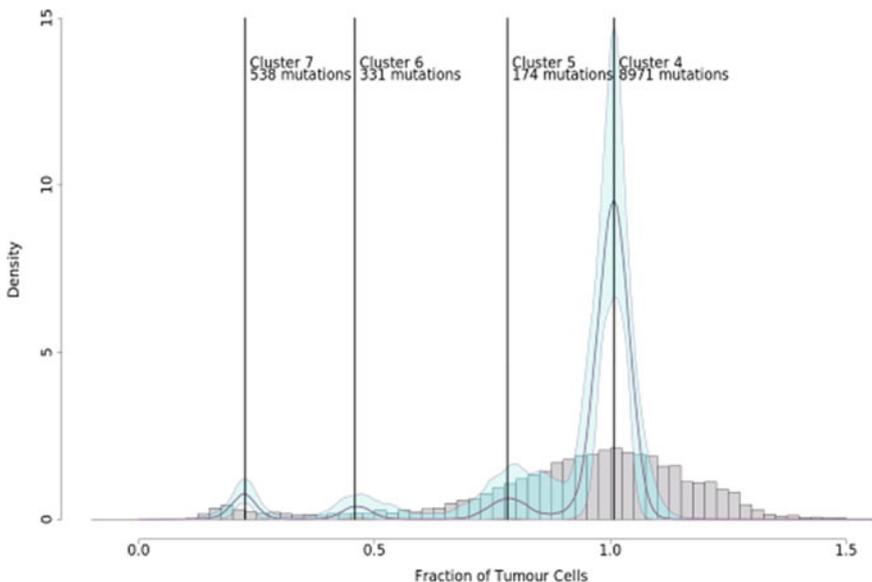
Fig. 6.2 An example of subclonal deconvolution using variant allele fraction. The clusters of mutations identify different populations of cancer cells, with the mutations in the largest peak being present in all of the cancer cells sampled

The best data on which to attempt clonal deconvolution is high-depth whole genome sequencing, where adequate coverage to call tumour variants in addition to germline single nucleotide polymorphisms (SNPs) allows finer discrimination of subclonal mutations and changes in copy number number [73]. In the PCAWG analysis, subclonal deconvolution was possible in 34 cases of head and neck cancer, suggesting that only 25% of these had no subclones identifiable. Single sample whole genome or, to a lesser extent, whole exome sequencing can also be used to infer the order in which genomic aberrations were acquired [73]. Mutations and copy number changes that are identified as subclonal must be preceded by clonal changes, and mutations that can be ascribed to a particular copy number can be inferred to have occurred before or after a particular gain (Fig. 6.3) [73]. In the PCAWG cohort of head and neck cancer (n = 57) events that occurred early in evolution using this approach included loss of 9p, along with mutations in *NOTCH1* and *TP53*, consistent with previous studies of pre-malignancy [58–60].

Sampling and sequencing multiple areas of a cancer can allow more detailed ordering of the observed genomic events [74, 75], categorising them into shared or private, and capture additional populations that would be missed with a single sample [76, 77]. This can also shed light on heterogeneity within a single cancer and provide potential clues to drivers of convergent evolution, as exemplified in the TRACERx study of lung cancer [76, 78, 79]. There are few studies that have directly assessed the sub-clonal heterogeneity of HNSCC with multiple sampling of the same tumour, and these are all from oral cavity cancers, where resection is the primary treatment and thus multi-regional samples easier to obtain. One small study of 5 patients with oral
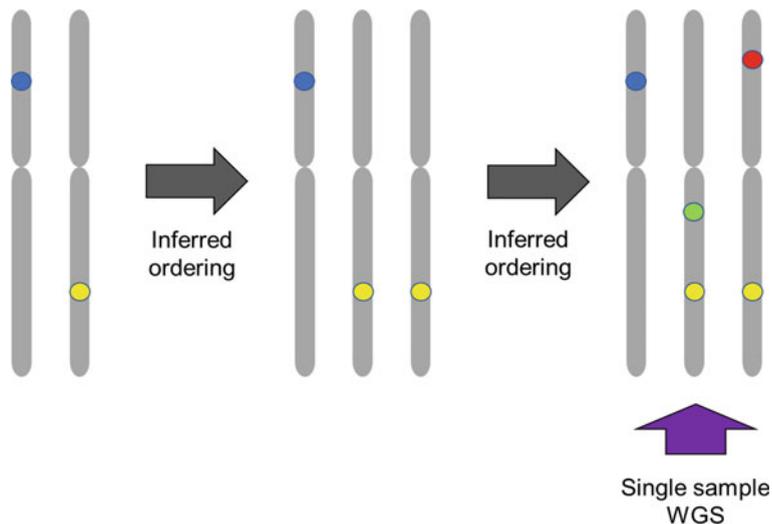
Fig. 6.3 Example of inferring timing of genomic events. Data from a single sample are observed (far right). Subsequently it can be inferred that the yellow mutation was acquired before the copy number gain, whereas the green and red mutations were acquired after

cavity SCC using whole exome sequencing found that the vast majority of mutations were conserved in the ~3 areas they sampled, though did not systematically examine copy number [80]. Another study of oral cavity SCC examined 44 biopsies from 13 cancers using shallow-depth whole genome sequencing to compare regions for copy number, finding relatively low levels of variation [81]. This seems at odds with the inferred sub clonal architecture seen in PCAWG, though all the datasets in question are probably too small to draw any firm conclusions. A further area of uncertainty is whether significant differences exist between primary HNSCC and lymph node metastases, with only a few studies directly examining this. One study using whole exome sequencing of matched primary and lymph node metastases in 13 patients found most mutations were shared (86%) [82], with another study involving single cell RNA sequencing of 5 cases of matched primary and lymph node suggesting tumours exhibiting signalling consistent with partial epithelial to mesenchymal transition were more associated with lymph node metastases. Larger studies along these lines will be needed to clarify intra-tumour heterogeneity and the molecular relationship between primary HNSCC and lymph node metastases.

Evolution and Considerations for Therapy in HNSCC

Developing Biomarkers and Therapies Informed by Evolution

There are currently no licenced therapies for HNSCC that incorporate genomic or evolutionary elements, but there are avenues along these lines that have shown some promise for the future. As discussed above, the PCAWG evolutionary timing analysis for the 57 patients in the head and neck cancer cohort highlighted with high confidence the loss of 9p as an early, clonal event in HNSCC [2]. Loss of 9p has been identified at a high prevalence within premalignant oral cancer lesions and associated with a higher risk of progression to malignancy [57, 58, 83], further highlighting its potential functional importance. A major pan-cancer study that included the HNSCC TCGA cohort found an association between aneuploidy and reduced host immune response, with this further associating to poorer outcomes on immune checkpoint inhibitors in a melanoma cohort [84]. Seeking to explore the immune consequences of 9p loss in HNSCC, William et al. examined a cohort of 188 cases of HPV-negative oral pre-malignant disease for copy number changes including 9p21.3 in addition to CD3+ , CD8+ and CD68+ cells assessed with multicolour immunofluorescence to characterise the immune infiltrate [85]. In this cohort, loss of 9p21.3 was not associated with increased infiltrate, although chromosomal gains such as trisomy and tetrasomy were. Recapitulating their analysis in the TCGA dataset, inferring immune infiltrate from RNA expression, the investigators observed that 9p loss in HNSCC was associated with reduced T cell infiltrate, so-called 'immune-cold' tumours, an effect that appeared to be driven by cases where there was 9p loss at the chromosomal arm level, rather than the more focused 9p21 loss typically associated with deletion

of *CDKN2A* and the *IFNA1* genes. Interestingly, these associations appeared to hold only for more advanced cases of HNSCC. The authors hypothesized that loss of 9p may be an important switch leading to a change in the microenvironment from immune-hot to immune-cold. Examining 9p as a potential biomarker, the investigators looked at 9p loss in a mixed clinical cohort treated with immune checkpoint inhibitors, finding that loss of 9p was associated with poorer prognosis, an effect not observed in an unrelated observational chemotherapy cohort [85].

A further example of evolutionary considerations applied to therapy is provided by the development of *HRAS* mutation-targeted therapies in HNSCC. In HNSCC, mutations in *HRAS* occur in 4–8% of cases, clustered around the activating hotspots in codons 13, 13 and 61 [18, 19, 86]. Tipifarnib is a farnesyl transferase inhibitor, a family of drugs that were developed to indirectly target the oncogenic activity of Ras through preventing its farnesylation, a key step in its localisation to the cell membrane and a pre-requisite for activation of its signalling. Though results in *KRAS* and *NRAS*-mutated cancers were disappointing, preclinical data suggested efficacy for tipifarnib in *HRAS*-mutant HNSCC cell line and xenograft models [87] leading to the KO-TIP-001 trial, an open label, phase II study of tipifarnib in *HRAS*-mutated HNSCC [88]. After an ad hoc analysis of the first 16 patients recruited to the trial, the protocol was amended to limit eligibility to patients with a variant allele fraction of >20% in their cancers, with 11/20 evaluable patients experiencing at least a partial response for an overall response rate of 55%. Selection of patients with specific mutations based on high variant allele fraction increases the chance that the mutation in question is clonally dominant, in theory improving the rationale for targeting the change, as subclonal populations with different molecular characteristics can exhibit varying responses to treatment [89]. This consideration of how clonally dominant specific mutations are may well be important when considering targeted therapies.

Moving beyond single molecular alterations, it is also possible that more abstracted evolutionary processes could be used to inform treatment in HNSCC. Tumour mutational burden (TMB) is effectively a composite output of the sum of mutational signatures acting upon a cancer genome, and potentially of neoantigen burden, and has been put forward as a candidate biomarker for response to immune checkpoint inhibitors [90]. This is supported by a large meta-analysis of over 1000 patients treated with immunotherapy which identified tumour mutational burden as the strongest predictor of response [91]. Two retrospective cohorts of HNSCC treated with immune checkpoint inhibitors have also demonstrated improved outcomes with high tumour mutational burden [92, 93]. Though these data seem to confirm a signal for tumour mutational burden and improved outcome on immune checkpoint inhibition in HNSCC, it is uncertain how this can be usefully integrated into the clinic, not least as there is debate around how best to define TMB [90]. Moreover, with ~20% of HNSCC qualifying as 'high' [94] and most patients with HNSCC eligible for access to checkpoint inhibitor therapy in the first or second line based on CPS anyway, it is not clear how TMB would be best integrated into the standard of care pathway.

Considering copy number signatures as a potential biomarker for treating HNSCC, Essers et al. examined a cohort of 173 patients with HPV-negative oropharyngeal,

laryngeal and hypopharyngeal cancers who were treated with definitive chemora-
diotherapy and performed low coverage whole genome sequencing to assess copy
number signatures as defined by Macintyre et al. [12, 95]. Subsequent analysis and
validation in the TCGA cohort identified a number of clinically relevant associations
with copy number signatures. High signature 1 and 6 were associated with better
and worse outcomes respectively, with 5 and 7 associated with increased frequency
of distant metastases. Further work is needed to understand better the underlying
biology that is driving these associations, but this study provides support for the
concept of investigating evolutionary processes pertaining to copy number variation
within the clinical paradigm.

Recurrent and Metastatic HNSCC—Evolution on Treatment

A knowledge of the genomic landscape of recurrent and metastatic HNSCC is an
important starting point for understanding the relationship between molecular char-
acteristics and treatment outcomes. Unfortunately, the genomic landscape of recur-
rent/metastatic HNSCC remains poorly defined at present, with available datasets
limited by small size and heterogenous cohorts. The largest available cohort of recur-
rent/metastatic head and neck cancer with genomic characterisation comes from
Memorial Sloan Kettering, with sequencing data from 151 patients using a 410 gene
panel for mutations combined with low-depth whole genome sequencing for copy
number [96]. Of these, 53/151 were HNSCC, the rest being accounted for by other
head and neck malignancies, limiting the scope for characterising HNSCC. Nonethe-
less, some interesting comparisons could be made with primary HNSCC, including
increased frequency of *TERT*-promoter mutation in HPV-negative HNSCC, most
notably in tongue SCCs where it was seen in 91% (10/11) cases. HPV-positive
tumours were observed to have fewer subclonal populations than HPV-, though data
from targeted sequencing such as used in this study are not optimal for assessing
this. Of note, recurrent/metastatic HPV-positive tumours were found to have a higher
prevalence of features more commonly associated with HPV-negative disease, such
as whole genome doubling and concurrent loss of 3p with *TP53* mutation, suggesting
these might be associated with a poorer prognosis 'HPV-negative-like' phenotype.
The other available cohort to consider with regards the genomic landscape of recur-
rent/metastatic HNSCC is the Hartwig Foundation whole genome sequencing project
for metastatic cancer, which included 42 cases of head and neck cancer in its pan-
cancer analysis [97]. The spectrum of mutations and copy number variations in this
set does not appear to depart significantly from the landscape of treatment-naïve
HNSCC, but is too limited by size to make a meaningful comparison. It is likely
that more will be learned as these cohorts of recurrent/metastatic HNSCC grow and
genomic assays become more accessible.

The question of which genomic aberrations, if any, select for treatment resis-
tance is best examined by using longitudinal matched tumour samples with matched
clinical annotation. Due to the logistical challenges presented by generating these

datasets, the available data are mostly limited to small cohorts, though these nonetheless present a valuable resource for hypothesis generation. Hedberg et al. examined a cohort of 10 HNSCC patients with whole exome sequencing from matched primary and metachronous recurrence samples, one of these cancers being HPV-positive [82] and five of them treated with radiotherapy. All the recurrences were in the upper aerodigestive tract and 9/10 were within 12 months of the initial treatment. Comparison of genomic profiles for primary versus metastasis was possible in 8 patients. Provocatively, only 60% of mutations were shared between the primary and metachronous occurrences, suggesting significant biological differences. In this particular study, many of the recurrences were from a distinctly separate anatomical site in the upper aerodigestive tract, inviting the hypothesis they had arisen from a distinct, but related population of either premalignant or treatment resistant cells. Similarly, a slightly larger cohort of 19 mainly HPV-negative patients subjected to the lower resolution technique of targeted sequencing with a 257 gene panel also found a significant proportion of the cohort had a markedly different mutational profile on relapse (31.9%, 6/19) [98]. Data on HPV-positive disease are even more limited, but a cohort of 7 matched primary/recurrence samples also showed substantial variation in the mutational spectrum between primary and relapse [99]. Focusing on patients treated with definitive chemoradiotherapy, de Roest et al. conducted low-coverage whole genome sequencing with targeted sequencing of 12 genes in 10 HPV-negative paired primary and relapse samples [81]. Here again, significant differences were seen in the genomic profiles of the primary versus the relapsed disease samples, even to the point where an algorithm trained on multi-regional data from primary cancers designated many of the relapses as 'genetically unrelated', even in some cancers which had relapsed within a few months of treatment [81].

The largest cohort of primary/relapse patients comprises 38 patients with HPV-negative disease, who had relapsed more than 6 months but less than 3 years after radiotherapy [100]. Using whole exome sequencing on DNA derived from FFPE samples, the investigators complemented their genomic analysis with paired RNA sequencing, additionally categorising the cancers sampled before and after relapse using the transcriptomics profiles described by Keck et al. [101]. Additionally, they performed single cell RNAseq on three patient-derived in vitro models. In addition to finding heterogeneity of transcriptional subtypes at a single cell level in the in vitro models, the investigators found variation in the longitudinal transcriptional subtypes observed between primary and relapsed, with a relative reduction in the frequency of the inflamed-mesenchymal subtype [100]. Of note, those patients in whom different transcriptional subtypes were observed tended to have longer time to relapse. Paired genomic analysis was conducted in 28 pairs and 79% were inferred to be genetically related, with an overall overlap of 70.9% of the top 20 mutated genes seen in paired samples [100]. Taken all together, these studies suggest a complex evolutionary relationship between primary and relapsed HNSCC, with some molecular elements that remain consistent with others changing or being selected through the treatment process. More insights will follow if analyses such as the above can be scaled in size and ideally integrated into clinical trials for high-quality clinical annotation.

Circulating Tumour DNA

Unpicking questions related to evolution in cancer on treatment ideally requires longitudinal tissue sampling, a process that involves discomfort and a degree of clinical risk for patients undergoing these procedures. However, in patients with cancer, a proportion of their cell-free DNA is derived from cancer, and as such can be used as a 'liquid biopsy' for the purposes of molecular characterisation [102–104]. This potentially offers a major opportunity to overcome some of the challenges of longitudinal tissue biopsies, with the blood tests required for plasma DNA collection being substantially less invasive than tissue biopsies. In addition to ease of sampling, circulating tumour DNA (ctDNA) may confer additional advantages over tissue biopsies for certain analyses, particularly with regards assessment of heterogeneity, where ctDNA may provide representation of a number of different metastatic deposits, rather than a single sample from a tissue biopsy [105, 106]. Novel analytical techniques have been able to move beyond the calling of mutations and copy number in ctDNA and integrate other characteristics such as fragmentation pattern and inferred nucleosomal occupancy to allow further exploration of tumour biology [107, 108]. That said, there are a number of technical challenges inherent in ctDNA analysis, the fraction of cell-free DNA that is derived from a patient's cancer is often extremely low, especially in localised disease, meaning sampling effects can dominate assessments [109] and variant calling, particularly of copy number, can be challenging.

For HNSCC, ctDNA has predominantly been investigated as a tool to predict relapse, within the context of minimally residual disease. One particularly attractive area for this is in HPV-positive and EBV-positive disease, where the presence of viral DNA in the plasma provides a much easier target to differentiate for detection than mutated human DNA [110–114]. However, there are also data supporting this approach in non-virally driven HNSCC [115, 116]. There are few data at the present time for using ctDNA analysis to track longitudinal evolution of HNSCC on treatment, and this is an opportunity that needs to be exploited in the future.

Conclusions

Cancer cannot be understood without evolution. Evolutionary concepts are critical to understanding both how cancer develops and how it responds to treatment. Thanks to technological advances in sequencing and the efforts of international academic consortia we now have an approximate outline of the genomic landscape of treatment-naïve HNSCC, a starting point to unpick how those cancers develop, and why they respond so differently to treatment. The next steps are to improve our knowledge of recurrent/metastatic disease and begin to associate molecular characteristics with clinical phenotypes. More longitudinal studies of cancer evolution on treatment will be important in achieving these goals, with circulating tumour DNA a useful tool in

taking this forward. To ultimately improve patient outcomes we need biologically-directed clinical trials embedded in a paradigm of forwards and reverse translation.

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Chapter 7
Sensitivity and Specificity of Extranodal Extension: Unlocking One of the Strongest Prognostic Factors in Head and Neck Cancer

Shao Hui Huang, Ionut Busca, Eugene Yu, Ezra Hahn, and Brian O'Sullivan

Introduction

The lymph node (LN) capsule is a natural barrier for tumor progression. When tumor breaches the LN capsule (termed extranodal extension [ENE] by the 8th edition TNM [1, 2]), it presents a challenge to disease clearance regionally but more importantly, also augments risk of distant metastasis (DM). Presence of ENE could be a consequence of long-growing ignored tumor, but more likely represents an aggressive tumor phenotype [3].

ENE can be detected on pathology specimens, inferred from imaging, or be indirectly evident via clinical examination. Extent of ENE reflects incremental tumor

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invasion. The initial stages of ENE can only be detected under the microscope (namely pathologic ENE, pENE). When ENE continues, it can eventually become visible on radiologic imaging (namely radiologic ENE, rENE). When ENE further progresses to invade skin with hallmark changes of skin ulceration or dermal edema (e.g., peau d'orange) and/or adjacent soft tissue structures (e.g., muscles, nerve, and vessels) causing fixation and neurovascular impairment, it will result in obvious clinical features consistent with ENE (namely clinical ENE, cENE).

ENE in head and neck cancer (HNC) was first described by Willis [4] in autopsy material from a head and neck epidermoid carcinoma in 1930. Its prognostic importance was confirmed in subsequent studies [5–7]. Convincing evidence demonstrates that ENE is one of the strongest prognostic factors for both viral-related and unrelated HNC. Unequivocal rENE carries prognostic significance beyond traditional cN classification and has the potential for risk stratification and future N classification [8–15]. Detection of ENE may also directly impact clinical care and treatment planning. If ENE can be identified before surgery, it can help predict the likelihood of needing more intense adjuvant approaches including triple modality treatment (postoperative chemoradiotherapy, postop-CRT) due to presence of pENE. Therefore, early recognition of its presence can triage appropriate treatment recommendations. This is especially relevant for many HPV-positive oropharyngeal cancer (OPC) patients where equipoise concerning disease control has emerged between primary (chemo-)radiotherapy (RT/CRT) and transoral surgery (TOS) due to an important focus on functional preservation. However, the sensitivity of rENE for pENE remains unsatisfactory.

In this chapter, we summarize pathological, radiological, and clinical signs of ENE and their relationships. Since rENE may have a broader implication in pretreatment risk stratification and treatment selection, we propose a means to augment sensitivity and specificity of rENE for pENE detection. Finally, we review emerging data on biomarkers that are associated with ENE.

Pathological, Radiological, and Clinical Signs of ENE

ENE refers to tumor invasion through the nodal capsule into perinodal fat and beyond. It can invade through a single LN or involve 2 or more adjacent LNs to form a coalescent nodal mass. It can also destroy the entire nodal structure and manifest as a soft tissue deposit within nodal regions without associated clearly identifiable LN(s).

The extent of pENE has been categorized differently by various authors [2, 16–19]. Carter et al. [16] in 1985 classified ENE as "*microscopic*" (microscopic breaks in the lymph node capsule, only evident on histologic examination) versus "*macroscopic*" (spread of tumor into identifiable structures within the specimen) pENE. The latter may also be evident with clinical and radiological assessment. However, this classification is somewhat rudimentary since much "microscopic" ENE also carries prognostic significance. Yamada et al. [19] later classified pENE into three types: "*Type*

A"—few tumor cells outside the LN capsule; "*Type B*"—microscopic invasion of the tumor cells into perinodal fat tissue, with capsular destruction, and "*Type C*"—macroscopic tumor invasion into perinodal fat or muscle tissue. However, this classification is ambiguous in practice since specific descriptions of the pathologic assessment of "microscopic" vs "macroscopic" were not provided. Lewis et al. [18] classified pENE into four grades: "*Grade 1*"—tumor reaching LN capsule with thickening of the overlying capsule; "*Grade 2*"—tumor extending ≤1 mm into perinodal issue; "*Grade 3*"—tumor extending >1 mm beyond nodal capsule; and "*Grade 4*"—soft tissue deposit without residual nodal architecture. The latter is probably related to the effacement of the entire nodal capsule by tumor or due to tumor foci escaping from the lymphovascular pathway. However, "*Grade 1*" pENE category in this classification does not truly reflect the essence of ENE. The 8th edition American Joint Committee on Cancer (AJCC) TNM (TNM-8) [1, 2] recommended directly measuring the distance from the breached nodal capsule to the farthest extent of tumor to quantify pENE extent as "*microscopic ENE (micro-ENE)*" (≤2 mm) versus "*major-ENE*" (>2 mm) (Fig. 7.1A–C). When tumor destroys the entire nodal architecture with only a soft tissue deposit in the neck tissue, it represents the most advanced form of pENE and should be considered as "*major-ENE*" (Fig. 7.1D).

The cutoff of "micro-pENE" versus "major-pENE" varies in the literature. For example, the ECOG 3311 trial [20] used a 1 mm cutoff where ≤1 mm pENE is considered "intermediate" risk and eligible to receive postoperative RT alone with either 50 Gy or 60 Gy in HPV-positive OPC following TOS. Wreesmann et al. [21] used receiver operator curve (ROC) analysis at specific time points and identified a prognostic cutoff for ENE extent at 1.7 mm in oral cavity squamous cell carcinoma (OSCC). Similarly, Mamic et al. [22] reported a 1.9 mm cutoff by ROC analysis for prognostically important ENE in 174 cN0 OSCC who underwent surgery with elective neck dissection. Arun et al. [23] found that a 2 mm cutoff did not show prognostic significance in 212 OSCC patients, whereas a 5 mm cutoff demonstrated significant differences in overall survival (OS) and disease-free survival (DFS).

Similar to pENE, the grading of rENE definition is also evolving [13, 24–27]. It is now recognized that rENE can manifest in any individual LN or affect multiple adjacent LNs to form an inseparable nodal mass. Chin et al. [28] recently proposed clearly defining rENE extent into three grades: "*Grade 1*" rENE—tumor breaching the nodal capsule of an individual LN characterized by unambiguously ill-defined

| A. No ENE | B. Micro-ENE | C. Major-ENE with effacement of partial nodal capsule | D. Major-ENE with effacement of entire nodal capsule (soft tissue deposit) |
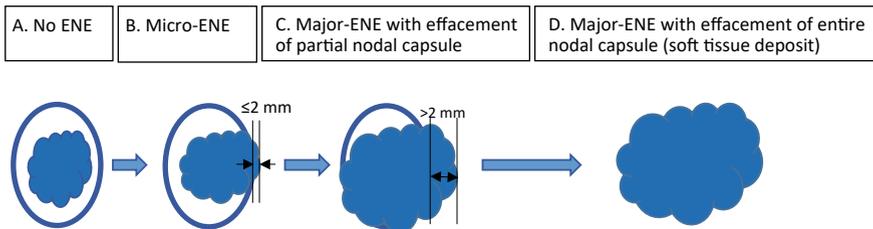
Fig. 7.1 Schematic depiction of various extent of pENE described in literature

nodal border(s), but confined to perinodal fat; "*Grade 2*" rENE—tumor invasion through two or more inseparable adjoining LNs exhibiting unambiguous effacement of any component of their internodal plane(s) (implying replacement by tumor) [1], invariably resulting in a lobulated appearing nodal mass; "*Grade 3*" rENE—tumor invasion beyond perinodal fat to overtly invade or encase adjacent structures, e.g. skin, muscle, and neurovascular structures (Fig. 7.2). Interestingly, other terms, such as "*conglomerate*" and "*matted*", have been used to describe an aggregation of multiple juxtaposed LNs, without necessarily adhering to or fusing into each other, to form a compact mass. We prefer the term "*coalescent*" to describe two or more adjoining LNs consuming each other into an inseparable mass; this is characterized by unequivocal effacement of internodal planes that forges multiple LNs into a single entity.

cENE has been introduced as a new N-classifier in the TNM-8 for non-viral related HNC [2]. It represents the most overt form of ENE and refers to detectable ENE by clinical examination. When ENE is advanced, clinical signs emerge. *Peau d'orange* is a clinical sign of dermal infiltration with edema and, along with ulceration, is indicative of tumor invading skin. "Fixation" of a nodal mass during palpation is a clinical sign of tumor infiltration of deeper fascial structures and musculature. Brachial plexopathy is often a sign of tumor invasion to neural structures, but like any clinical findings, cENE should be interpreted in context. Thus, cENE can also

Definition	Schematic Depiction	Radiologic Example
Grade 1: Tumor breaching the nodal capsule of an individual LN characterized as unambiguously ill-defined nodal border(s), but confined to perinodal fat		
Grade 2: Tumor invasion through two or more inseparable adjoining LNs exhibiting unambiguous effacement of any component of their internodal plane(s) (implying replacement by tumor), invariably resulting in a lobulated appearing nodal mass		
Grade 3: Tumor invading beyond perinodal fat to overtly invade or encase adjacent structures, e.g., skin, muscle, carotids, parotids, and neurovascular structures		
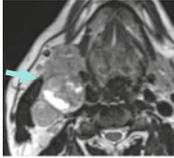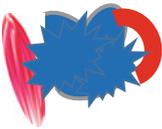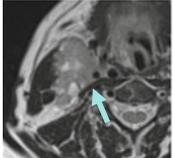
Fig. 7.2 Definition and extent of radiologic extranodal extension

be subjective. Fixation of an upper neck mass can sometimes be caused by advanced primary tumor extension rather than a nodal mass [29]. Therefore, TNM-8 mandates cENE to be supported by rENE [2].

Sensitivity and Specificity of rENE for pENE

Since pENE is identified microscopically, it is regarded as the most sensitive and objective way of identifying ENE; comparatively, subjectivity exists for rENE and cENE detection. Hence, pENE often serves as a gold standard to examine the accuracy of rENE and cENE. It is understandable that not all pENE would have rENE since the method of assessment differs significantly, using the "naked eye" on one hand compared to the microscopic in the other. Studies in HPV-negative HNC showed that only about 50% pENE may have signs of rENE [10] and only about 50% rENE could have evidence of cENE [29]. Despite low sensitivity, when more stringent criteria are used for rENE declaration, the specificity is generally high [8].

The sensitivity and specificity of rENE for pENE depends on pENE extent, rENE grade and the level of certainty a radiologist has adopted for declaration. Blasco et al. [30] showed that the sensitivity of rENE is higher in identifying major pENE compared to micro-pENE. Chang et al. [31] found that rENE carried a higher hazard ratio (HR) for OS compared to major-pENE or micro-pENE (2.27 vs 1.06 vs 0.49). Hu et al. [24] showed that the higher the certainty of rENE declaration, the higher the inter-rater concordance; and a higher grade of rENE is associated with less ambiguity for a radiologist to declare rENE. Of importance, "suspicious" rENE did not carry prognostic difference vs no rENE while those with a high certainty of rENE patients had lower distant control (DC).

Studies have consistently shown that rENE is one of the strongest prognostic factors for survival in HPV-negative HNC (HR 1.3–3.3), nasopharyngeal cancer (HR 1.6–1.9), and HPV-positive oropharyngeal cancer (OPC) (HR 3.9). A recent meta-analysis showed that rENE-positive HPV-positive OPC patients suffered a much higher risk of death (HR 2.6) versus rENE-negative HPV-positive OPC, mainly related to increased risk of DM (HR 3.8), and the HR of rENE for survival was even higher than that of pENE (HR 2.6 vs. 1.9). This is likely because, on average, unequivocal rENE recognizes a worse version of extranodal disease than pENE which includes cases with minimal extent of ENE lacking the same prognostic significance under contemporary treatment. Therefore, rENE can serve an important role in clinical care and risk stratification in HNC and has been used as an exclusion factor in some de-intensification trials.

The priority of sensitivity over specificity and vice versa depends on the clinical scenario. For clinical care, such as triaging cases of T1-2N1 OPC to surgery vs radiotherapy, a high sensitivity of rENE in identifying pENE would be important to avoid potential triple modality treatment which is ordinarily recommended if pENE is detected; therefore, a relatively modest level of certainty (>50%) may be used for declaration of rENE before treatment assignment. For staging, the preservation of

prognostic importance to minimize dilution due to the inclusion of uncertain cases (or those with less important minimal disease) is needed, and a high level of certainty (>90%) should be maintained for rENE declaration.

Radiology assessment has always played an essential role in clinical decision making and staging of HNC patients. Standardization of taxonomy describing nodal features and the certainty for their declaration will help facilitate clear communication and interpretation of radiology reports. Chin et al. [28] studied interrater concordance by two radiologists assessing 7 nodal features frequently highlighted in the literature in 413 HPV-positive OPC patients and found that variation existed in interpretation regarding radiologic nodal features. Clearly defined nomenclature results in improved interrater reliability when assessing radiologic nodal features, especially for coalescent adenopathy and ENE. A multicenter study by Hoebers, et al. [32] showed that a learning curve exists for rENE assessment. Reliability of rENE assessment across institutions improved after consolidation of rENE operational definitions. Higher levels of certainty were associated with higher inter-rater agreement. The authors propose a strategy to augment the reliability of rENE ascertainment including: high certainty for declaration, consolidating operating definitions, and sharing experience among the radiology community [32].

Artificial Intelligence and Machine-Learning in Identifying rENE

Recognizing the limitations of imaging interpretation by human eyes, some researchers have turned to artificial intelligence and machine-learning with automated detection algorithms to improve interrater concordance [33]. Kann et al. [33] trained on a dataset of 2875 CT-segmented LN samples with corresponding pENE data and derived an algorithm which predicted pENE with area under the receiver operating characteristic curve (AUC) of 0.91 (95%CI: 0.85–0.97). The subsequent validation study from two different datasets (one from an external institution and the other from The Cancer Genome Atlas (TCGA) Head and Neck Squamous Cell Carcinoma imaging data repository) and showed improved AUC compared to radiologists' assessment. Moreover, the diagnostic accuracy of the radiologists improved when receiving assistance from the detection algorithm.

Although Kann's work shows promise of artificial intelligence in enhancing sensitivity and objectivity in recognizing rENE and improving rENE-pENE correlation, it is not ready for routine clinical practice. In part this is because it relies on modelling processes for prediction of a status among a group of patients, rather than declaring its presence in an individual case. For deep-learning performance evaluation, the authors used the primary endpoint of AUC. Whether AUC is the optimal endpoint for developing models to guide clinicians on treatment recommendation is uncertain since AUC measures the overall "goodness-of-fit" of the model. In clinical situations, specific requirements often dictate the priority for either high sensitivity or

high specificity and AUC rarely provides adequate information in this regard. If the objective is to avoid tri-modality treatment in TOS-eligible patients, identifying ENE before treatment with high sensitivity is important. Conversely, a false-positive declaration of ENE may prompt chemoradiotherapy when surgery-alone may have been sufficient. One can argue for a high sensitivity test to identify any ENE to avoid tri-modality treatment. In contrast, one could also argue for a high specificity test to identify only those cases bearing prognostically important major ENE for staging and treatment recommendation. This fits the quintessential staging rule of the UICC and AJCC: when there is doubt, a lower stage (i.e. less ominous prognostic level) should be assigned [34].

Biomarkers Associated with ENE

ENE has been proven to be associated with aggressive phenotypes in many cancers including HNC. Several biomarkers (whether protein, RNA, DNA, or epigenetic markers) have been reported to be associated with presence of ENE [3] and mostly in OSCC population. Podoplanin is a small mucin-type transmembrane glycoprotein that promotes local invasion and metastasis through the regulation of tumor cell migration and epithelial–mesenchymal transition [35, 36]. Lee et al. [37] recently found that almost all (93%) ENE-positive OSCC patients had podoplanin expression in the peri-nodal stroma of metastatic LNs compared to 47% in ENE-negative patients, and the intensity of podoplanin was also higher in ENE-positive patients. Noda et al. [38] examined clinical features associated with the tumor microenvironment in 186 surgically treated OSCC patients and 83 matched biopsy specimens, and found that ENE-positive patients had a high tumor budding pattern, low tumor-infiltrating lymphocytes, and an immature desmoplastic reaction in the primary site. Gieber-Netto et al. [39] observed that tumors carrying high-risk TP53 mutations had a significantly increased risk of developing ENE in OSCC. Similarly, Sandulache et al. [40] analyzed TCGA OSCC dataset and found that pENE-positive patients had the highest proportion of high-risk TP53 mutations while wild-type TP53 was highly representative in pN0 patients. Dhanda et al. [41] performed microarray and immunohistochemistry staining of primary tumors in 102 OSCC patients and found that high or intermediate expression of both SERPINE1 and SMA at the primary site was strongly associated with the presence of ENE. In addition, expression of both SERPINE1 and SMA was associated with poor OS.

It is important to point out that all these studies are retrospective and conducted on OSCC sites. It is unclear if these observations can be replicated in more diverse disease sites.

Clinical Implication of ENE

Compelling evidence demonstrates that the presence of pENE carries prognostic significance in both HPV-negative and HPV-positive HNC, and mainly affects DM [3, 9, 37]. As mentioned earlier, recent data shows that micro-pENE and major-pENE have different prognostic importance [17, 20, 21]. Wreesmann et al. [21] showed that OSCC patients with ≤1.7 mm pENE had similar 5-year DFS versus no ENE while patient with >1.7 mm pENE had much lower DFS. De Almeida et al. [17] analyzed 348 OSCC patients and found that patients with micro-pENE (≤2 mm from the nodal capsule) had no difference in locoregional control (LRC, 72 vs. 74%, p = 0.86) compared to patients with no ENE, while DC was only slightly lower (80 vs. 86%, p = 0.17). In contrast, patient with major-pENE had significantly worse 5-year OS (16 vs. 45%, p = 0·002), DFS (15 vs. 42%, p = 0·004), and DC (58 vs. 80%, p = 0·005) compared to patients with minor ENE, although LRC was only marginally worse (61 vs. 72%, p = 0·07). More importantly, the addition of cisplatin chemotherapy improved DFS for patients with major-pENE but had no impact in patients with micro-pENE. The effect of cisplatin chemotherapy on DFS was mainly due to enhanced LRC but not DC. The ECOG 3311 trial [20] result shows that HPV-positive OPC with ≤1 mm pENE can be safely treated with reduced dose postop-RT without chemotherapy. Emerging data suggest that differentiating micro-pENE from major-pENE might impact treatment choice in the future, although more robust trial data are warranted.

Data on the prognostic value of rENE are also emerging. Almulla et al. [8] showed that within pENE-positive patients, rENE-negative status had less prognostic importance than rENE positive status. The meta-analysis of HPV-positive OPC by Benchetrit et al. [9] showed that both pENE and rENE were prognostic and mainly impacted on DM rather than LRC. Emerging data has consistently show that ENE is one of the most powerful prognostic factor for all HNC including OSCC [8], HPV-negative OPC [29], HPV-positive OPC [10, 27], and nasopharyngeal cancer (NPC) [12, 13, 24]. In viewing the prognostic importance of rENE, many authors have now proposed to include rENE in pre-treatment risk stratification and future staging [12, 13, 15, 27, 29]. Some clinical trials have also included rENE as an exclusion criterion when designing studies addressing treatment deintensification [42, 43].

As mentioned earlier, ENE affects DFS, mainly through increased risk of DM [17]. Effective systemic agents to address DM are needed. However, cisplatin, the most commonly used chemotherapeutic agent, seems insufficient to address this risk. Felon et al. analyzed a NCDB dataset of 14,071 HPV-positive OPC undergoing TOS treatment and found that both micro-pENE and major-pENE patients had inferior OS, but addition of cisplatin did not improve OS of pENE-positive patients although disease specific endpoints (locoregional or distant failure) were not reported. Huang et al. [27] also showed that cisplatin chemotherapy could improve LRC in HPV-positive OPC but could not fully negate the high DM risk of being rENE-positive.

Conclusion

Convincing evidence exists that ENE is one of the strongest prognostic factors for both viral related and unrelated HNC. It is mainly associated with a higher risk of DM with some additional influence on risk of locoregional recurrence. cENE and pENE are new N-category modifiers for non-viral HNC. Minor and major pENE may have different clinical importance. Similar to pENE, rENE should have a promising role in risk stratification of HNC. However, more work is needed to improve reliability of rENE assessment. Radiology reporting rENE needs to consider both sensitivity and specificity. To avoid inadvertent intense treatment combinations, including triple modality treatment, and to optimize treatment recommendations upfront, high sensitivity is important; to avoid falsely up-staging to the detriment of prognostication, high specificity is important. A standardized radiologic taxonomy and reporting template is warranted. Cisplatin appears to have insufficient effect in negating DM risk associated with ENE, novel systemic agents are needed to better address risk of DM in patients with ENE.

Disclosure Statements None

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Chapter 8
Proton Therapy for Head and Neck Cancer

V. Budach and A. Thieme

Introduction

Radiation therapy (RT) is a mainstay of treatment for patients with head and neck cancer (HNC). At present, the most common form of RT is external beam photon therapy. The development of intensity-modulated radiotherapy (IMRT) and more recently advanced forms of IMRT such as volumetric modulated arc therapy (VMAT) allowed improvements in dose conformality in target volumes and reduction of high doses in nearby healthy tissues and organs at risk (OARs). This resulted in a drastic reduction of the most common forms of RT-associated toxicity in HNCs such as xerostomia [1–4], and dysphagia [5]. However, technological advances in photon therapy to further optimize the dose distribution are reaching the limits imposed by the physics of photon radiation. In consequence, IMRT's usage of multi-angled radiation fields has led to a redistribution of the integral dose causing alternative toxicities such as fatigue by the low dose bath of the posterior cranial fossa [6]. Therefore, alternative methods of radiation delivery with distinct physical properties are required to further refine the therapeutic index of RT.

For decades, proton therapy (PT) offers attractive options for technological advances in RT, potentially leading to a reduction in treatment-related toxicities or an isotoxic dose escalation through dosimetric advantages over photon therapy. PT is the standard of care for skull-base tumours which are characterized by a challenging tumour location and proximity to critical structures. In recent years, the

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use of PT has expanded to numerous other head and neck disease sites such as nasopharynx, oropharynx, nasal cavity and paranasal sinus, periorbital, and salivary glands including reirradiation.

Physical Properties of Proton Therapy

Dosimetric Benefits of Proton Therapy

Photon and proton beams are different forms of ionizing radiation causing DNA damage in cancer cells. Both are elementary particles with different physical properties and energy deposition profiles in tissue favouring protons for treatment in cancer patients (Fig. 8.1). Photons are electromagnetic packets of energy, which are massless and have an infinite range in patient tissue. In contrast, protons have a physical mass and the range of a proton in patient tissue is a function of its initial energy. A monoenergetic proton beam releases most of the energy in the distal part of its path in a characteristic peak, the so-called Bragg Peak. By using a range of energies a spread out Bragg Peak (SOBP) can be created that allows highly conformal treatment of tumour target volumes. The absence of an exit dose beyond the target volume allows for precise sparing of adjacent OARs. Additionally, the entry portion of the proton beam receives less integral dose compared with a photon beam. In summary, proton beams offer several advantages over photon beams in cancer treatment, including the ability to more precisely spare surrounding healthy tissues and the potential to deliver lower integral doses to the patient.

PT uses passive scattering or active scanning techniques. The passive scattering beam technique was introduced first, using scattering devices to broaden the proton beam and a range-modulation device to create the SOBP. This technique requires patient individualized scattering devices, which are expensive to create and limit the ability of this technique for adaptive planning in case of excessive weight loss of the patient or changes of the anatomy. A more recent form of PT is the active scanning technique which uses magnets to deflect the proton beam. Using this technique, the radiation dose is delivered to the target volume layer by layer with protons of different energies. Inverse planning methods are used to deliver highly conformal doses to the target volume with either single field optimization (SFO) or multifield optimization (MFO) with MFO being generally more conformal than SFO. Intensity-modulated proton therapy (IMPT) takes advantage of MFO with each individual radiation field delivering an inhomogeneous dose to the target volume to minimize radiation exposure of OARs. Comparative HNC treatment plans with IMRT show dosimetric advantages of IMPT (Fig. 8.2). Several recent studies have confirmed the dosimetric advantages of IMPT for unilateral HNCs [8], oropharyngeal carcinoma (OPC) [9], adjuvant RT of OPC [10], and in cases of HNC re-irradiation [11].
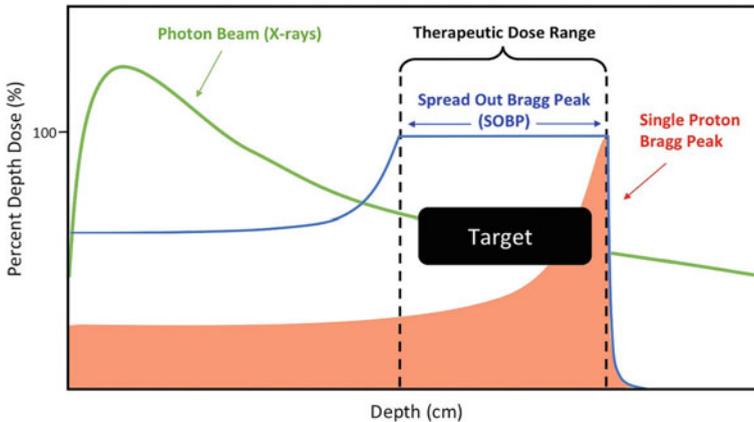
Fig. 8.1 Dose-depth curves comparing photon and proton beams. A single monoenergetic proton beam releases most of its energy in the so-called Bragg Peak (red curve). By variation of the Proton energies, a Spread Out Bragg Peak conformal to the tumour target volume can be created (blue curve). The energy deposition of a photon beam exponentially decreases with depth in the patient tissue and has an infinite range (green curve). [7]

Dosimetric Uncertainties of Proton Therapy for Head and Neck Patients

While the sharp dose fall-off beyond the Bragg Peak is considered to be a primary beneficial property of PT for OAR sparing, it is also the source of significant uncertainties in dose delivery and a possible cause of underdosage in the tumour volume. For instance, proton beams passing the nasal cavity or paranasal sinuses should be avoided due to variable fillings of these structures which can lead to significant distortions of the proton irradiation fields. A general approach for a robust PT treatment plan is the usage of MFO and careful selection of beam angles avoiding heterogeneous tissues. The dose distribution of PT is sensitive to the correct conversion of computed tomography (CT) Hounsfield units to proton stopping power [13, 14], image artifacts and interfraction, and interfield motion [15]. Uncertainties arise at multiple steps of the typical radiation oncology workflow and countermeasures exist (Fig. 18.3). Robust treatment plans that are clinically acceptable can be created when the aforementioned uncertainties are taken into account as part of multi-criteria optimization simulating these uncertainties or combinations thereof [16, 17]. Robust IMPT planning is based on the clinical target volume (CTV) without using margins for a planning target volume (PTV) [18] (Fig. 18.4). Instead of relying on precise proton ranges, robust optimization often relies on the sharp lateral penumbra of proton beams.

A further source of uncertainty is the relative biological effectiveness (RBE) of protons which is a factor multiplied by the proton dose to calculate the biological equivalent photon dose. Currently, a homogeneous value of 1.1 for the proton RBE is
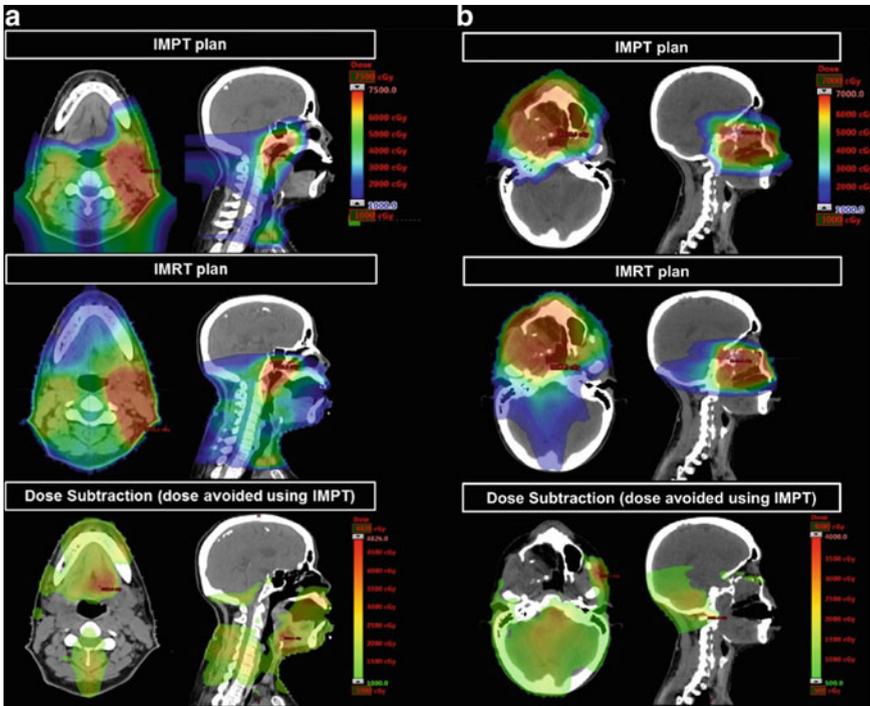
Fig. 8.2 Comparative treatment planning with IMPT and IMRT for two example HNC cases. (A) definitive RT of a nasopharyngeal carcinoma T1N1, (B) adjuvant RT of an adenoid cystic carcinoma of the hard palate T4N0. Dose substractions of both cases show a dosimetric advantage of IMPT compared with IMRT [12]. HNC: Head and neck cancer; IMPT: Intensity-modulated proton therapy; IMRT: Intensity-modulated radiation therapy; RT: Radiation therapy

used in clinical practice, but there have been studies that suggest a variability of RBE with higher values close to the Bragg Peak [19]. While the clinical relevance of a variable RBE is unclear especially in regards to normal tissue toxicity, some treatment planning systems allow for biological uncertainties optimization by locating higher RBE values inside the target volume while avoiding OARs.

Take Home Message for Physical Properties of Proton Therapy

- Protons have a different energy deposition profile than photons suitable for cancer treatment.
- Protons have several physical properties that are beneficial for normal tissue sparring: (1) release of most of the energy in the Bragg Peak, (2) steep dose fall-off beyond the Bragg Peak, (3) lower integral dose in the entry path, and (4) a sharp lateral penumbra.
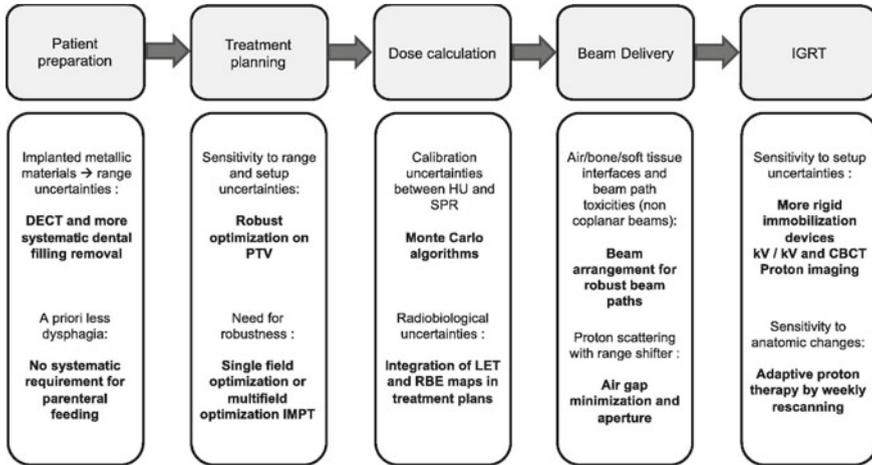
Fig. 8.3 **Causes of uncertainties of proton therapy and possible countermeasures to increase robustness** [20]. CBCT: Cone beam computed tomography; DECT: Dual-Energy computed tomography; HU: Hounsfield Unit; IGRT: Image-guided radiation therapy; IMPT: Intensity-modulated proton therapy; LET: Linear energy transfer; PTV: Planning target volume; RBE: Relative biological effectiveness; SPR: Stopping Power Ratio

- By using a range of energies a spread out Bragg Peak can be created which is highly conformal to the target volume.
- Proton therapy is subject to range uncertainties which can be successfully mitigated with robust optimization of the treatment plan.

Patient Selection for Proton Therapy

While protons, from a physical point of view, have more favourable properties for RT than photons, there is a lack of evidence from randomized controlled trials (RCTs) comparing IMRT vs IMPT and investigating differences in toxicity profiles. The "ALARA" principle states that ionizing radiation should be applied to humans "as low as reasonably possible" motivating the fast introduction of modern photon radiation techniques like IMRT and VMAT into clinical practice, because they allowed for better dose conformity to the target volume and sparing of OARs. Due to the significantly higher costs of PT, the question arises to what extent PT translates into a clinically relevant reduction of toxicities [21].

Alternative evidence-based approaches to RCTs rely on predicting RT related toxicities via Normal Tissue Complication Probability (NTCP) models, to identify patients who benefit most from PT (model-based selection) and to continuously validate this patient selection process (model-based validation).
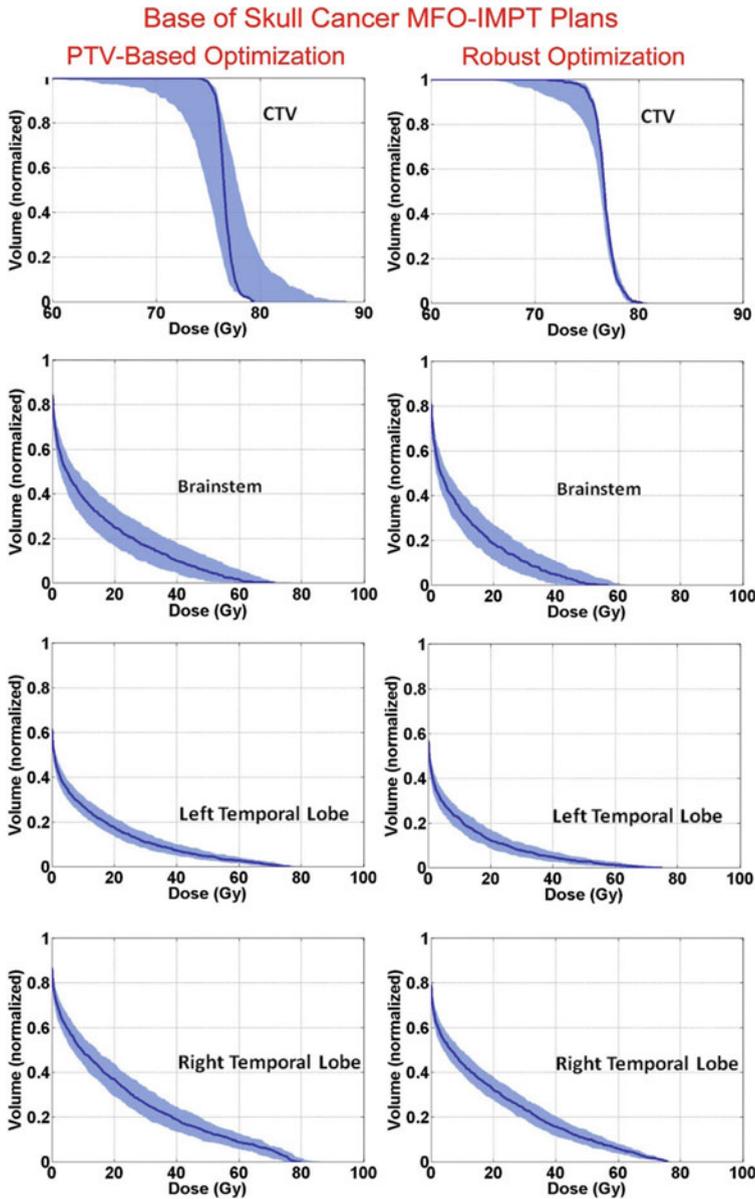
Fig. 8.4 Standard optimization involving a PTV vs robust optimization based on the CTV for a skull base cancer. Nominal DVH curves and DVH bands accounting for proton range uncertainties are shown for a treatment plan without (left column) and with robustness optimization (right column). Smaller variances of DVH bands of CTV coverage for the robustly optimized treatment and benefits in OAR sparing can be observed [17]. CTV: Clinical target volume; DVH: Dosevolume histogram; IMPT: Intensity-modulated proton therapy; MFO: Multifield optimization; OAR: Organ-at-risk; PTV: Planning target volume

Normal Tissue Complication Probability Models for Head and Neck Cancer

RT to the head and neck has various potentially severe acute and late side effects. The relationship between the dose distribution in OARs and the probability to develop RT-related side effects are described by NTCP models. In general, the probability of a side effect will increase with higher doses and larger volumes in the OAR to receive certain doses [22]. Side effects are assessed by medical healthcare professionals (investigator-reported outcomes) preferably in combination with direct reports of the patients (patient-reported outcomes (PROs)). Sophisticated grading scales have been developed for both investigator-reported outcomes such as the Common Terminology Criteria for Adverse Events (CTCAE) [23] and PROs such as the European Organisation for Research and Treatment of Cancer Quality of Life Head and Neck Module (EORTC QLQ-HN43) [24]. Most relevant dose-volume parameters vary from the observed side effect and OARs, e.g. the mean dose to the parotid glands for xerostomia [25], and in some cases may even depend on multiple dose-volumen parameters, e.g. the mean dose to the superior pharyngeal constrictor muscle and the mean dose to the supraglottic area for swallowing dysfunction [26]. The most reliable NTCP models are obtained from prospective clinical trials which are validated in an independent external cohort. Some models improve their predictive performance by considering patient factors (e.g. age) and treatment related factors (e.g. concomitant chemotherapy) which are then called multivariable NTCP models. The Quantitative Analyses of Normal Tissue Effects in the Clinic (QUANTEC) was an effort to accumulate the evidence for dose–response models and dose-volume constraints which was published in 2010 [27]. Since then more NTCP models have been developed which incorporate PROs and/or evaluated modern RT techniques for xerostomia [25, 28–30] (Fig. 8.5), dysphagia and feeding tube dependency [26, 31–33], hypothyroidism [34], laryngeal edema [35], emetogenesis [36] and acute mucositis [33].

Model-Based Approach

The general idea behind the model-based approach is patient selection for either IMPT or IMRT based on an expected reduction of RT-associated toxicities as predicted by NTCP models. A major challenge with this approach is that many NTCP models are based on patient cohorts which received photon therapy with outdated techniques and that their validity for IMPT have not been demonstrated. To this end, existing NTCP models have been verified with external validation cohorts receiving PT. While a drop in the performance of the NTCP models could be noticed, the models demonstrated robustness and generally remained to be valid [37].
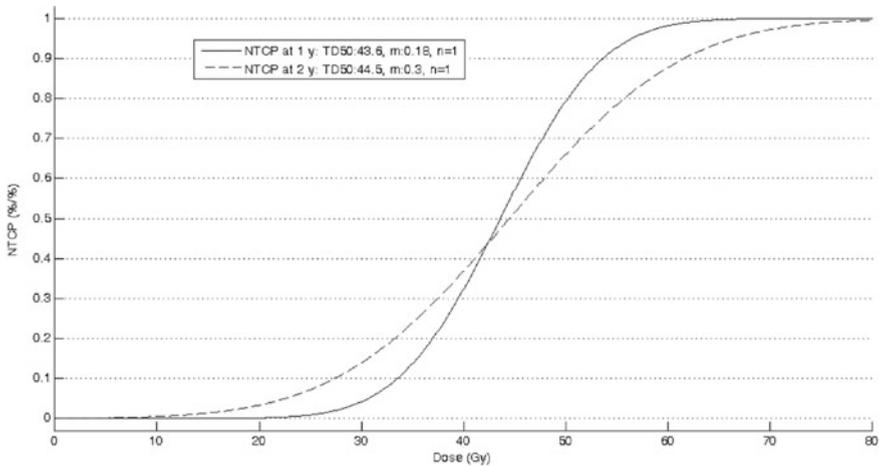
Fig. 8.5 NTCP curve for the parotid gland as function of the mean parotid gland. This curve is based on the objective measurement of the salivary excretion function assessed by quantitive scintigraphy. Complication was defined as a post-RT salivary excretion function ratio of <45%. The solid line represents NTCP after 1 year and dashed line after 2 years. NTCP: Normal Tissue Complication Probability; post-RT: post radiotherapy [29]

The model-based approach works with the following steps (Fig. 8.6):

(1) For every patient in silico planning comparative (ISPC) studies are created and the best photon (VMAT) and proton (IMPT) treatment plans are compared.
(2) NTCP models are used to predict the probability of the most relevant acute and late RT induced side effects for both treatment plans.
(3) It is determined to which extent the difference in dose (Δdose) translates into a large difference in complication probability (ΔNTCP) of acute and late side effects. This step is crucial since not all Δdose translate into ΔNTCP which can be the case in two situations: the VMAT treatment plan is already sufficiently optimized and has a low probability of complication which cannot be significantly improved with IMPT, or 2) both the IMPT and VMAT treatment plans are located at the upper end of the NTCP curve and the Δdose is too small to result into a lower complication probability.
(4) If a predefined threshold for ΔNTCP is reached, e.g. the probability of severe complication is 5% lower with IMPT than with VMAT, the patient is selected for treatment with IMPT (model-based selection).
(5) After treatment, actual complications in patients are observed and NTCP models are validated (model-based validation).

The model-based approach has been approved and accepted by the Dutch Health care institute for selection of patients for PT. In the National Indication Protocol Proton therapy (NIPP) the following ΔNTCP thresholds and CTCAE grades are used for patient selection: no ΔNTCP threshold for grade 1 side effects, ΔNTCP $\geq$ 10% for
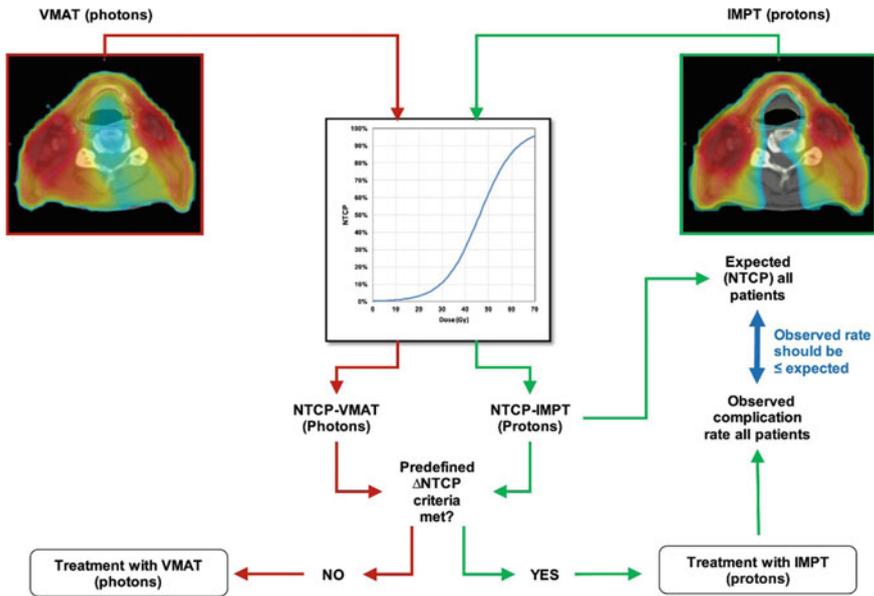
Fig. 8.6 Model-based selection of patients for VMAT or IMPT and validation pipeline. For a patient in silico planning comparative studies for VMAT and IMPT are created and evaluated by NTCP models in regards to their probability of RT-related side effects. If a certain threshold in difference in NTCP (Δ NTCP) is predicted, e.g. a 5% lower probability to develop severe xerostomia with IMPT, the patient is selected for this modality (model-based selection). After treatment, actual complications are observed and compared with NTCP predictions to further validate the process (model-based validation) [38]. IMPT: Intensity-modulated proton therapy; NTCP: Normal Tissue Complication Probability; VMAT: Volumetric modulated arc therapy

grade 2 or ΔNTCP $\geq$ 5% for grade 3 or higher. A further criterion for PT selection is the sum of ΔNTCPs of all grade 2 or higher side effects exceeding the threshold of 15%.

In a first evaluation of the model-based approach by Tambas et al. [39] 35% of patients (n = 221) with HNCs in distinct anatomical loci (oropharynx, larynx, nasopharynx, hypopharynx, oral cavity) and mostly higher stage (stage III/IV 83%) qualified for PT according to the NIPP thresholds. In the sub-group of patients with OPCs the PT qualification rate was with 65% even higher.

Randomized Controlled Trials for Proton Therapy

A RCT is the most scientifically reliable method of hypothesis testing and is considered the gold standard for evaluation of the efficacy of an intervention. There might be situations where a RCT should be preferred over the model-based approach: concerns regarding a decreased tumour control probability; concerns regarding increased side

effects, e.g. due to range uncertainties or an unknown RBE; in healthcare systems that require a RCT for reimbursement.

As pointed out by Widder et al. [40], including patients in a RCT who are unlikely to experience lower toxicity from PT due to a low Δdose and/or ΔNTCP, will only increase the noise and decrease the power of the study. For a particularly costly intervention like PT, even a positive RCT with an unselected patient cohort will provoke questions about patients who benefit most from PT in order to reduce costs in the health care system. In consequence, even in a setting of RCT, patient enrichment by the model-based selection is preferable to generate further evidence of the benefits of PT.

Take Home Message for Patient Selection for Proton Therapy

- Normal Tissue Complication Probability (NTCP) models can be used to estimate the probability of acute and late toxicities associated with photon and proton radiotherapy.
- The model-based approach assumes a clear dose-dependence for RT-related toxicities best described by the NTCP-models, which serve as a selection tool for comparative photon and proton treatment plans.
- The Netherlands consensus for model-based selection implies a reduction of $\geq 10\%$ and $\geq 5\%$ for a grade 2 or 3 side effects, respectively, which would qualify the patient for proton treatment.
- With the model-based approach, patient cohorts of randomized controlled trials can be enriched with patients who are likely to profit from proton therapy.

Outcomes After Proton Therapy of Head and Neck Cancers

Skull-Base Chordomas and Chondrosarcomas

Skull-base chordomas and chondrosarcomas are locally aggressive malignancies that belong to the group of sarcomas and are characterized by a close proximity to critical structures. Chordomas are rare malignancies with an incidence <0.1 per 100,000 [41]. Skull-base chordomas mostly arise from the clivus and often become clinically apparent with cranial nerve deficits, sensorimotor deficits, pituitary dysfunction, or hydrocephalus. Without treatment the average overall survival (OS) is short (6–24 months) [42]. Chondrosarcomas comprise a heterogeneous group of slow-growing sarcomas originating from cartilage-producing cells in areas of enchondral ossification and have an incidence of 0.2 per 100,000 [43]. Surgery is the primary treatment, however due to the location a gross total resection often cannot be achieved. In chordomas, surgery alone results in a high local recurrence rate of 58% [44]. Adjuvant

RT is of crucial importance to reach acceptable rates of local control (LC). Since the main site of recurrence is local and the chances of salvage surgery are remote, LC is directly associated with OS. A clear dose–response relationship with LC could be observed. Median PTV doses of <60 Gy, 60 Gy and 66.6 Gy resulted in a 5-year LC of 28% [45], 39% [46] and 50% [47]. Chordomas and chondrosarcomas have a relatively high radioresistance and RT should aim for target volume doses above 70 Gy for best responses. This is especially challenging at the skull-base since the optimal doses exceed the tolerance of proximal neural structures such as the brainstem, spinal cord, and optic nerves and chiasm.

Multiple studies have reported outcomes of PT and skull-base chordomas [48–56] and chondrosarcomas [51, 52, 55, 56]. Munzenrider et al. have published so far results for the largest patient cohort (n = 519) who received 66–83 Gy (RBE) as a combination of photon and proton RT. The median follow-up was 41 months. The 5-year LC and OS was 73 and 80% for chordomas and 98 and 91% for chondrosarcomas. Male chordomas patients had a significantly higher 5-years LC than females (81 vs. 65%, p = 0.035). The following significant toxicities were reported: three (0.8%) patients died from brain stem injury, 8 (2.2%) experienced temporal lobe injury (Fig. 8.7), hearing loss, cranial neuropathy, or endocrinopathy. More recent studies could confirm similar rates for LC [48, 50, 57] and higher grade toxicities [54, 58, 59]. In summary, PT has allowed for dose intensification that resulted in improved clinical outcomes and tolerable toxicity profiles.

Sinonasal Cancers

Sinonasal cancers (SC) are a heterogeneous group and comprise of malignancies from the nasal cavity and paranasal sinuses including the maxillary, ethmoid, frontal, and sphenoid sinuses and the middle ear. SCs are very rare with an incidence of 8.7 per 1.000.000 [61]. The histology is mostly squamous cell carcinomas (SCCs) followed by adenocarcinomas [62]. Risk factors for SCs are occupational exposures, e.g. wood dust, leather dust, formaldehyde, nickel and chromium compounds [63]. After mesothelioma, sinonasal cancers are the second most common malignancies in number of cases associated with occupational exposure [64]. Surgery is the preferred primary treatment of SCs and small tumours with complete gross tumour resection have an excellent prognosis. However, many SCs are detected at a later stage which makes complete resection difficult.

In a meta-analysis by Patel et al. [65] a subgroup analysis comprising 16 trials and 539 patients specifically compared PT with IMRT and found a significantly higher disease-free survival (DFS) at 5 years (hazard ratio (HR) = 1.44, 95% confidence interval (CI) = [1.01–2.05], p = 0.045) and locoregional control (LRC) at longest follow-up (HR = 1.26, 95%CI = [1.05–1.51], p = 0.011) in favour of PT.

A large study by Resto et al. [66] comprised 102 patients who received a combination of adjuvant photon RT and PT. The median total dose was 71.6 Gy (range 55.4–79.4 Gy) with a median of 57.1% delivered via protons (range 22.9%–84.8%).
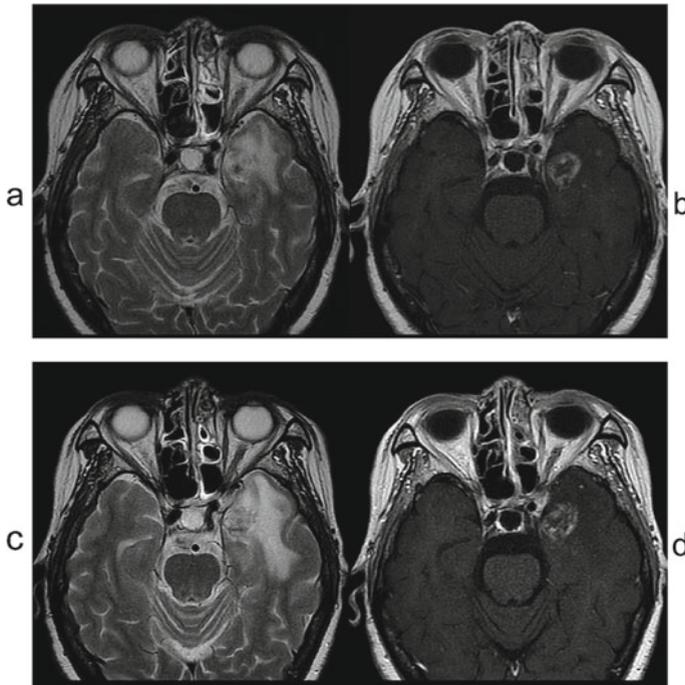
Fig. 8.7 MRI images of a temporal lobe radiation injury induced by proton therapy. An 81-year old woman received proton therapy for adenoid cystic carcinoma of the pterygopalatine fossa and developed temporal lobe radiation injury without symptoms and without requirement of treatment. (a) T2-weighted and (b) contrast-enhanced T1-weighted MRI images 30 months after RT showing marginal enhancement and edema in left temporal lobe; (c) T2-weighted and (d) contrast-enhanced T1 weighted MRI images 36 months after RT showing further development of radiation-induced changes [60]. MRI: Magnetic resonance imaging; RT: Radiation therapy

The study had a median follow-up of 5.1 years. The 5-year LC of patients with complete resection, partial resection and biopsy were 95%, 82% and 87%. The extent of surgical resection was associated with improved OS (p = 0.02), DFS (p = 0.009) and distant relapse (p = 0.03).

In a comparative study by Lewis et al. [67] VMAT and IMPT treatment plans for patients (n = 10) with SCs were created and dosimetric parameters compared (Fig. 8.8). IMPT was superior for dosimetric parameters of the brain (mean, V10, V30), brainstem (max dose/D0.01), ipsilateral cochlea (V30), contralateral cochlea (mean), contralateral lacrimal gland (mean), contralateral parotid (mean), spinal cord (max dose/D0.01) and inferior for the ipsilateral eye (mean) and ipsilateral lens (mean). The secondary malignancy risk with VMAT was 3.35 times higher (95%CI = [1.92,5.89]) than with IMPT. The authors conclude that IMPT better spared OARs not immediately adjacent to the target volume and reduced the risk of secondary malignancies.
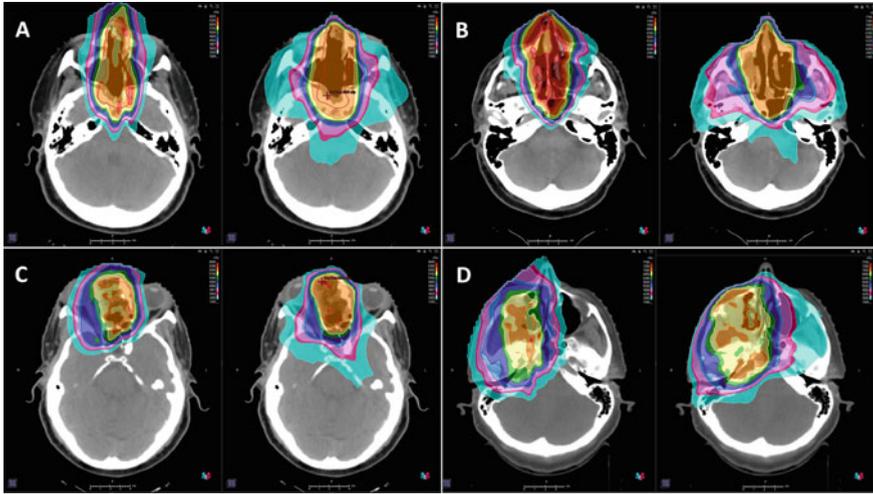
Fig. 8.8 Representative slices of IMPT vs VMAT treatment plans for sinonasal cars. IMPT plans are on the left and VMAT plans on the right of each panel. (A) A high conformality of IMPT and low dose bath of VMAT can be observed; (B) high conformality, but dose hot spots of IMPT in the multiple sinuses; (C) superior ipsilateral eye and lense sparing of VMAT; (D) superior contralateral OAR sparing of IMPT [67]. IMPT: intensity-modulated proton therapy; OAR: organ-at-risk; VMAT: volumetric modulated arc therapy

In a study by Pasalic et al. [68], patients (n = 64) with SCs of mostly advanced stage (T4 disease 46%) and mostly olfactory neuroblastoma as histology (28%) received PT and were evaluated for toxicities by physician-assessed toxicities (PATs) and PROs. The 3-year LC, DFS and OS were 88%, 76%, and 82%. PATs were assessed with CTCAE and PROs with the Xerostomia-Related Quality-of-Life Scale (XeQoLS), MD Anderson Dysphagia Inventory (MDADI), and Functional Assessment of Cancer Therapy (FACT) scales. No late grade 3 or higher PATs were observed. Significant changes in PROs from baseline were observed in the acute and sub-acute phase, but no chronic sequelae.

Periorbital Tumours

Periorbital tumours refer to malignancies in proximity to optic structures, including the nasopharynx, the nasal cavity and paranasal sinuses, and the dura of different histologies. Surgery and adjuvant RT are often indicated in the presence of high risk features like positive resection margins, bone invasion, high-grade disease, positive lymph nodes and/or perineural invasion. Historically, periorbital tumours were treated with orbit exenteration in order to ensure a margin-negative resection. Orbit-sparing RT treatments are an alternative to orbital exenteration which aim to preserve visual function and maintain high rates of LC. The complex anatomy of this region

and the proximity to critical structures such as the globe, cornea, lacrimal gland and duct system, tumours of the periorbital locations are particularly difficult to treat with RT.

In a study by Holliday et al. [69], patients (n = 20) with periorbital tumours were treated with global-sparing surgery and PT. The median radiation dose was 60 Gy (RBE) (range: 50–70 Gy) and 11 patients received concomitant chemotherapy. After a median follow-up of 27 months, LC was 100% (1 regional and 1 distant relapse). Toxicities were graded by CTCAE. There were 3 (15%) occurrences for grade 3 epiphora and 3 (15%) for grade 3 exposure keratopathy (damage to the cornea caused by prolonged exposure to air and instability of the tear film due to incomplete eye lid closure). Patients experiencing these toxicities had a higher maximum dose to the ipsilateral cornea (median 46.3 Gy (RBE) vs. 37.4 Gy (RBE), p = 0.017). Visual acuity decreased in 4 patients (20%).

In the study by El-Sawy et al. [70], patients (n = 14) received treatment for periorbital tumours (lacrimal sac or nasolacrimal duct carcinoma). Globe-sparing treatment was conducted in 10 patients and 4 patients received orbit exenteration. 13 patients received postoperative RT as IMRT (n = 5) or PT (n = 7) (median dose 60 Gy). Globe sparing was successful in all 10 patients after a median follow-up of 27 months. 9 patients (90%) maintained or improved their baseline visual acuity.

Damico et al. (2021) [71] evaluated 17 patients with tumours in paranasal sinuses, nasal cavity, or nasopharynx within 2 cm of the eye and optic apparatus that were treated with passive scatter PT and had comparative VMAT plans available. Median follow-up was 19.7 months. 14 patients received globe-sparing surgery and post operative RT, 3 received definitive RT. PT significantly reduced mean doses to the optic nerves and chiasm, pituitary gland, lacrimal glands and cochlea. Only 1 patient experienced grade 3 late toxicity (hearing impairment). The 18-month cumulative incidence of local failure was 19.1% and 1-year OS was 80.9%.

Additional studies are warranted for this entity to evaluate optimal patient setup, IMPT planning specifications, and dose tolerance limits of OARs.

Salivary Gland Cancer

Malignancies of the salivary glands are rare with incidences varying between 0.05 and 2 per 100.000 [72]. Tumours are mostly adenocarcinomas of the parotid which is the largest salivary gland. The etiology of salivary gland cancer is largely unknown. The primary treatment is surgery followed by postoperative RT for adverse features. Unilateral RT benefits from IMPT versus IMRT due to the absence of the exit dose (Fig. 8.9).

Bhattasali et al. [73] reported on nine patients with unresectable node-negative head and neck adenoid cystic carcinoma (ACC) who received definitive IMPT and concurrent cisplatin. The prescription dose was 70 Gy (RBE) in 33 fractions. Median follow-up was 27 months (range 9.2–48.3 months). 4 patients had complete response
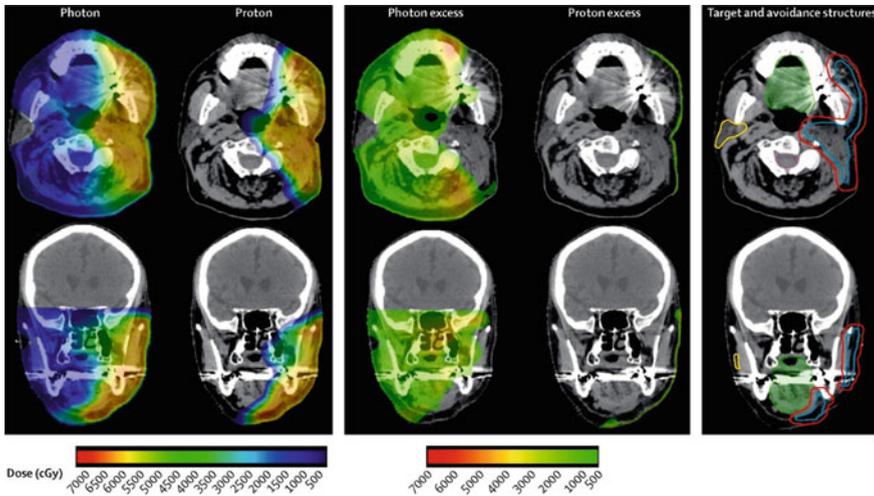
Fig. 8.9 Postoperative RT plans for treatment of a salivary duct carcinoma of the left accessory parotid gland comparing photons and protons. Prescriped dose is 66 Gy (RBE). Dose distributions of photon and proton treatment plans (left), plan differences with excess doses (middle) and contours of target volume and OARs (right) are shown. Color scales are in cGy (RBE) and minimum dose shown is 500 cGy. PT achieves better sparing of midline and contralateral OARs and an increased skin dose can be observed. Colors of contours: Green = oral cavity. Yellow outline = parotid gland. Magenta outline = spinal cord. Blue outline = clinical target volume. Red outline = planning target volume [76]. OARs: Organs-at-risk; RBE: Relative biological effectiveness; PT: Proton therapy; RT: Radiation therapy

(CR), 4 patients partial response (PR) and 1 patient showed progression. 5 patients experienced grade 3 toxicities and one patient grade 4 optic nerve disorder.

In a study by Romesser et al. [74], 41 patients with either major salivary gland cancer or cutaneous SCC were either treated with IMRT (n = 18, 43.9%) or passively scattered PT (n = 23, 56.1%). Gross disease was treated with normofractionated 70 Gy (RBE), close or microscopically positive margin with 66 Gy (RBE), high-risk volumes such as the tumour bed with 60 Gy (RBE). A reduction of grade 2 or greater acute dysgeusia (5.6 vs. 65.2%, p < 0.001), mucositis (16.7 vs. 52.2%, p = 0.019), and nausea (11.1 vs. 56.5%, p = 0.003) in favour of PT was observed.

Zakeri et al. [75] treated 68 patients with major salivary gland tumours with IMPT. Patients with positive margins received 66 to 70 Gy (RBE) and close margins/clear margins with 60 to 66 Gy (RBE) to the postoperative bed. Oncological outcomes were excellent with 3-year rates of LC, progression-free survival (PFS), and OS of 95.1% (95%CI = [89.9%,100.0%]), 80.7% (95%CI = [70.2%,92.7%]), and 96.1% (95%CI = [90.9%,100.0%]). Acute grade 3 dermatitis was observed in 9 (13.2%) patients. One patient developed late grade 3 osteoradionecrosis of the mandible.

Oropharyngeal Cancers

In the study by Tambas et al. [39], evaluating the model-based approach, 65% of OPC patients were predicted to benefit from IMPT. OPC with association of human papillomavirus (HPV) have a rapid increase in incidence. Since this patient cohort has a particularly good prognosis, improvements of late toxicities is one of the most important considerations. Current RCTs use de-escalation protocols for total radiation doses, target volumes, and combinations with systemic treatments to reduce morbidities with the aim to not sacrify oncologic outcomes. PT can provide other measures for a substantial reduction of radiation injury. There is a growing body of studies demonstrating that PT offer unique chances for dose reductions in virtually all organs (Fig. 8.10) and tissues at risk, thereby decreasing acute toxicity and long-term morbidity without compromising the radiation dose to target volumes and oncologic outcome (Table 8.1).

A case-matched analysis by Blanchard et al. [77] evaluated patients with IMPT (n = 50) and IMRT (n = 1000). 20% of patients received unilateral irradiation. It
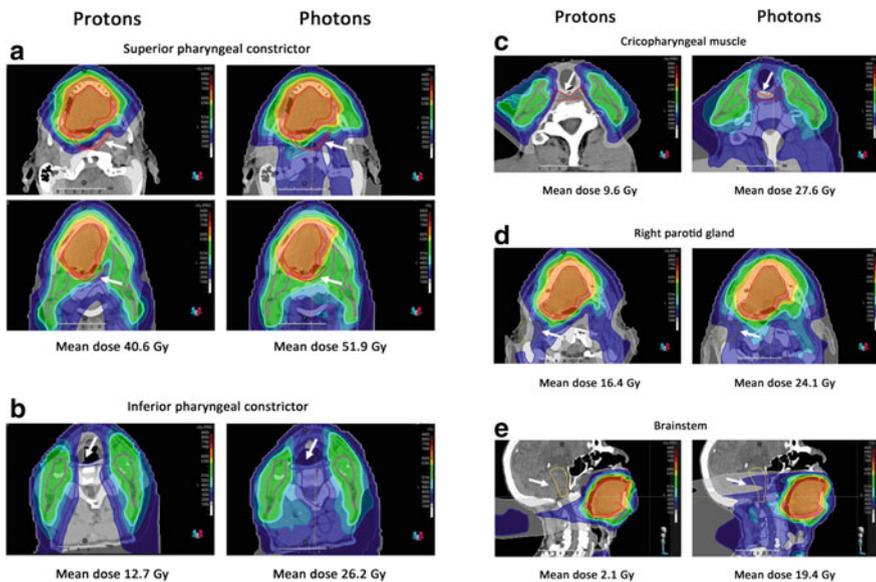
Fig. 8.10 Comparison of proton and photon treatment plans of a patient with cT4N0M0 OPC. Patient is 47 years old and receives chemoradiotherapy with 70 Gy (RBE) for HPV-positive OPC involving the base of the tongue, tongue and floor of the mouth. (A) Mean dose to the superior pharyngeal constrictor is 40.6 Gy for protons vs 51.9 Gy for photons; (B) Mean dose to the inferior pharyngeal constrictor is 12.7 Gy for protons versus 26.2 Gy for photons; (C) Mean dose to the cricopharyngeal muscle is 9.6 Gy for protons vs 27.6 Gy for photons; (D) Mean dose to the right parotid gland is 16.4 Gy for protons vs 24.1 Gy for photons; (E) Mean dose to the brainstem is 2.1 Gy for protons vs 19.4 Gy for photons [87]. HPV: Human papillomavirus; OPC: Oropharyngeal cancer; RBE: Relative biological effectiveness

Table 8.1 Selection of proton studies for oropharyngeal cancer. Modified from Blanchard et al. [12]

References	Study type	Site/Stage (percentage)/edition	Technique (dose)	Comparison with IMRT	CCT	Patients (n)	Follow-up (median)	Outcomes	Toxicity
Slater et al. [84]	Retro	OPC/ II (10.3%) III (27.6%) IV (62.1%)/ AJCC 4th	Cobalt (50.4 Gy) + PSPT boost (25.5 Gy RBE)	No	No	29	28 mo	5 y: LRC 88%, DFS 65%	2 y actuarial incidence of grade ≥3 16%
Gunn et al. [85] Blanchard et al. [77] Sio et al. [81]	Pro	OPC/ I (2%) III (18%) IVA (74%) IVB (6%)/ N/A	IMPT (70 Gy RBE)	Yes	Yes	50	30 mo	3 y: LRC 91%, OS 94.3%	Reduced use of gastrotomy tube or severe weight loss at 3 mo and 1 y; less subacute impairment of quality of life
Takayama et al. [86]	Pro	OC/ III (24%) IVA (73%) IVB (3%)/ UICC 7th	Photon (36 Gy) + PSPT boost (28.6–39.6 Gy RB), no surgery	No	Yes	33	43 mo	3 y: LC 86.6%, RC 83.9%, OS 87%	No grade ≥3 osteonecrosis
Manzar et al. [78]	Pro	OPC/ N/A (1.3%) I (1%) II (2.3%) III (7.5%) IVA (80.3%) IVB (5.6%) IVC (2.0%)/ AJCC 7th	VMAT/IMPT (70 Gy RBE definitive, 60–66 Gy RBE adjuvant)	Yes	Yes	305	12 mo (IMPT) and 30 mo (VMAT)	1 y: OS 92.6%	IMPT had lower feeding tube placement, less hospitalization during 60 days post-RT and less narcotic use

Table 8.1 (continued)

References	Study type	Site/Stage (percentage)/edition	Technique (dose)	Comparison with IMRT	CCT	Patients (n)	Follow-up (median)	Outcomes	Toxicity
Bagley et al. [79]	Retro	OPC/ III (20%)/ IV (80%)/ AJCC 7th	IMPT (median 69.3 Gy RBE)	No	Yes	69	N/A	N/A	Significant improvement of xerostomia-related PROs at 10 wks post-RT

AJCC: American Joint Committee on Cancer; CCT: concomitant chemotherapy; IMPT: Intensity-modulated proton therapy; IMRT: Intensity-modulated radiation therapy; LC: Local control; LRC: Locoregional control; mo: Month; N/A: Not available; OC: Oral cancer; OPC: Oropharyngeal carcinoma; OS: Overall survival; Pro: Prospective study; PSPT: Passive scattered proton therapy; RBE: Relative biological effectiveness; RC: Regional control; Retro: Retrospective study; UICC: Union for International Cancer Control; wks: weeks; y: Year

could be demonstrated that IMPT significantly decreased the necessity for feeding tube placement during treatment (odds ratio (OR) = 0.53; p = 0.011) and resulted in a significant reduction of the composite endpoint of grade 3 weight loss or feeding tube placement at 3 months (OR = 0.44) and 1 year (OR = 0.23; p < 0.05). There was no difference in OS or PFS between the study arms.

Several studies have evaluated PROs and could demonstrate the benefits of PT, including significant reductions in mucositis, xerostomia, dysgeusia, nutrition, dental problems, fatigue, and physical function [78–81].

The largest PROs study to date is a comparative analysis by Manzar et al. [78] reporting PATs and PROs of patients receiving IMPT (n = 46) or VMAT (n = 259) with either 70 Gy (RBE) definitively or 60–66 Gy (RBE) postoperatively. In the cohort receiving unilateral RT (n = 44), significant improvements for IMPT could be identified in PROs including dry mouth, sticky saliva, and taste (p < 0.05). Improvements in PATs could be observed for IMPT in regards to mucositis, pain, weight loss, and fatigue, while VMAT induced less mucosal infection and dermatitis. IMPT was associated with a relative risk reduction of 22.3% for narcotic use at the end of treatment. Feeding tube dependency within 30 days of RT was significantly lower among patients treated with IMPT (19.6% versus 46.3%, OR = 0.27, 95%CI = [0.12,0.59], p = 0.001). Additionally, a significantly lower rate of acute hospitalization was observed in the IMPT-arm (OR = 0.21, 95%CI = [0.07,0.6], p = 0.009). No difference in the 1-year OS could be detected between the study-arms (VMAT 91.3% vs IMPT 92.6%, p = 0.98).

A study by Bagley et al. (2020) [79] evaluated patients (n = 69) treated for OPC with IMPT in regards to PROs for xerostomia using the Xerostomia-Related QoL Scale (XeQoLS). Greatest xerostomia-related impairment was recorded at 6 weeks on treatment, followed by a 49% improvement 10 weeks after RT. PROs improved subsequently but remained above baselines after 2 years. Late xerostomia PRO scores were correlated with the mean oral cavity dose (p = 0.038), baseline score (p = 0.001), stage (p = 0.008) and N status (p = 0.006).

The current evidence in support of PT, particularly the benefits as assessed by PROs, warrants further investigation via RCTs: The "Randomized Trial of IMPT versus IMRT for the Treatment of Oropharyngeal Cancer of the Head and Neck" (NCT01893307) is a non-inferiority phase II/III RCT comparing IMPT with IMRT for OPC [82]. The primary endpoint is PFS at 3 years, with secondary endpoints of PATs and PROs. The "TOxicity Reduction using Proton bEam therapy for Oropharyngeal cancer (TORPEdO)" trial is a multicenter, phase III RCT of IMRT versus IMPT for OPC [83]. The primary endpoints are PROs as physical toxicity composite score, and feeding tube dependency or severe weight loss at 12 months after treatment.

Nasopharyngeal Cancers

Nasopharyngeal cancers (NPC) are chemoradiosensitive, and, therefore, RT plays a crucial role in both the definitive and adjuvant settings. This particular region

includes critical neurological structures that can be affected by the high doses of RT which can result in hearing impairment, optic neuropathy, or temporal lobe necrosis [88]. Several studies demonstrated improved target volume coverage and reduced dose to OARs with IMPT vs IMRT and helical tomotherapy [89, 90]. Studies with clinical evidence on oncological outcomes and toxicities after PT are summarized in Table 8.2.

A phase II study by Chan et al. [91] evaluated patients (n = 23) with stage III-IVB NPCs treated with PT. Prescribed dose was 70 Gy (RBE) in 35 fractions. The chemotherapy regimen consisted of 3 cycles of concurrent cisplatin (100 mg/m2) on days 1, 22, and 43 followed by adjuvant cisplatin (80 mg/m2) on day 1 and fluorouracil (1,000 mg/m2/d) on days 1 through 4 every 4 weeks for 3 cycles. Toxicity was graded with CTCAE. At a median follow-up of 28 months, none of the patients had local or regional relapse. 2-year DFS and OS were 90% and 100%. Grade 3 hearing impairment was present in 29% and weight loss in 38% of patients. 48% of patients required feeding tube placement during treatment.

Lewis et al. [92] published a study for a cohort of 10 NPC patients treated with platinum-based concurrent chemoradiation using IMPT (prescribed dose of 70 Gy (RBE) in 33 fractions) and treatment plan comparison with IMRT. Median follow-up of this study was 24.5 months (range, 19–32 months). 2-year LRC and OS were excellent with 100% and 88.9%. Acute grade 3 toxicity dermatitis (n = 4) and acute grade 3 mucositis (n = 1) were reported. No patient experienced late grade 3 or higher toxicities. The dosimetric comparisons revealed significant differences in OAR mean doses in favour for IMPT in 13 out of 29 evaluated OARs.

A 2:1 case-matched analysis with patients (n = 20) receiving IMRT for NPC found a significantly lower rate of feeding tube placement with IMPT (20% vs. 65%; p = 0.02) [93].

Beddok et al. [94] analyzed patients (n = 17) with stages III–IVa NPC, who received a definitive treatment with a combined photon and proton-boost therapy and concurrent chemotherapy. Patients with stage III and IVa were 12% and 88%. The prescribed doses were 70–78 Gy (RBE). Median follow-up was 98 months. After 2-,5- and 10-years LRC was 94%, 86% and 86% and OS 88%, 74%, and 66%. Three patients (17.6%) developed distant metastasis. Late grade 3 toxicities were observed in regards to hearing loss (n = 4, 23.5%) and osteroradionecrosis (n = 1, 5.9%). One patient died from necrosis-induced nasopharynx bleeding.

Take Home Message for Outcomes after Proton Therapy of Head and Neck Cancers

- Skull base tumours: Proton therapy is the standard of care and allowed for dose intensification resulting in improved clinical outcomes and tolerable toxicity profiles.

Table 8.2 Selection of proton studies for nasopharyngeal carcinoma. Modified from Blanchard et al. [12]

References	Study type	NPC stage (percentage)	Technique (dose)	Comparision with IMRT	CCT	Patients (n)	Follow-up (median)	Outcomes	Toxicity
Chan et al. [91]	Pro	III (N/A) IVB (N/A)	PSPT (70 Gy RBE, upper neck only)	No	Yes	23	28 mo	2 y: LRC 100%, DFS 90%, OS 100%	Grade ≥3: Hearing loss 29%
Lewis et al. [92] Holliday et al. [93]	Pro	II (22%) III (56%) IVA (22%)	IMPT (70 Gy RBE)	Yes	Yes	10	24 mo	2y: LRC 100%, OS 88.9%	Less gastrostomy tube in IMPT patients compared to IMRT (p = 0.02)
Beddok et al. [94]	Retro	III (12%) IVA (88%)	PSPT + 3DRT/IMRT (70–78 Gy RBE cummulative)	No	Yes	17	98 mo	2 y: LRC 94%, OS 88%	Grade ≥3: Hearing loss 23.5%, Osteoradionecrosis 5.9%, Nasopharynx bleeding 5.9%

CCT: Concomitant chemotherapy; IMPT: Intensity-modulated proton therapy; IMRT: Intensity-modulated radiation therapy; LRC: Locoregional control; mo: Month; N/A: Not available; NPC: Nasopharyngeal carcinoma; OS: Overall survival; Pro: Prospective study; PSPT: Passive scattered proton therapy; RBE:Relative biological effectiveness; Retro: Retrospective study; y: Year

- Periorbital tumours: Proton therapy is part of orbit-sparing multidisciplinary concepts, and further studies are warranted to find optimal parameters and dose constraints for IMPT.
- Salivaryry gland cancer: Proton therapy delivers excellent oncological outcomes and favourable toxicity profiles for unilateral radiation.
- Oropharyngeal cancers: Competitive dose planning studies showed protons offering unique chances for dose reductions in virtually all organs-at-risk with the possibility of toxicity reduction without dose de-escalation in the target volumes. Toxicity reduction is of particular importance in HPV-positive patients with a good prognosis. Randomized phase III trials comparing IMPT with IMRT are underway.
- Nasopharyngeal cancers: Proton therapy offered dosimetric advantages at critical neurological structures and excellent oncological outcomes.

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Chapter 9
Treatment De-Escalation of HPV-Positive Oropharyngeal Cancer—Lessons Learnt from Recent Trials

Hisham Mehanna

Introduction

Human papillomavirus (HPV)—mediated oropharyngeal cancer (OPC) has been rapidly increasing in incidence over the past few decades [1, 2]. As HPV-positive OPC often affects younger people and demonstrates high survival rates, this means that patients will usually live with the morbidity of treatment for decades to come. As a result, the concept of de-escalation of treatment has found widespread acceptance as a possible solution to this problem.

Why De-escalation?

The seminal RTOG 0129 trial showed that HPV-positive OPC demonstrates considerably better overall survival than HPV-negative disease. It also described three risk groups, depending on HPV and smoking status. The lowest-risk patients demonstrated especially high rates of cure with over 90% three-year survival [3]. These patients, who are often younger than the traditional HPV-negative head and neck cancer patients, may often live for many decades with the long-term toxicities of their treatment. Importantly, it is widely acknowledged that treatment with cisplatin increases the number of acute serious toxicities by a factor of two, compared to radiotherapy alone [4]. It also demonstrates long-term and lasting toxicities such as swallowing dysfunction [4, 5].

Therefore, the concept of de-escalation has gathered momentum over the last decade. This concept espouses the reduction of treatment doses, or the use of alternative treatments to reduce toxicity, whilst maintaining the excellent survival rates

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demonstrated with standard chemoradiotherapy. Treatment de-escalation can take place in many forms; including the substitution of cisplatin for other potentially less toxic agents, for example cetuximab; the introduction of induction instead of concomitant chemotherapy with radiotherapy; the reduction of the dose of radiotherapy; the elimination of chemotherapy altogether; and the use of single modality treatment, either surgery or radiotherapy alone, instead of combination therapy. As a result, many studies were initiated, now almost a decade ago. The results of the first studies to report have raised some interesting, and indeed at times disturbing, conclusions which necessitate a re-assessment and reconsideration of our strategies.

It is important at this point to ask whether the concept of de-escalation is supported by patients. This question was elegantly addressed by the work by Brotherston et al. [6] who interviewed patients with OPC who had been treated with chemoradiotherapy about their preferences for de-escalation. Ninety-nine percent of the respondents favoured de-escalation of treatment if that did not result in a difference in overall survival. However, if it reduced survival then only 69% of the patients interviewed would support any form of de-escalation, and only up to a detriment of no more than 5% in survival rates. Eighty-one percent would prefer to avoid chemotherapy rather than radiotherapy. Therefore, from that study we surmise that patients are supportive of de-escalation, but only if it has a minimal effect on survival and efficacy of treatment [6].

Results of Recent De-escalation Trials

Several de-escalation studies have been reported in the last five years. The first to report were the De-ESCALaTE [7] and RTOG 1016 [8] randomised controlled trials. Later the TROG 12.01 study also reported [9]. These compared concurrent cisplatin with cetuximab, in conjunction with radiotherapy. Cetuximab had been reported in the Bonner trial [10] to improve survival when added to radiotherapy, with relatively little reported additional toxicity. Cetuximab was therefore widely considered to be less toxic than cisplatin. However, the De-ESCALaTE [7], RTOG 1016 [8] and TROG 12.01 [9] studies all showed similar and surprising results. These studies demonstrated a significant additional benefit from cisplatin, compared with cetuximab, both loco-regional control and overall survival. The RTOG 1016 trial [8] demonstrated an estimated overall survival at five years of 84.6% (95% CI, 80.6–88.6%) for cisplatin compared to 77.9% (95% CI, 73.4–82.5%) for cetuximab. Similarly, the De-ESCALaTE study [7] showed overall survival at two years of 97.5% for cisplatin, compared to 89.4% for cetuximab, demonstrating an adjusted hazards ratio of 5.0 (95% CI 1.7–14.7; log-rank P = 0.0012). This difference in survival was seen even in the lowest risk HPV-positive OPC, that is when excluding T4 and N3, in the De-ESCALaTE study. In this latter group, there was a two-year overall survival difference of 5.2% between the two groups with a hazards ratio of 4.3 (95% CI, 0.9–19.8; log-rank P = 0.0431), in favour of cisplatin. The TROG12.01 study [9] showed a significant difference in 3-year failure-free survival rates, which were

93% (95% CI, 86–97%) in the cisplatin arm and 80% (95% CI, 70%-87%) in the cetuximab arm (hazard ratio = 3.0 [95% CI, 1.2–7.7]); $P = 0.015$.

Importantly, in all the studies there was no significant difference in toxicity between the two arms. For example, in the De-escalate study [7] the incidence of all grade or severe (grade 3–5) toxicity, both in the acute and late phases between the two groups. The mean number of overall severe (grade 3–5) toxicity events per patient was 4·8 [95% CI 4·2–5·4] with cisplatin vs 4·8 [4·2–5·4] with cetuximab; p = 0·98). The mean number of late severe toxicity events was 0·41 (0·29–0·54) with cisplatin and 4·82 (4·22–5·43) with cetuximab. The types of toxicity differed between the arms, as would be expected.

The next randomised study to report was NRG HN002 [11]. This phase II study randomised patients into accelerated intensity modulated RT (IMRT), at a dose of 60 Gy in 5 weeks, with weekly cisplatin (40 mg/m^2/week), against IMRT alone. Both of the arms were experimental, because of the reduced radiotherapy dose, and there was no comparison with standard chemoradiotherapy regimens. The hypothesis was that an arm would be taken forward into a larger phase III study if it achieved a historical control rate of progression-free survival (PFS) rate at two years of equal or more than 85%. The arm also had to show a mean one-year MD Anderson Dysphagia Inventory (MDADI) composite score of more than 60, demonstrating acceptable swallowing toxicity. The concomitant cisplatin and IMRT arm reached the pre-specified criterion, with a two-year PFS of 90.5%. However, the IMRT alone arm demonstrated a two-year PFS of 87.6% (P = 0.23) and therefore did not meet the pre-specified endpoint. It again demonstrated the importance of the addition of cisplatin even in the lowest risk p16 positive patients [11].

More recently, the ECOG 3311 phase II study [12] was published. This randomised study looked at the role of dose de-escalation of adjuvant radiotherapy, in conjunction with cisplatin. Patients with HPV-positive OPC received transoral surgery. If, on post-operative histology, they were low risk, they were not given any adjuvant treatment, and if they were high risk, they were recommended adjuvant chemoradiotherapy. If they were determined to be at intermediate risk of recurrence and were recommended adjuvant radiotherapy alone, they were randomised to receive either 50 or 60 Gy post-operative radiotherapy. The results of the two randomisation arms were very similar, with the lower dose radiotherapy 50 Gy arm showing a two-year PFS of 94.9% (90% CI, 91.3–98.6) and for the standard 60 Gy arm 96.0% (90% CI = 92.8–99.3). This study showed the possibility of reducing the dose for post-operative patients who did not require cisplatin and is the first evidence of the possibility of de-escalation [12]. The Pathos trial [13] is looking to demonstrate the same as the ECOG 33–11 trial, but also extend de-escalation to the high-risk group of patients by eliminating cisplatin from the experimental arms. This study has progressed from the phase II stage, and is now recruiting in phase III.

There have been other de-escalation studies, but these have all been phase II, non-randomised studies and are therefore difficult to draw conclusions from. Some have shown interesting, and possibly promising results, but they should all be considered hypothesis generating, and all require validation in randomised phase III studies.

Lessons Learnt from De-escalation Studies

The first lesson that was learnt from these de-escalation studies was that concomitant chemoradiotherapy with cisplatin is a highly effective treatment for patients with HPV-positive OPC. Substitution of cisplatin or its elimination appear to have a significant detrimental effect in several studies. This is the case even in very low risk patients, as demonstrated in the HN002 and the sub analysis of the De-ESCALaTE study [7, 11]. It should therefore be clearly understood and widely appreciated that de-escalation of treatment in patients with HPV-positive OPC can have detrimental effects on patients' survival, and therefore must be studied in a highly controlled manner.

The second lesson follows on from the first one. Because of the potential for significant detriment, studying de-escalation should be in the context of randomised phase II trials. Running phase III trials from the start, without a preceding phase II study, should be avoided and strongly discouraged. We saw that the De-ESCALaTE study [7] demonstrated a similar result to RTOG 1016 [8]. However, it utilised considerably fewer patients: 334 compared to 844 respectively. This meant that fewer patients came to harm as a result of being part of the study, whilst demonstrating the same effect. This was also demonstrated in both HN002 (which randomised 308 patients) and in the randomisation arms of ECOG 3311 (which randomised 359 patients). Indeed, the TROG 12.01 study [9] showed a significant difference in failure free survival with only 189 patients randomised. Therefore, when it comes to de-escalation in the context of HPV-positive OPC, it would appear that studies randomising approximately 300–350 patients are sufficient to demonstrate significant differences between the two arms. Once the results of the phase II studies are available, then the phase III studies should be started, and not before.

An example of good practice is NRG HN005, which is now taking the results of HN002 into a new phase II study, which is comparing the successful arm of HN002 against the standard of care arm (concomitant cisplatin and radiotherapy), and against a third, new experimental arm of 60 Gy with nivolumab 240 mg in six cycles. This study would then progress to a randomised phase III study, comparing one or two experimental arms against the standard chemoradiotherapy arm. Very recently, a press release by NRG announced that the cisplatin plus 60 Gy arm has been suspended because it has not met its prespecified non-inferiority criteria.

The third lesson is that because concomitant cisplatin and radiotherapy are such an effective treatment for HPV-positive OPC, consideration of other ways of reducing toxicity, as alternatives to de-escalation, should be. These we term 'harm minimisation techniques', rather than de-escalation. Such strategies could include the use of proton therapy for selected oropharyngeal cancers instead of IMRT, which is currently being tested in the TORPEDO study. Other studies are looking at reducing the volume of radiotherapy delivered; for example, the INFIELD study is looking at elective volume de-intensification [14]. Similarly, the Canadian CCTG HN11 trial, currently recruiting, is exploring the elimination of radiotherapy to the contralateral side of the neck following a SPECT CT scan, compared to elective bilateral neck

radiotherapy, which is the standard practice in many centres. Another study, the phase II AVOID study is examining omitting radiotherapy to the resected primary tumour bed after transoral robotic surgery, if there are negative surgical margins. The mean dose received to the primary site as a result of radiation delivered to the neck is therefore greatly reduced—around 36.9 Gy. These trials have the potential to demonstrate reduced toxicity, whilst maintaining high survival outcomes, and should be supported through recruitment.

Conclusions

In conclusion, de-escalation of HPV-positive OPC can result in patient harm, and must be undertaken in highly-controlled phase II trials. If no detrimental signals are seen, then these treatments can be tested further in phase III trials. Different modalities of harm reduction—what we call 'harm minimisation' should also be considered. Recently a framework for de-intensification of treatment for HPV-positive OPC patients has been published by the Head and Neck Cancer International Group, which provides guidelines on how best to achieve safe evaluation of new de-escalation treatments [15].

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Chapter 10
Treatment Intensification in Locoregionally Advanced Head and Neck Squamous Cell Carcinoma: What Are the Options and for Whom?

Jan B. Vermorken

Introduction

About two-thirds of the patients with head and neck squamous cell carcinoma present with locoregionally advanced disease (LA-HNSCC). These patients have a 5-year overall survival (OS) of approximately 50% and improved tailoring of existing treatment modalities is thought to have important influence on outcome [1]. For unselected patients with LA-HNSCC current treatment guidelines recommend multimodal treatment, including concurrent chemoradiotherapy (CCRT) or surgery followed by radiotherapy (RT) with/without chemotherapy (CT). Moreover, induction chemotherapy (ICT) followed by RT/CCRT is an alternative larynx preservation approach in patients with advanced laryngeal or hypopharyngeal carcinomas [2, 3]. The choice of treatment largely depends on primary tumor site, resectability and the expertise of the hospital where the patient is being treated.

For the CT part of the CCRT, the National Comprehensive Cancer Network (NCCN) guidelines categorize two systemic therapies during conventionally fractionated RT as "category 1" in the definitive CCRT setting: high-dose cisplatin (100 mg/m^2 for 3 cycles) and 3 cycles of carboplatin/5-fluorouracil. In the postoperative setting, CCRT with "cisplatin" without further specification is recommended for high-risk non-oropharyngeal cancer patients. Although weekly low-dose cisplatin for a long time was classified as a category 2B category, recent prospective randomized studies have shown that weekly low-dose cisplatin (40 mg/m^2 for 7 cycles) is a good alternative for the high-dose cisplatin regimen, i.e. showing the same efficacy, but with less toxicity [4, 5]. For patients not eligible or not tolerating cisplatin there are other alternatives, such as carboplatin with or without fluorouracil, taxanes or

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cetuximab, although with uncertainty about equal efficacy [6–8]. Independent prognostic factors include performance status, tumor (T) stage, nodal (N) stage, human papillomavirus (HPV) status (in oropharyngeal cancer) and the treatment itself.

Standard of Care Concurrent Chemoradiotherapy (CCRT)

The individual patient-based Meta-Analysis of Chemotherapy in Head and Neck Cancer (MACH-NC) was practice-changing in 2000 in that it became clear that concurrent chemoradiation had more impact on outcome than sequential use of chemotherapy and RT (whether as neoadjuvant or adjuvant), showing an absolute survival benefit of 8% at 5 years with CCRT [9]. Further updates of this MACH-NC, with a different statistical approach (Peto analysis), showed an absolute benefit of 6.5% at 5 years (26.1% → 33.6%) and 3.6% at 10 years (17.3% → 20.9%) and a decreasing effect with increasing age [10, 11]. Using single agent platin showed the strongest relative risk reduction in comparison to RT alone (hazard ratio [HR] 0.74, 95% confidence interval [CI, 0.67; 0.82], p = 0.006).

Both accelerated and hyperfractionated RT have been proposed as an alternative for adding chemotherapy to RT, thereby avoiding the typical chemotherapy-induced toxicities and possibly reducing the RT enhancing effect of CT on normal tissues. The individualized patient-based Meta-Analysis of Radiotherapy in squamous cell carcinoma of Head and Neck (MARCH) showed a survival benefit with altered fractionation (3.4% at 5 years; hazard ratio [HR] 0.92, 95% CI, 0.86; 0.97, p = 0.003) with hyperfractionated RT showing the greatest benefit and again showing a decreasing effect with increasing age [12]. A further update of MARCH confirmed the survival benefit with altered fractionation versus standard fractionating (absolute benefit at 5 years 3.1%, at 10 years of 1.2%). However, that analysis showed that survival benefit was restricted to hyperfractionated RT with an absolute benefit of 8.1% at 5 years [13]. In the same update, conventionally fractionated RT plus concomitant CT versus altered fractionation RT alone showed a significantly worse OS with altered fractionation radiotherapy (HR 1·22, 1·05–1·42; p = 0·0098), with absolute differences at 5 years of −5·8% (−11·9 to 0·3) and at 10 years of −5·1% (−13·0 to 2·8). Both moderately accelerated chemoradiotherapy (70 Gy in 6 weeks plus 2 cycles of 5 days' concomitant carboplatin and 5-fluorouracil regimen) and very accelerated RT alone (64.8 Gy [1.8 Gy twice daily] in 3.5 weeks) were inferior to conventional CCRT (70 Gy in 7 weeks plus 3 cycles of 4 days' concomitant carboplatin and 5-fluorouracil regimen) in a prospective randomized phase III study of the Groupe d'Oncologie Radiothérapie Tête Et Cou (GORTEC-99–02) [14]. For the moment, it is unclear whether hyperfractionated RT equals cisplatin-based CCRT.

Toxicity of Standard of Care Concurrent Chemoradiotherapy

Acute Toxicities

Using single day fractionation RT alone (70 Gy at 2 Gy/day; arm A) versus the same RT plus high-dose cisplatin (100 mg/m^2 every 3 weeks on days 1, 22 and 43; arm B) versus a third arm with 3 cycles of cisplatin/5-fluorouracil and split course RT (arm C) in an Intergroup phase III trial in patients with unresectable HNSCC, grade 3–5 acute toxicity was significantly higher in arm B versus arm A (89 vs. 52%). This mostly concerned nausea/vomiting (16%), mucositis/dysphagia (45%), leukopenia (42%), anemia (18%), renal toxicity (8%) and skin toxicity (7%). Feeding tubes were needed in 52% [15]. In that study all four disease sites were represented, but 50–60% of disease sites in the different arms consisted of oropharyngeal squamous cell carcinomas (OPSCC).

To determine the contributions of chemotherapy and radiotherapy to larynx-preserving treatment, the Radiation Therapy Oncology Group (RTOG) and the Head and Neck Intergroup conducted a randomized trial (RTOG 91–11) in patients with advanced laryngeal cancer to investigate three radiation-based treatments: induction cisplatin plus 5-fluorouracil followed by RT if there was a response to the CT, RT with concurrent cisplatin (100 mg/m^2 three-times every three weeks) and RT alone [16]. The rate of high toxic effects (grade 3–4) was highest in the CCRT arm (82%) versus 81% in the ICT arm and 61% with RT alone. This mostly concerned nausea/vomiting (20%), mucositis/stomatitis (43%), hematologic toxicity (47%), pharyngeal or esophageal toxicity (35%), skin reaction in the radiation field (7%), renal or genitourinary (4%) and neurologic side effects (5%).

We explored the efficacy, toxicity and compliance of three-weekly high-dose cisplatin in three meta-analyses of aggregate data, separately evaluating chemoradiotherapy based on conventional and on altered fractionations in the definitive and the post-operative settings [17, 18]. Among 31 prospective trials utilizing conventionally fractionated RT, model-based estimates of 5-years OS were 39 and 51% in the definitive and adjuvant setting, respectively. Relative to RT alone, patients treated with the combined regimen experienced more grade 3–4 acute toxicity [17]. Of those treated in the definitive setting, about 40% developed severe mucositis, about 25% had severe swallowing problems and at least 20% showed severe bone marrow suppression. As a result of that, only about two-thirds of these patients could receive all three planned cycles of high-dose cisplatin, while 92% received at least two cisplatin cycles [17]. In the same setting, the outcome with altered fractionation and high-dose cisplatin was better, showing an estimated 5-year survival of 57%. In this situation 92% of all planned cisplatin cycles (i.e. two) could be given. Also with altered fractionation RT severe mucositis occurred in around 40%, but severe dysphagia occurred in 40%, and again around 20% had severe bone suppression [18]. The early toxic effects are typically transient, but for a number of tissues data have been presented supporting the concept that severe early effects may be causally related to the subsequent late effects [19].

Late Toxicities

Late toxic effects in patients become manifest after latent periods ranging from months to years and include radiation-induced fibrosis, atrophy, vascular damage, neural damage, and a range of endocrine and growth-related effects [20]. There has been a lack of adequate reporting on late toxicities [17, 21]. Despite this limitation, the earlier mentioned meta-analysis on prospective trials reported an overall prevalence of grade 3–4 late toxicity of 20% with the use of conventionally fractionated RT in combination with concurrent high-dose cisplatin (10% xerostomia, 10% dysphagia and 5% subcutaneous fibrosis) [17]. For altered fractionation RT plus high-dose cisplatin the overall prevalence of grade 3–4 late toxicity was 43% (6% xerostomia, 12% dysphagia and 2% subcutaneous fibrosis).

In a study that was specifically set up to report on late toxicities in a subset of patients who participated in three previously reported RTOG trials of CCRT for LA-HNSCC, all being cisplatin-based (RTOG 91–11, 97–03 and 99–14), the data were more impressive [22]. Of the 230 assessable patients, 99 (43%) experienced severe late toxicities (grade 3–5), 27% showed pharyngeal dysfunction, 13% were feeding-tube dependent >2 years post-RT and 12% showed laryngeal dysfunction. Extremely worrying was the fact that there were 10% (unexplained) deaths. The long-term follow-up data from RTOG 91–11 showed that the survival curves were diverging after 4.5 years of observation in favor of the ICT arm and showed more noncancer-related deaths with CCRT, which likely related to the more frequently occurring and more severe late toxic effects with this treatment approach [16]. In the above mentioned RTOG analysis of Machtay et al., older age, advanced T-stage and larynx/hypopharynx primary site were strong independent risk factors and neck dissection after CCRT was associated with an increased risk of these complications. In a later analysis, additionally, it was found that higher point dose estimates to the hypopharynx (superior and/or inferior) were also associated with an increased risk of severe late toxicity [23].

In support of the Machtay data are the data of the long-term outcome and morbidity after treatment with accelerated RT to a dose of 68 Gy and weekly cisplatin (40 mg/m^2) in 77 LA-HNSCC patients treated between May 2003 and December 2007 at the departments of Radiation Oncology and Medical Oncology of the Radboud University Nijmegen Medical Center in the Netherlands [24]. The radiation treatment technique used was three-dimensional (3D) conformal radiotherapy (from May 2003 to July 2006) and thereafter intensity-modulated radiation therapy with simultaneous integrated boost (IMRT-SIB). In that study, surviving patients were invited to a multidisciplinary late morbidity clinic to evaluate late toxicity. Of the 43 patients still alive, 32 participated in the late morbidity evaluation, with a median follow-up of 44 months (range, 14–68 months). The majority of patients had a least one RTOG/European Organization for Research and Treatment of Cancer (EORTC) grade 2 late toxicity (53%). Grade 3 toxicity of one or more organs or tissues was observed in 12 patients (38%), mostly fibrosis of subcutaneous tissue and xerostomia. Five patients (16%) experienced grade 4 toxicity, 2 laryngeal necrosis, 3 osteoradionecorisis of the mandible. The 5-year actuarial rates of overall grade 3–4

toxicity were 52 and 25%, respectively. Toxicity was significantly correlated with tumor site, with oropharyngeal and oral cavity tumors having more grade 3–4 toxicity than hypopharyngeal tumors. There was also a significant correlation with T-stage, higher T-stages having more grade 3–4 toxicity. Radiologic evaluation demonstrated impaired swallowing in 57% of the patients, including 23% with silent aspiration. Both studies stress the dangers of swallowing difficulties with dysphagia and aspiration following CCRT [22, 24]. These side effects are being seen as major obstacles in intensifying treatment from RT to CCRT (with cisplatin). Identifying those patients as early as possible seems pertinent [25]. Subjective assessment using a systematic scoring system in the Dutch study indicated normalcy of diet in only 15.6% of the patients. Nevertheless, despite all this, quality-of-life questionnaires used in the Dutch study showed that the overall quality-of-life was good; yet, the majority of the patients had a high score on the symptom scale "dry mouth" and "sticky saliva". The study also showed a significant correlation between dysphagia and xerostomia and subcutaneous fibrosis. Therefore, prevention of xerostomia by sparing the parotid and/or submandibular glands might reduce the incidence of dysphagia. It is expected that with contemporary studies utilizing IMRT these late side effects might be less. Examples of that are the two recent prospective randomized phase III de-escalation studies in p16-positive OPSCC, using normofractionation RT in the De-ESCALaTE study and accelerated IMRT in RTOG 1016, and high-dose cisplatin in the control arm and cetuximab in the experimental arm [26, 27]. Apart from 13% ear and labyrinth disorders and 12% gastrointestinal disorders, all remaining severe late toxicities in the control arm of the De-ESCALaTE study occurred in 1–3% (at 24 months). In the control arm of the RTOG 1016 study, overall prevalence of grade 3–4 late toxicity was 20%, with only 2% severe xerostomia, 4% severe dysphagia and no subcutaneous fibrosis at a median follow-up of 4.5 years. The lower overall prevalence of severe late toxicity in RTOG 1016 than in the meta-analysis (20 vs. 43%) may pertain to the use of IMRT in this study.

Is Single Day Three-Weekly High-Dose Cisplatin Optimal to Enhance the Effect of Radiation?

This has remained the most crucial question. The use of high-dose cisplatin during RT has been built on the results of four large phase III trials published between 2003 and 2004 and these data have been supported by the outcome of the two above mentioned de-escalation trials (De-ESCALaTE and RTOG 1016), all showing superiority of this high-dose cisplatin-based CCRT approach versus RT alone or versus cetuximab plus RT [28]. However, the disadvantage of this high-dose cisplatin regimen during RT is that a substantial proportion of patients do not receive the planned number of three cycles due to toxicity issues and therefore alternative cisplatin administration schedules have been studied, and for those patients ineligible for cisplatin, other

cytotoxics (e.g. carboplatin, taxanes, low dose gemcitabine), targeted agents (e.g. cetuximab, despite inferior outcome) or hypoxic modification have been investigated.

An important aspect in this discussion is the impact of the cumulative dose of cisplatin. In a recent systematic review, Strojan et al. [29] showed that the cumulative delivered dose of cisplatin is prognostic for survival, even beyond the 200 mg/m^2. Although the critical cumulative dose is not exactly known, several other studies have suggested or indicated that minimally a cumulative dose of 200 mg/m^2 is needed for obtaining a survival benefit [21]. Strojan's additional observation that the benefit of a higher cumulative dose was independent from the type of cisplatin schedule is intriguing in light of data from clinical pharmacology studies showing cisplatin-induced toxicities are not only dose related, but at a specific dose also peak dose related [30, 31], i.e. the higher the dose and the shorter the infusion time, the more toxicity was experienced. Vermorken et al. found free platinum clearance correlating with creatinine clearance and, in addition, observed that patients experiencing nephrotoxicity also showed an increased incidence of ototoxicity [31, 32]. In their studies in patients with normal renal and hepatic functions, these investigators found the area under the concentration–time curves (AUCs) of free platinum species (the active component) to be identical for cisplatin infusions of different duration when utilizing the same dose [32, 33]. These observations support the clinical impression that, contrary to toxicity, antitumor activity of cisplatin is not dependent on the method of administration. In that context prolonged infusion over 24 h, or splitting up the dose over 4 or 5 days are worth studying with the hope to induce less toxicity.

The German ARO 96–3 trial, comprising 440 patients with high-risk HNSCC, compared CCRT with cisplatin (20 mg/m^2/day, on days 1–5 and 29–33) plus 5-fluorouracil (600 mg/m^2/day, on days 1–5 and 29–33) to RT alone (66 Gy/33 fractions/6.6 weeks) in the postoperative setting [34]. The incidence of grade 3 or higher acute toxicity was higher during CCRT than during RT alone, but lower when compared with the grade 3 or higher acute toxicity observed in the earlier mentioned meta-analysis utilizing high-dose cisplatin and conventionally fractionated RT: mucositis 20.8% versus 42% and leucopenia 4.4% versus 19% in the meta-analysis [17]. However, it should be realized that, contrary to the meta-analysis, the cumulative cisplatin dose in the ARO 96–3 study did not go beyond the 200 mg/m^2. Moreover, although the 5-year OS favored CCRT over RT alone (58.1 vs. 48.6%), this did not reach significance. A second study of interest in that respect is the GORTEC 2015–2 study [35]. The trial, stratified for postoperative or definitive CCRT, compared standard of care (SOC) cisplatin dose (100 mg/m^2 three-times every three weeks) to fractionated high dose (FHD) cisplatin (25 mg/m^2/day, on days 1–4 three-times every three weeks) concomitantly with RT in 124 patients with LA-HNSCC (oropharynx 51%, p16+: 43%). Definitive RT included 70 Gy/7 weeks, postoperative RT 66 Gy/6.5 weeks. The median delivered cumulative dose (primary endpoint) was 291 mg/m^2 (interquartile: 256–298) with FHD cisplatin and 280 mg/m^2 with SOC cisplatin (p = 0.03). Overall, 50 (35%) grade 3–4 acute toxicities occurred with FHD cisplatin versus 91 (65%) with SOC cisplatin (p < 0.001). Efficacy endpoints OS, progression-free survival (PFS) and locoregional control (LRC) were identical. The authors considered FHD cisplatin concomitantly with RT worth further study.

Table 10.1 Phase III trials comparing radiotherapy given concurrently either with three-weekly high-dose or with weekly low-dose cisplatin (non-exhaustive list)

Author, year	Therapy intent	Study arms	Inclusion period	ITT pop.	Planned schedule	Planned Cum. Dose
Tsan, 2012 [36]	Adjuvant	Weekly 3-weekly	2008-2010	55	7×40 3×100	280 300
Noronha, 2018 [37]	Adjuvant (93%), Def.	Weekly 3-weekly	2013-2017	300	$6–7 \times 30$ 3×100	180–210 300
Kyota, 2022 [4]	Adjuvant	Weekly 3-weekly	2012-2018	261	7×40 3×100	280 300
Sharma, 2022 [5]	Definitive	Weekly 3-weekly	2018-2021	278	7×40 3×100	280 300

Cum = cumulative, Def. = definitive, ITT pts = Intent-to-Treat population, () = reference

The low-dose weekly cisplatin during RT has gained ground also in the Western world, now that some recent prospective randomized trials have indicated this treatment schedule to be noninferior to the SOC high-dose cisplatin during RT [4, 5]. This is particularly the case for high-risk patients treated in the postoperative setting (see below). The data are still premature for the definitive setting. Moreover, the comparison of high-dose three-weekly cisplatin versus low-dose weekly cisplatin concomitantly with RT in HPV-associated OPSCC was still not reported (see below). Nevertheless, this regimen, at least the weekly dose of 40 mg/m^2 is now recommended in official guidelines [2]. Crucial in this comparison of three-weekly high-dose cisplatin versus weekly low-dose cisplatin is whether the cumulative dose at the end of treatment is comparable or not (Table 10.1).

We learnt from the meta-analysis on studies using conventionally fractionated RT, both in the adjuvant setting and in the definitive setting that severe acute toxicities with the weekly regimen occurred significantly less frequently than with the high-dose three-weekly cisplatin regimen and there was no suggestion of any difference in outcome [17]. The two prospective randomized trials reported at the previous THNO meeting were, as described, not conclusive [28]. In the small randomized study, reported by Tsan et al. [36] a cumulative dose of 200 mg/m^2 could be delivered to significantly more patients in the high-dose arm. Nevertheless, the weekly low-dose regimen was found more toxic, in particular with respect to severe mucositis (38.5 vs. 75.0%, p = 0.012). However, contrary to the patients in the three-weekly high-dose treatment arm, those in the weekly low-dose cisplatin arm did not receive adequate hydration schemes. The Indian phase III study, reported by Noronha et al. [37], made use of a weekly low-dose of 30 mg/m^2 during RT and compared this with a three-weekly high-dose cisplatin regimen during RT. This led to a difference in cumulative cisplatin dose between both arms of the study (Table 10.1). Two hundred seventy-nine of the 300 enrolled patients (93%) received chemoradiotherapy in the adjuvant setting. After a median follow-up of 22 months, OS was not significantly different between both arms, but the three-weekly regimen did generate a better LRC (58.5 vs. 73.1%, p = 0.014). This was obtained at the cost of more severe acute

toxicity (71.6 vs. 84.6%, p = 0.006), in particular, vomiting, infection, hearing loss, hyponatremia and myelotoxicity.

The Japanese Clinical Oncology Group (JCOG) performed a multi-institutional open-label phase II/III study, in which patients with postoperative high-risk LA-HNSCC were randomly assigned to receive either chemoradiotherapy with three-weekly cisplatin (100 mg/m^2) or weekly cisplatin (40 mg/m^2) to confirm the noninferiority of the weekly regimen [4]. Primary endpoint of the phase II part of this study was the proportion of treatment completion, and that of the phase III part was OS. After a median follow-up of 2.2 years, CCRT with weekly low-dose cisplatin proved to be noninferior to three-weekly high-dose cisplatin in terms of OS with a HR of 0.69 (99.1% confidence interval [CI], 0.374 to 1.273, p < 1.32, one-sided p for noninferiority = 0.0027). As expected, grade 3 or more neutropenia and infection were less frequent in the weekly cisplatin arm, as was any grade of hearing impairment, including tinnitus and renal impairment. Grade 3–4 dysphagia occurred in 12% in the weekly cisplatin arm and in 19% in the three-weekly cisplatin arm. This academic trial will change practice for high-risk patients in the postoperative setting. However, there is insufficient data on the p16-positive OPSCC patients to make a firm statement on that subset of patients.

Another study of interest on this topic was recently presented at the 2022 Annual meeting of the American Society of Clinical Oncology (ASCO) by Sharma and colleagues [5]. This concerned a multicentric Indian non-inferiority study, comparing three-weekly high-dose cisplatin to weekly 40 mg/m^2 cisplatin in the definitive CCRT setting (Table 10.1). Among the primary disease sites, OPSCC comprised 59.6%, with 13% of those tested for p16 being positive. The primary objective was the comparison of the 2-year LRC rates. Two hundred seventy-eight patients were randomized. Treatment interruptions (p = 0.035), hospitalizations (p = 0.004), use of additional intravenous fluids (p < 0.001), mucositis (p = 0.029), myelosuppression (p = 0.0212), renal toxicity (p < 0.001), vomiting (p = 0.002) and hyponatremia (p = 0.004) were all significantly more frequent in the high-dose cisplatin arm. LRC rates at 2 years were 57.69% in the high-dose cisplatin arm and 61.53% in the weekly cisplatin arm of the study. There was no significant difference in median time to locoregional failure, OS, and PFS. Again, no information could be given on the p16-positive OPSCC patients. Moreover, a large number of patients were treated with 2D radiotherapy in this study. The study needs further update and finally peer-review.

Both studies are not conclusive on what is the best CCRT option for patients with HPV/p16-positive OPSCC. This aspect is being covered in the ongoing NRG-HN009 study. In this phase II/III study two cohorts will be assessed for this important question, i.e. patients with p16-positive OPSCC/cancer of unknown primary (target number of patients in phase III is 500) and patients with non-OPSCC/p16-negative OPSCC (target number in phase III is 750), and should be able to definitively answer this important question (Fig. 10.1).
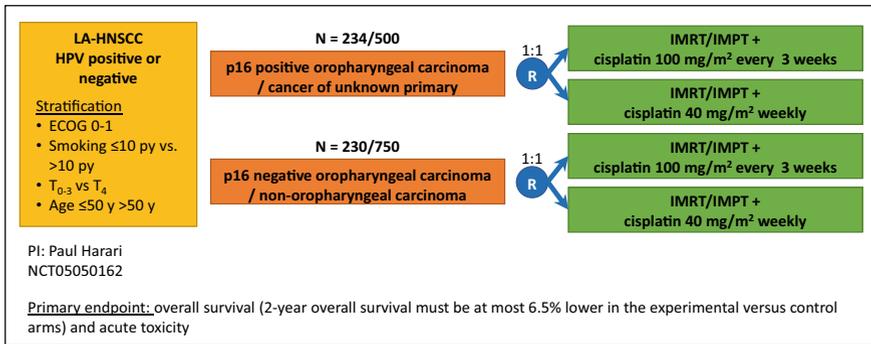
Fig. 10.1 NRG-HN009: Randomized phase II/III trial of radiation with high-dose cisplatin every three weeks versus radiation with low-dose weekly cisplatin for patients with LA-HNSCC

Concurrent Chemoradiotherapy in HPV-Positive Oropharyngeal Cancer Patients

After the first description of a causative association between infection with high-risk HPV and oral squamous cell cancer in 1983 by Syrjanen et al., it was Maura Gillison who clearly indicated that HPV-positive OPSCC was a distinct molecular, clinical and pathologic entity with a markedly improved prognosis [38, 39]. This observation was further substantiated by the retrospective subgroup analysis of the OPSCC patients in the RTOG 0129 study (a study in which all LA-HNSCC patients were treated with high-dose cisplatin based CCRT), showing that the HPV-positive OPSCC patients had a considerably better survival than the HPV-negative OPSCC patients [40]. In that study, Ang et al., using a recursive-partitioning analysis, could classify the patients on the basis of four factors (HPV-status, pack-years of tobacco smoking, tumor stage and nodal stage) in three risk groups: a low-risk category with a 3-year survival rate of 93%, an intermediate category with a 3-year survival rate of 70.8% and a high-risk category with a 3-year survival rate 46.2%. Low-risk was defined as HPV-positive with low tobacco exposure ($\leq$10 pack-years, regardless of T- or N-classification) or >10 pack-years and one ipsilateral node <6 cm (regardless of T-classification); intermediate-risk was defined as both HPV-positive and >10 pack-years and advanced nodal disease (multiple ipsilateral, $\geq$1 contralateral or any node >6 cm), as well as HPV-negative with low tobacco exposure and <T4; high-risk was reserved for HPV-negative with >10 pack-years or T4. Basically, it can be summarized that all HPV-positive OPSCC are either low- or intermediate-risk. The observed differences in the RTOG-0129 trial remained consistent with longer follow-up (5-year estimates of OS and PFS in low-risk group 87.6% and 80.3%, respectively). External validation of the risk groups in RTOG-0552 showed similar results, i.e. significant differences between the three risk groups, but the 5-year estimate of the PFS in the low-risk group in RTOG-0522 was lower than in RTOG-0129 (5-year estimates in RTOG-0522 were for OS 88.1%, for PFS 72.9%). However, in a subgroup of very good-risk patients in

RTOG-0522 (p16-positive, $\leq$10 pack years and T1-2 with ipsilateral $\leq$6 cm nodes or T3 without contralateral or >6 cm nodes), 5-year OS and PFS were 93.8% and PFS 82.2%, respectively [41]. These data suggest that caution is indicated in selecting patients for treatment de-escalation and support a more stringent definition of low-risk than that defined by RTOG-0129.

Considering that future treatment of patients with HPV-positive OPSCC might become different from those with HPV-negative OPSCC, selection of the ideal candidates for treatment-de-escalation and treatment intensification become imperative. In that respect, future novel approaches will likely feature radiographic, proteomic and genomic biomarkers to define prognostic groups and guide treatment selection with greater precision. In the same line of thinking, the availability of a diagnostic test that can reliably select OPSCC tumors that are caused by HPV, becomes indispensable. This has been highlighted in the Key Concepts from the Sixth THNO meeting [42]. Several de-escalation approaches are under study, and will not be further discussed in the current chapter. However, an important question remains "which cisplatin regimen in the control arms of de-escalation studies should be applied, should it be the three-weekly high-dose cisplatin approach or the weekly low dose cisplatin approach?" and "which regimen should be preferred when evaluating treatment intensification approaches in selected locoregionally advanced OPSCC patients and non-OPSCC patients?".

As mentioned earlier, there have been two recent randomized de-escalation trials reported (De-ESCALaTE and RTOG 1016) in which high-dose cisplatin/RT was compared to cetuximab/RT [26, 27]. A third study, contrary to the first two, included only low-risk patients with HPV-positive OPSCC [43]. This randomized trial, comparing cisplatin/IMRT to cetuximab/IMRT, was executed by the Trans-Tasman Radiation Oncology Group (TROG 12.01, Table 10.2). The TROG 12.01 study included patients with American Joint Committee on Cancer (AJCC)/tumor, node, metastasis (TNM) 7th edition stage III (excluding T1-2N1) or stage IV (excluding T4 and/or N3 and/or N2b-c if smoking history >10 pack years and/or distant metastases). The primary outcome was symptom severity assessed by the MD Anderson Symptom Inventory—Head and Neck (MDASI-HN) questionnaire. Quality-of-life was assessed with the Functional Assessment of Cancer Therapy—Head and Neck (FACT-HN) questionnaire. There proved to be neither a significant difference between the arms in symptom severity, nor were there significant differences between both arms in the FACT-HN total score or any of the FACT-HN subscales. With respect to acute toxicity, more dermatitis and acneiform rash were seen in the cetuximab arm and more febrile neutropenia, emesis, dry mouth and fatigue in the cisplatin arm. The number of grade 3 or higher acute events per patient as measured by the T-score (using the TAME method of reporting, [44]) was higher with cisplatin (4.35 vs. 3.82 in the cetuximab arm), but this was not statistically significant. Although there was more grade 2 or higher hearing impairment and tinnitus in the cisplatin arm, the Hearing Handicap Inventory (HHIA-S) did not reveal a significant difference between the arms, though there was a statistically significant deterioration in both arms over time. It is interesting to compare these data with the observations in the De-ESCALaTE study (using 3×100 mg/m^2) and in RTOG 1016

Table 10.2 Concurrent chemoradiotherapy with cisplatin in HPV (p16) associated locoregionally advanced oropharyngeal squamous cell carcinoma

Author, year	Ang criteria [40]	IMRT	Cisplatin schedule (mg/m^2)	Compliance (%)	Cum. Dose ($\geq$200 mg/m^2) (%)	Outcome
Gillison 2018 [27]	Low/intermediate risk	AFRT	2 × 100	93	93	5-yr OS 84.6%
Mehanna 2018 [26]	Low-risk +T4 & N3	CFRT	3 × 100	38	84	2-yr OS 97.5%
Rischin 2021 [43]	Low-risk	CFRT	7 × 40	49	91	3-yr FFS 93%

IMRT = intensity modulated radiotherapy, Cum = cumulative, AFRT = altered fractionated radiotherapy, CFRT = conventionally fractionated radiotherapy, OS = overall survival, FFS = failure-free survival, yr = year, () = reference

(using 2 × 100 mg/m^2). In De-ESCALaTE, late severe ear and labyrinth disorders occurred in 13% of patients with high-dose cisplatin (versus 5% with cetuximab) while in RTOG 1016 late severe hearing impairment was recorded in only 6.3% with high-dose cisplatin (versus 2.1% with cetuximab). Quality-of-life studies done in these trials did not show a striking difference between the cisplatin containing arms and the cetuximab containing arms. In De-ESCALaTE, the mean quality-of-life score measured by the EORTC Core Quality of Life questionnaire (QLQ C30) showed substantial drop at 3 months but that recovered rapidly. At 12 months and 24 months, a significant difference in role functioning was observed in favour of cisplatin (difference in mean scores of 8.32 points, p = 0.0173). However, none of the differences reached the minimal clinically important difference of 10 points. Of interest is the observation in a substudy of the TROG 02.02 study [45]. TROG 02.02/HeadSTART compared RT (70 Gy/7 weeks) given concurrently with three cycles of either cisplatin (100 mg/m^2) or cisplatin (75 mg/m^2) plus tirapazamine, a bioreductive agent that can enhance the cytotoxic effects of ionizing radiation in hypoxic cells. Quality-of-life was comparable between both arms of the study. However, Ringash et al. noticed in a subset of 200 OPSCC patients with known p16 status that p16-positive OPSCC patients overall had a better quality-of-life at baseline but showed a more dramatic drop in quality-of-life at 2 months, which was recovered by 12 months, with even superior scores than observed among the p16-negative patients. It was speculated whether this effect was primarily related to physical injury or to the shock of the diagnosis and change in lifestyle and self-image associated with the quite aggressive cancer therapy.

The survival data, as shown in Table 10.2, clearly show the unprecedented favorable outcome of the HPV-associated locoregionally advanced OPSCC patients. O'Sullivan and colleagues demonstrated that patients with T4 and N3 disease had poorer survival due to a high rate of distant metastases that ultimately led to these patients being classified as stage 3 in the AJCC/TNM version 8 HPV-related

OPSCC staging. The investigators in the De-ESCALaTE study performed a post-hoc subgroup sensitivity analysis. In 276 patients with AJCC/TNM version 8 stage I or II disease, a significant difference in 2-year OS was observed: 98.4% for the cisplatin group and 93.2% for the cetuximab group. (p = 0.0431). The 58 patients with AJCC/TNM 8 stage III (T4 or N3) disease that were allowed in this study showed a larger 2-year OS detriment with cetuximab (67.1%) than with cisplatin (93.3%; p = 0.0304). Interestingly, the study overall showed significantly fewer distant metastases with high-dose cisplatin (3 vs. 9%, log rank p = 0.0092). That was also the case in the TROG 12.01 study, showing a 3-year freedom from distant failure rate with cisplatin 97%, with cetuximab 88% (HR, 4.1; 95% CI 1.2–14.9; p = 0.018). So, seemingly there is not much difference in the effect on distant metastases whether using three-weekly high dose cisplatin or weekly low-dose cisplatin, albeit moderate.

Taking together, the above-mentioned data indicate that cisplatin-containing CCRT is the standard approach for fit patients with LA-HNSCC when there are no absolute contra-indications for cisplatin. This is true for both non-OPSCC primary disease sites, HPV-negative OPSCC as well as HPV-positive OPSCC. Weekly low-dose cisplatin (40 mg/m^2 × 7) is not inferior to three-weekly high-dose cisplatin (100 mg/m^2 × 3) in the postoperative setting, but the available data (abstract only) in the definitive setting needs to be peer-reviewed first. Although there are no direct comparisons between the two approaches in HPV/p16-positive OPSCC, the suggestion is that both are effective (also for the effect on distant metastases). Overall, the data suggest that acute toxicity is worse with the tri-weekly high dose, both hematologic (neutropenia) and nonhematologic (severe nausea/vomiting, nephrotoxicity), but in some studies more dysphagia and weight loss have been encountered with the weekly regimen. Overall, late toxicity seems to be lower with the weekly regimen (ototoxicity, renal toxicity). Late pharyngeal toxicity is a major threat with both CCRT regimens, and early detection and taking early measures to reduce the risk of aspiration pneumonia is essential. A direct comparison of both approaches in HPV/p16-positive tumor would still be of academic interest.

Treatment Intensification Beyond Concurrent Cisplatin-Based Chemoradiotherapy (summarized in Table 10.3)

Candidates for treatment intensification are fit LA-HNSCC patients, both in the definitive setting and in the adjuvant setting who are candidates for CCRT without absolute contra-indication for cisplatin [46] but still have a poor outcome:

1. HPV/p16-positive OPSCC patients who do not belong to the category with the most favorable outcome (who are candidates for de-escalation approaches).
2. HPV/p16-negative OPSCC patients and non-OPSCC LA-HNSCC patients.

A separate group are the patients with LA-HNSCC with oligometastatic disease.

Table 10.3 Intensification strategies of potential interest, beyond cisplatin/RT

• Combining altered fractionation radiotherapy with chemotherapy
• Adding more cytotoxic chemotherapy – Induction chemotherapy (ICT) – Adjuvant chemotherapy (ACT)
• Adding targeted therapy – Targeting the epidermal growth factor receptor (EGFR) – Targeting the Inhibitor of Apoptosis Proteins (IAPs)
• Adding approaches that increase the radio-sensitivity of hypoxic cells – Nitro-aromatic sensitizers (nimorazole) – Hyperthermia (HT)
• Adding immunotherapy (immune checkpoint inhibitors)

Combining Altered Fractionation Radiotherapy with Chemotherapy

As discussed earlier, the MARCH meta-analysis showed that altered fractionation RT was associated with a significant OS benefit compared with conventional fractionation RT [13]. However, the OS benefit was restricted to hyperfractionated radiotherapy. A recent updated individual patient data network meta-analysis, evaluating both MACH-NC and MARCH combined, comprised 115 randomized trials, in a patient population that in great majority was HPV/p16-negative. It compared 16 different treatments, and of those different approaches hyperfractionated RT with concomitant chemotherapy (HFCRT) ranked as the best treatment, when compared with locoregional therapy alone (P score for OS 97%; HR 0.63 [95% CI 0.51–0.77] [47]. In the comparison of HFCRT versus conventionally fractionated platinum-based CCRT (the present standard), the HR was 0.82 (95% CI 0.66–1.01) for OS and the corresponding HR for event-free survival (EFS) was significant with a HR of 0.80 (95% CI 0.65–0.98). The investigators did not analyze toxicity data because the data available in MACH-NC and MARCH were different, with very few toxicities in common [47]. It is therefore important to mention here, that the meta-analysis on altered fractionation with weekly low-dose cisplatin versus two times 100 mg/m^2 showed that compared to the weekly low-dose cisplatin, the high-dose cisplatin regimen not only improved OS (p = 0.0185), but was more compliant with respect to receiving all planned cycles of cisplatin (71 vs. 95%, p = 0.0353) and demonstrated less complications in terms of severe (grade 3–4) acute mucositis and/or stomatitis (75 vs. 40%, p = 0.0202) and constipation (8 vs. 1%, p = 0.0066) and severe late subcutaneous fibrosis (21 vs. 2%, p < 0.0001) [18].

Although in the above mentioned network meta-analysis, ICT with TPF (docetaxel, cisplatin and 5-fluorouracil) followed by conventionally fractionated platinum-based CCRT ranked fourth for OS, according to a sensitivity analysis restricted to trials mandating the use of granulocyte colony-stimulating factor, ICT with TPF followed by CCRT ranked second after HFCRT for OS and first for EFS.

As TPF followed by CCRT probably is more commonly used in clinical practice than HFCRT, this network meta-analysis partly supported the use of ICT with TPF followed by CCRT in practice for selected patients with advanced disease, in good clinical condition and minor comorbidities. However, it should be further tested in clinical trials. Clearly, as stated by the authors, the results of this network meta-analysis are a decision-supporting tool rather than a decision-making tool [47].

Adding More Cytotoxic Chemotherapy

Induction Chemotherapy

The original MACH-NC analysis and also the two later updated versions did not suggest a major role for induction chemotherapy in the treatment of patients with LA-HNSCC [9–11]. However, in the two more recent network meta-analyses, in particular with the use of the TPF regimen, the position of this so-called sequential approach has become stronger [47, 48]. Only one of five moderately sized individual trials comparing TPF followed by CCRT versus CCRT alone, presented at THNO-7, showed survival benefit of the sequential approach over CCRT alone, but all five trials showed an increase in toxic events with the sequential approach [49]. Only two of the five studies showed fewer distant metastases in the ICT arms. This positive effect on distant metastases was also confirmed in two meta-analyses, but still did not lead to a significant effect on OS [50, 51]. A better selection of patients who are at risk for developing distant metastases therefore seems appropriate. Features such as low neck nodes and matted nodes (a proxy for extranodal extension) are of interest. Burningham et al. [52] reported on the prognostic impact of matted lymphadenopathy (ML) in 417 OPSCC patients treated with definitive CCRT. Patients were stratified into favorable OPSCC (p16-positive with ≤10 pack-years smoking history, n = 220) and unfavorable OPSCC (p16-negative and/or >10 pack-years, n = 197). ML had only a significant negative impact on OS and PFS in the unfavorable group, with a 3-year OS for patients with and without matted nodes being at 56% and 74%, respectively (HR 1.61, 95%CI 1.01–2.58). On multivariate Cox regression, patients with ML experienced significantly worsened OS (HR 1.65, 95% CI 1.03–2.65) and PFS (HR 1.94, 95% CI 1.28–2.93). The cumulative incidence of distant metastases was also higher with ML (31 vs. 9%, adjusted HR 3.3, 95% CI 1.71–6.48). ML had no prognostic importance in patients with favorable OPSCC. Similar results had been reported in a retrospective analysis of 321 patients treated with three cycles of docetaxel/cisplatin followed by CCRT (with weekly cisplatin) [53]. Lower neck node involvement (level IV, Vb and supraclavicular regions, p = 0.008) and poor response to ICT (p < 0.001) were associated with significantly inferior distant metastases-free survival [53].

Huang et al. very elegantly discusses the prognostic significance of the different forms of extranodal extension (pathologically [pENE], radiologically [rENE] and clinically [cENE]) in Chap. 7 of this book. Emerging data have consistently shown

that ENE is one of the most powerful prognostic factors for all head and neck cancers, including OPSCC (HPV/p16-positive and HPV/p16-negative) and nasopharyngeal cancer (NPC). The role of rENE is becoming more prominent in selecting patients that need additional systemic treatment to have sufficient effect on distant metastases. Huang et al. were not impressed by the effect of cisplatin on negating distant metastases in patients with ENE. Therefore, additional systemic therapy for that purpose needs to be explored, whether cytotoxic, targeted or immunologic, whether in the induction setting or in the adjuvant setting. For protein expression biomarkers of aggressive disease that could help in a better selection of those that may benefit from ICT, see also Chap. 11 of 7th Critical Issues in Head and Neck Oncology, 2021 [49].

Roughly 15% of head and neck cancer patients will present initially with distant metastases, but a portion of these will have only few discrete lesions. A review of this so-called oligometastatic disease status is beyond the scope of this chapter. Suffice to say, there are no specific treatment guidelines for oligometastatic HNSCC patients [54]. However, the increasing sophistication and clinical experience with stereotactic body radiotherapy has made definitive local treatment to these sites a reasonable option (see also Nevens and Szturz, Chap. 15). When it concerns patients with synchronous oligometastatic disease, aggressive treatment of the primary disease site is essential, leading to better survival outcome compared to patients treated with systemic therapy alone. With that in mind, strategies may emerge combining ICT and upfront metastasis-directed treatment prior to locoregional therapy for the primary tumor [55]. However, for the moment there is a lack of data on this.

Adjuvant Chemotherapy

The data on adjuvant chemotherapy is very scarce. Patients who were given cytotoxic therapy in the adjuvant setting after CCRT had difficulties to tolerate that and 50% or more had to stop treatment early. The original MACH-NC analysis in 2000 and the later updated versions in 2009 and 2021 clearly indicated that adjuvant chemotherapy had no established role [9–11]. However, recent reports on the benefit of adjuvant capecitabine after CCRT (with/without prior ICT) in patients with locoregionally advanced NPC have reactivated the discussion [56, 57], (Table 10.4). In particular, the data on the use of metronomic capecitabine in that respect was intriguing, showing only 17% grade 3 or higher adverse events associated with this approach [57], which seems less than what is usually seen with cisplatin/5-fluorouracil in the adjuvant setting after CCRT (43%, [58]) or with cisplatin/gemcitabine after CCRT (80%, [59]).

Metronomic chemotherapy is the chronic administration of chemotherapeutic agents at relatively low, minimally toxic dose (one tenth to one third of the maximum tolerated dose [MTD]) with no prolonged drug-free breaks. Several mechanisms of actions have been proposed, including inhibition of the nutrition supply for tumor growth, inhibition of tumor angiogenesis, immune system modulation and cellular dormancy mechanisms [60].

Table 10.4 Capecitabine as adjuvant therapy after concurrent chemoradiotherapy in patients with high-risk locoregionally advanced nasopharyngeal carcinoma

Author, year	Treatment arms	Capecitabine dose schedule (mg/m^2)	No. of pts	Follow-up median (months)	Outcome survival & tox. (percentages)
Miao et al., 2021 [56]*	CCRT + AC	1000 bid × 14 days, every 21 days × 8	90	44.8	3-yr FFS 87.7 G3-4 tox. 57.8
	CCRT	no capecitabine	90		3-yr FFS 73.3 G3-4 tox. 51.1
Chen et al., 2021 [57]**	CCRT + AC	650 bid for 1 year	204	38	3-yr FFS 85.3 G3-4 tox. 17
	CCRT	no capecitabine	202		3-yr FFS 75.7 G3-4 tox. 6

CRT = concurrent chemoradiotherapy with cisplatin high-dose, AC = adjuvant chemotherapy, FFS = failure-free survival. *Including AJCC/TNM 7th edition TNM stages III-IVb and one of the following features: T3-4N2 or T1-4N3, or pre-treatment plasma EBV DNA >20,000 copy/ml or gross primary volume >30 cm^3 or a SUVmax>10.0 by 18FDG PET-CT within the primary tumor or multiple neck nodes, with any larger than 4 cm; randomization at the start of CCRT. **Including AJCC/TNM 8th edition TNM stages III-IVA, excluding T3-4N0 and T3N1 disease; randomization after the CCRT

There have been several promising reports suggesting a beneficial effect of maintenance metronomic chemotherapy, also in non-NPC HNSCC patients, most of them being retrospective data [61–65]. In these studies, use has been made most frequently of fluoropyrimidine derivatives, such as tegafur-uracil (UFT) and S-1, which can be given orally. Another popular combination is low dose oral methotrexate (15 mg/m^2) once a week and celecoxib 200 mg twice daily. All these treatments are mostly applied for a duration of one year to 18 months. So far, there have been no solid data of prospective randomized phase III trials and there are no meta-analyses on the use of these agents in the adjuvant setting. Nevertheless, it seems worth continuing to investigate this further.

Adding Targeted Therapy

Targeting the Epidermal Growth Factor Receptor (EGFR)

The epidermal growth factor receptor (EGFR) is a cell-surface receptor belonging to the ErbB family of receptor tyrosine kinases. Overexpression of EGFR, which is frequently found in HNSCC, is correlated with poor outcome [66, 67]. Advances in understanding of the EGFR signaling pathways in cancer have led to the development of anti-EGFR agents including monoclonal antibodies (mAbs) and small-molecule tyrosine kinase inhibitors (Table 10.5).

Table 10.5 EGFR-targeting agents investigated in patients with HNSCC (non-exhaustive list)

Monoclonal antibodies				Toxicity
Cetuximab	IMC225	Chimeric human/murine	IgG1	Skin
Matuzumab	EMD72000	Humanized mouse	IgG1	Skin
Nimotuzumab	h-R3	Humanized mouse	IgG1	System/hemodynamic
Zalutumumab	2F8	Human	IgG1	Skin
Panitumumab	ABX-XGF	Human	IgG2	Skin
Tyrosine kinase inhibitors				
Gefitinib	ZD1839	Reversible	EGFR	Skin/gastrointestinal (GI)
Erlotinb	OS-I774	Reversible	EGFR	Skin/GI
Lapatinib	GW-572016	Reversible	EGFR/ErbB2	Skin/GI/systemic
Afatinib	BIBW-2992	Irreversible	Pan Her[a]	Skin/GI/systemic
Dacomitinib	PF-00299804	Irreversible	Pan Her[a]	Skin/oral/GI/systemic

[a]EGFR/Her2/Her4 (from Szturz P and Vermorken JB. In: J. Bernier (ed), Head and Neck Cancer, 2016, pp711-729)

Cetuximab is a recombinant human/mouse chimeric mAb that binds the extracellular portion of the EGFR and interferes with binding and receptor activation by the natural ligands of EGFR. In addition, cetuximab not only hinders the binding of the natural ligands of EGFR, thereby inhibiting downstream signaling pathways and inducing apoptosis, but also has an immunological effect (antibody-dependent cellular cytotoxicity [ADCC]), thereby acting as a bridge between tumor cells expressing EGFR and immune cells such as CD16-positive natural killer (NK) and dendritic cells [68, 69]. Cetuximab is the only anti-EGFR mAb approved for the treatment of HNSCC in the US and Europe. The approval was based on the results of the EXTREME study in first-line recurrent/metastatic (R/M) HNSCC setting, comparing the platinum/5-fluorouracil combination (PF) versus PF plus cetuximab and the IMCL-9815 phase III registration trial in LA-HNSCC, comparing RT plus weekly cetuximab versus RT alone, both showing significant survival benefit [70–72]. At the time that these trials were performed there was no recognition of the important role of HPV, and stratification by HPV/p16 status had not been done. Subsequent p16 and HPV substudies performed in these two trials showed that, while p16 and HPV are prognostic biomarkers in patients with LA-HNSCC and R/M-HNSCC, it could not be shown that they are predictive for the outcomes of the described cetuximab-containing trial regimens [73]. This is remarkable, considering there is evidence that EGFR inhibitors, including cetuximab have minimal activity as single agents in R/M HPV-positive OPSCC compared with HPV-negative HNSCC [74].

The number of studies evaluating CCRT plus anti-EGFR treatment (with anti-EGFR given in the concurrent or adjuvant settings or in both) versus CCRT alone

is limited [75–81]. Anti-EGFR mAbs are not used as adjuvant therapies for LA-HNSCC and small tyrosine kinase inhibitors are not effective adjuvant therapies, as shown in two large phase III trials [78, 80]. A systematic review and meta-analysis of randomized trials published between 2005 and 2016 (not including the more recent nimotuzumab trials) concluded that for stage III/IV patients, anti-EGFR mAb plus RT can improve OS compared with RT alone, while replacement of chemotherapy with EGFR mAb or adding EGFR mAb to combined chemotherapy and RT did not [82]. Nimotuzumab, originally developed in Cuba, now approved in 30 countries, including countries in Asia, South America and Africa, is a humanized immunoglobulin G1 (IgG1) mAb that has demonstrated a unique clinical profile, where antitumor activity was observed in absence of severe skin, renal or gastrointestinal mucosa toxicities, commonly associated with anti-EGFR targeting antibodies. It is hypothesized that higher binding and internalization of mAbs in the tumor together with a low level of internalization in nontumor tissue is obtained when there is intermediate affinity (10^{-9} to 10^{-8} M) to the receptor [84]. For panitumumab and cetuximab this binding is high (5×10^{-11} and 1×10^{-10} M for panitumumab and cetuximab, respectively), while for nimotuzumab there is an intermediate binding capacity (about 1×10^{-9} M). In addition, this mAb also induces ADCC and complement dependent cytotoxicity.

The largest component in the above-mentioned meta-analysis of anti-EGFR agents administered concurrently with standard therapies was the RTOG 0522 study [75]. Patients included in RTOG 0522 had stage III & IV (excluded T1N+ , T2N1) squamous cell cancer of the oropharynx, larynx and hypopharynx and were randomized to receive altered fractionation with concomitant boost (AFX-CB: 72 Gy/42 fractions/6 weeks) and cisplatin (100 mg/m^2, twice every 3 weeks) or the same CCRT plus cetuximab (400 mg/m^2 × 1, then 250 mg/m^2/week). Details on outcome are in Table 10.6. Patients were stratified by tumor site (larynx vs other), nodal stage (N0 vs N1-N2b vs N2c-N3), Zubrod performance status, use of IMRT (yes vs. no) and receipt of pretreatment fused positron emission tomography/computed tomography scan (PET-CT, yes vs. no). The combined treatment led to more interruptions (26.9 vs. 15.1%) of the RT and induced more grade 3–4 mucositis (43.2 vs. 33.3%), rash, fatigue, anorexia and hypokalemia than CCRT alone, but not more late toxicity. There were no significant differences in outcome between the two arms, with the exception of a better OS for younger patients with the addition of cetuximab. Moreover, when the investigators looked specifically in the OPSCC cohort for whom p16 status was known, there was a trend in better outcome with the addition of cetuximab in the p16-negative cohort, but not (or even the opposite) in the p16-positive cohort, and this was true for both PFS and OS.

In the Tata Memorial Center trial [81], patients with oral cavity tumors were also allowed into the study, as were patients with a Karnofsky performance status ≥70. Stratification occurred for primary disease site (OPSCC vs. other), stage (stage III versus IV), age (≤60 vs. >60 years) and radiation technique (conventional vs. other). Radiation techniques included the standard 2D technique, a 3D conformal technique and IMRT. Gross tumor and lymph node disease received 70 Gy/35 fractions/7 weeks, and in both arms of the study cisplatin was dosed at 30 mg/m^2 weekly during RT.

Table 10.6 Anti-EGFR monoclonal antibodies plus concurrent chemoradiotherapy versus concurrent chemoradiotherapy alone in locoregionally advanced HNSCC: results of two large phase III trials

Author/year	Treatment arms	No. pts	mAge years	No. OPSCC (%)	RTI (%)	PFS (%)	OS (%)
Ang 2014 [75]	CCRT CCRT + Cetux	447 444	57 58	313 (70.0) 312 (70.3)	15.1 26.9	3-yr 61.2 3-yr 58.9	3-yr 72.9 3-yr 75.5
Patil 2019 [81]	CCRT CCRT + Nimo	268 268	54 55	135 (50.4) 134 (50.0)	26.9 29.9	2-yr 50.1[a] 2-yr 61.8[a]	2-yr 57.7 2-yr 63.8

[a]HR, 0.69 (95% CI, 0.53–0.89; p = 0.0044), CCRT = concurrent chemoradiotherapy, Cetux = cetuximab, Nimo = nimotuzumab, mAge = median age, OPC = oropharyngeal cancer, RTI = radiotherapy interruptions, PFS = progression-free survival, OS = overall survival, yr = year, () = reference

In the combined arm, nimotuzumab was administered weekly intravenously as a 200 mg flat dose in 250 mL normal saline over 60 min without premedication.

The primary endpoint of the Tata Memorial Center trial was PFS and that endpoint was reached (Table 10.6). The addition of nimotuzumab improved also LRC (HR 0.67; 95% CI 0.50–0.89; p = 0.006) and disease-free survival (DFS) (HR 0.71; 95% CI 0.55–0.92; p = 0.008) and showed a trend towards improved OS. Grade 3–5 adverse events were similar between the two arms, except for a higher incidence of mucositis in the combined arm (66.7 vs. 55.8%, p = 0.01).

Although the patient and treatment characteristic differed between both trials (in the Indian trial there were more younger patients, less OPSCC patients, more p16-negative OPSCC patients [69.5 vs. 26.8%]), there seemed to be a trend in having a positive effect on survival with the addition of both cetuximab and nimotuzumab in p16-negative OPSCC patients. This positive effect became more clear in a subgroup analysis which has been reported separately for the Tata Memorial trial [84]. Of the 269 patients in the Patil study with OPSCC (see Table 10.6), p16 testing was feasible in 212, of whom 187 were p16 negative (88.2%). Of these 187 patients, 91 were in the CCRT arm and 97 in the CCRT plus nimotuzumab arm. The arms were balanced for patient and disease characteristics. The interaction test for HPV status (positive and negative) was significant for PFS (p = 0.000), LRC (p = 0.007) and OS (p = 0.002), but not for DFS (p = 0.072). The 2-year PFS was 31.5% in the CCRT arm versus 57.2% in the combined arm (HR 0.54; 95% CI 0.36–0.79, p = 0.002). The 2-year LRC was 41.4% in the CCRT arm versus 60.4% in the combined arm (HR 0.61; 95% CI 0.40–0.94, p = 0.024). The addition of nimotuzumab also led to an improved OS at 2 years from 39.0 to 57.6% (HR −0.63, 95% CI 0.43–0.92, p = 0.018). This trial seems to indicate that treatment intensification in HPV-negative OPSCC patients with the use of nimotuzumab is feasible when using very low weekly cisplatin doses. It is unclear whether this also works when high-dose cisplatin during RT is utilized. In order to further identify patients who might benefit from this combined treatment, Patil et al. [85] performed a biomarker study in a subgroup

(n = 404, 206 treated with CCRT, 198 with CCRT + nimotuzumab) of the 536 patients enrolled in the Tata Memorial trial (Table 10.6). This cohort consisted only of HPV-negative cases. The investigators assessed the expression of EGFR, phosphorylated EGFR dimers (pEGFR; a surrogate marker of EGFR activity) and hypoxia-inducible factor 1α (HIF1α; because of increased sensitivity of HNSCC cells to cetuximab under hypoxia in vitro) by immunohistochemistry and EGFR gene copy change by fluorescence in situ hybridization (FISH). Multivariate analysis revealed HIF1α as an independent negative prognostic factor. Moreover, interestingly, outcomes (PFS, LCR, OS) were significantly improved with the addition of nimotuzumab in patients with high HIF1α, but not in those with a low HIF1α expression [85].

The addition of anti-EGFR mAbs to CCRT in patients with locoregionally advanced HPV/p16-negative OPSCC and non-OPSCC seems of interest, and this may be true for both cetuximab and nimotuzumab. Further selection of patients by using molecular markers might be the way to proceed.

Targeting the Inhibitor of Apoptosis Proteins

Inhibitors of apoptosis proteins (IAPs) are a class of proteins that negatively regulate apoptosis and modulate immune and inflammatory responses, processes that are frequently dysregulated in cancer [86]. IAPs are frequently overexpressed in various cancers, including HNSCC, and have been shown to increase the resistance of cancer cells to apoptosis and prevent cell death induced by anticancer treatments, such as chemotherapy and radiotherapy. Cellular IAPs, including cIAP1 and cIAP2, play a critical role in regulating death receptor-mediated apoptosis and modulating nuclear factor kappa B (NF-κB) pathways, driving immune and inflammatory responses. X chromosome-linked IAP (XIAP) plays a central role in the inhibition of apoptosis in both death receptor-mediated and mitochondria-mediated pathways by directly inhibiting members of the caspase family. The critical role of IAPs in primary and secondary resistance to anticancer agents has led to evaluation of IAP inhibitors as therapeutic targets.

Xevinapant (Debio 1143, also known as AT-406 and SM-406) is a first-in-class, potent, oral, small-molecule antagonist of IAPs, including XIAP, cIAP1 and cIAP2, with the potential to enhance the antitumor activity of cisplatin and radiotherapy. The radiosensitizing effect of xevinapant is mediated through caspase activation and tumor necrosis factor (TNF), interferon gamma (IFN$_\gamma$), CD8 T cell-dependent pathways [86, 87], (Fig. 10.2). Used as a single agent at doses up to 900 mg/day on days 1–5 or 400 mg/day on days 1–14 every 3 weeks could be given without reaching a MTD. Dose limiting toxicities (DLT) included elevations of transaminases, which were not dose-related [88]. When the drug was given in combination with CCRT (with high-dose cisplatin) in a phase I study, doses were escalated from 100 to 200 mg and to 300 mg, given for 14 days every 3 weeks. Two of the six patients treated at the 200 mg dose level experienced DLT (grade 3 tubular necrosis, grade 3 aspartate aminotransferase/alanine aminotransferase increase, grade 4 febrile neutropenia and
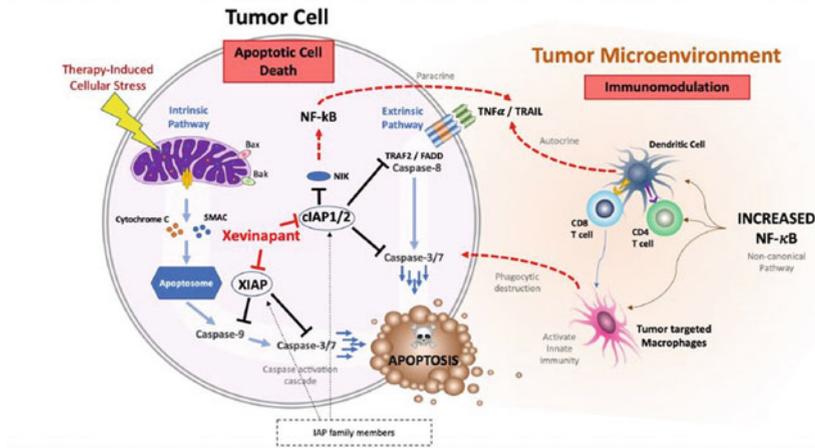
Fig. 10.2 Xevinapant unleashes the cancer cell death cascade and enhances antitumor immune response

grade 3 lipase increase). This dose was therefore considered the MTD and the recommend dose for phase II studies. The next step was a randomized phase II study, which was executed by the GORTEC [87]. The patients included in this study were aged 18–75 years, had histologically confirmed treatment naïve LA-HNSCC (stage III, IVa and IVb, limited to T ≥ 2, N0-3 and M0 (AJCC/TNM 7th edition) originating from the oral cavity, pharynx (OPSCC p16-positive or p16-negative) and larynx, Eastern Cooperative Oncology Group (ECOG) performance status 0–1, a tobacco smoking history of more than 10 pack-years, no diseases or conditions associated with chronic inflammation and adequate organ functions. The protocol design is shown in Fig. 10.3. The primary endpoint was the proportion of patients with LRC at 18 months after chemoradiotherapy termination, and the aim was reaching >20% difference in LRC rate at that time (with 0.8 power at 0.2 significance level). In the first report, the median follow-up was 25 months [87]. LRC at 18 months after chemoradiation was achieved in 26 of 48 patients (54%) in the xevinapant arm and in 16 of 48 patients (33%) in the placebo arm. Grade 3 or more toxicity was reported in 85% of patients in the xevinapant arm and in 87% in the placebo arm. Most common grade 3–4 adverse events were dysphagia (59 vs. 21%), mucositis (31 vs. 21%) and anemia (35 vs. 23%) in the xevinapant and placebo arms, respectively. Median PFS (secondary endpoint) was not reached for the xevinapant group and was 16.9 months for the placebo group (HR 0.37 [95% CI 0.18–0.76], p = 0.0069). There was no significant difference in OS between both groups at 24 months (73% with xevinapant versus 65%; HR 0.65 [0.32–1.33], p = 0.243). However, the data became even more promising at 3 years follow-up with at that time also a significant difference in survival (66% for the xevinapant group and 51% for the placebo group (HR 0.49 [95% CI 0.26; 0.92], p = 0.0261) (Fig. 10.4A and B) [89].
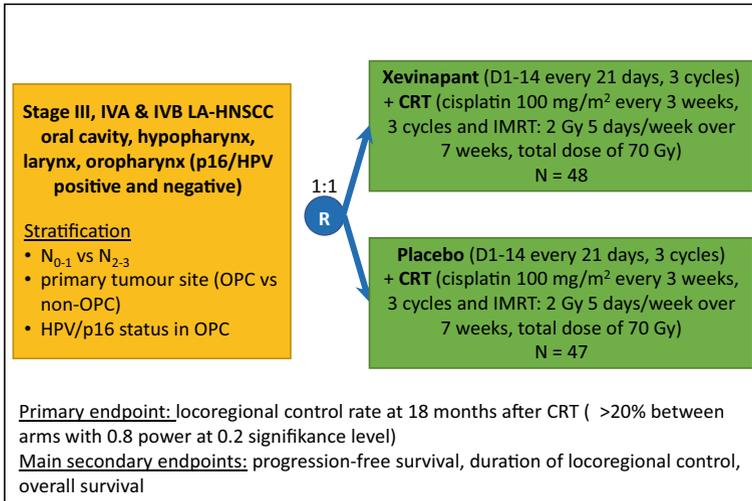
Fig. 10.3 Xevinapant randomised phase II trial: study design. A double-blind, placebo-controlled multicenter study [87]

(a)
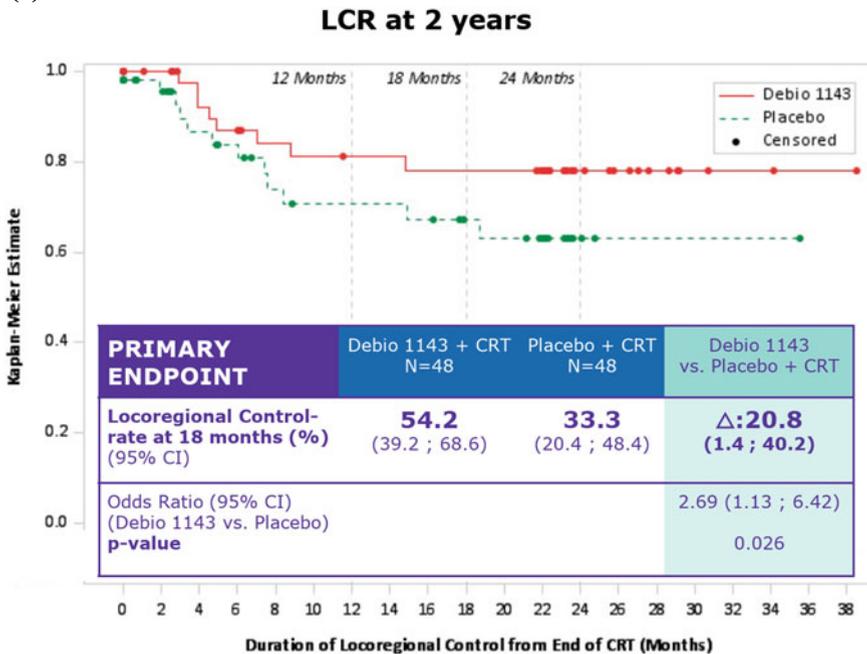
Fig. 10.4 A Locoregional control at 18-month timepoint. Sun et al. Lancet Oncol 2020 [87]. B Progression-free survival and overall survival at 3 years. Bourhis et al. Ann Oncol 2020 [89]

(b)
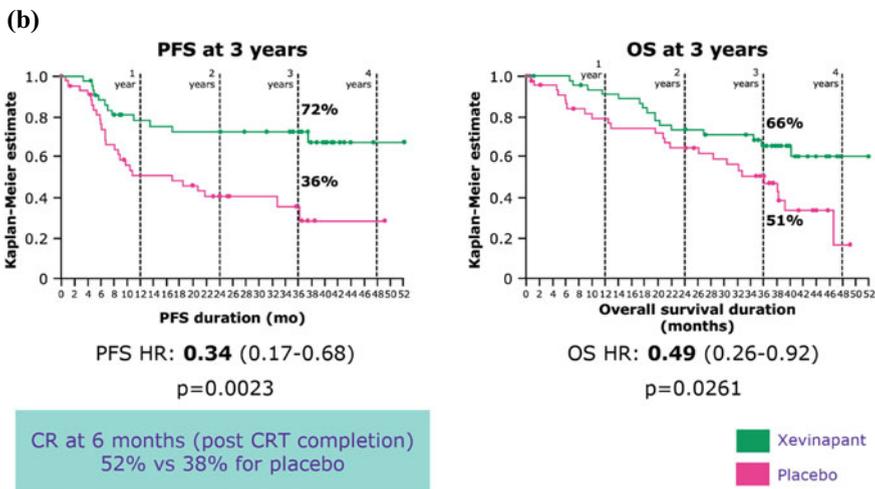
Fig. 10.4 (continued)

In conclusion: these differences are unprecedented for this poor-risk patient population and needs further study. A phase III trial (TrilynX study; NCT04459715) is recruiting similar poor risk patients to confirm these promising data. Details on the study can be obtained in a recent publication [90].

Adding Approaches that Increase the Radio-Sensitivity of Hypoxic Cells

Adding Hypoxic Sensitizers (Nimorazole)

One of the major hurdles in radiation oncology is radioresistance due to heterogeneous hypoxic areas in most solid tumors, including HNSCC, irrespective of their size and histological characteristics [91]. Various efforts and methods to overcome hypoxia-induced radiation resistance have been summarized by Elming et al. in 2019 [92] (Table 10.7). Basically, as indicated, they include improving oxygen availability, increasing radiosensitivity of hypoxic cells, killing the hypoxic cell population or modifying the radiation treatment either by increasing the dose to the hypoxic areas (dose painting) or utilizing radiation of a higher LET (linear energy transfer) in which the oxygen enhancement ratio is reduced [92]. Hyperthermia (HT; heat treatments of 39–45 °C) induces many of these effects and is therefore being considered as one of the best agents for eliminating hypoxia.

The beneficial effect of giving radiotherapy with hypoxic modification is supported by a meta-analysis [93]. Criteria for inclusion in that meta-analysis included curative treatment with RT alone with randomization to a hypoxic modifier

Table 10.7 Approaches for dealing with hypoxia[a]

Approach	Examples
Increasing oxygen delivery	
– High oxygen content gas breathing	hyperbaric oxygen carbogen
– Altering hemoglobin	transfusion, erythropoietin
– Reducing fluctuations in the flow	nicotinamide, pentoxifylline
– Decreasing oxygen consumption	metformin, phenformin
– Increasing blood flow	*hyperthermia*
Radio-sensitizing hypoxic cells	
– Nitroimidazoles	misonidazole, nimorazole, etanidazole
– *Hyperthemia*	
Preferentially killing hypoxic cells	
– *Hyperthermia*	
– Bioreductive drugs	tirapazamine, banoxantrone, evofosfamide
Vascular targeting therapies	
– Angiogenesis inhibitors	bevacizumab, tyrosine kinase inhibitors
– Vascular disruptive drugs	combretastatin, OXi4503, *hyperthermia*
Radiation-based approaches	
– Dose painting	
– High LET (linear energy transfer) radiation	

[a]Adapted from Elming et al. [92].

which should be known only to influence hypoxic radioresistance and have no other cytotoxic effect. Thus, studies involving chemotherapy, either as part of primary therapy or as intended hypoxic modifier or HT were not included. The same was true for studies with hemoglobin modification, these were also not included. Overall, hypoxic modification did result in a significant benefit in LRC (odds ratio [OR] 0.71, 95% CI 0.63–0.80, p < 0.001), disease-specific survival (OR: 0.73, 95% CI 0.64–0.82, p < 0.001) and to a lesser extent in OS (OR: 0.87, 95% CI 0.77–0.98, p = 0.03). The risk of distant metastases was not significantly influenced. Important was the observation that the radiation related late complications were not influenced by the overall use of hypoxic modification (Fig. 10.5).

After a first experience with misonidazole in the Danish Head and Neck Cancer Study Group (DAHANCA 2), showing better LRC with RT plus misonidazole than RT alone in patients with pharynx and supraglottic larynx carcinoma (no benefit in glottic lesions) at the cost of unacceptable peripheral neurotoxicity in 26% of the patients, nimorazole (1-(N-β-ethyllmorpholine)-5-nitro-imidazole) was tested in the DAHANCA 5 study [94]. This concerned a randomized double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary RT in supraglottic larynx and pharynx cancers. Overall, the nimorazole group (n = 219) showed a significantly better LRC rate (primary endpoint) than the placebo group (n = 195), 49% versus
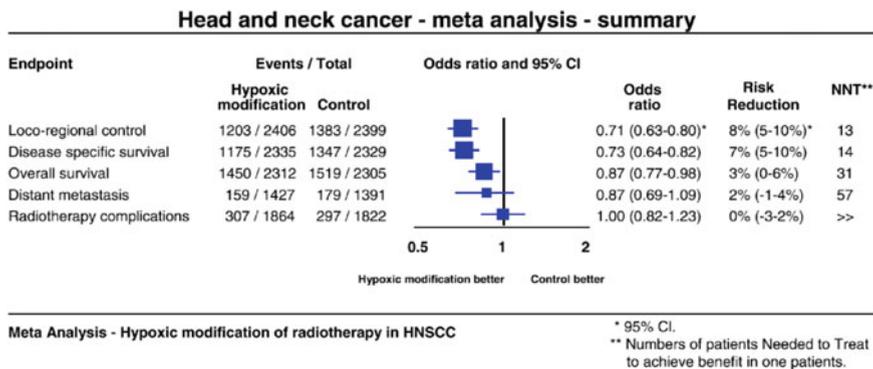
Fig. 10.5 Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck. Overgaard J. Radiother Oncol 2011; 100: 22–32 [93]

33% (p = 0.002) and also disease specific survival was significantly improved (52 vs. 41% at 5 years, p = 0.01). However, OS was not significantly different and late RT-related morbidity occurred in 10% of surviving patients, irrespective of nimorazole treatment [94]. Although these observations are promising, hypoxic modification found little following [95], except for the use of nimorazole in Denmark. As tumors display variable degrees of hypoxia, it is becoming increasingly clear that patient selection is an important factor in the evaluation and interpretation of clinical trials. Of the several different methods for measuring hypoxia, fluoromisonidazole (FMISO) and Fluoroazomycin arabinoside (FAZA)-PET are examples of functional, non-invasive imaging techniques, and PET measured hypoxia proved to be robust and showed a strong impact on LRC and OS in HNSCC patients treated with (chemo)radiotherapy [96]. Other methods include oxygen electrode measurements, exogenous hypoxia markers and endogenous hypoxia markers [97, 98]. Toustrup et al. developed a 15-gene hypoxia classifier, which was validated in 323 DAHANCA 5 patients of whom they had access to sufficient formalin-fixed paraffin-embedded (FFPE) pre-treatment tumor biopsies for gene expression classification. On the basis of this classifier, tumors were classified as either "more" hypoxic (n = 114 [35%]) and as "less" hypoxic (n = 209 [65%]). Patient characteristics in the two groups were grosso modo comparable and the relative number of p16-positive tumors was equally distributed between the two groups [97]. The "more" hypoxic group had a significant benefit of hypoxic modification with nimorazole compared with placebo in terms of LRC (5-year actuarial values of 49 vs. 18%, p = 0.001) and disease specific survival (48 vs. 30%, p = 0.04). "Less" hypoxic tumors had no significant effect of hypoxic modification. Contrary to HPV-negative tumors, HPV-positive tumors had a substantially better outcome in response to RT, which was irrespective of hypoxic modification [97].

The DAHANCA group has further tested the feasibility of hyperfractionated, accelerated RT with concomitant weekly low-dose cisplatin and nimorazole (HART-CN) in locoregionally advanced, HPV-negative squamous cell carcinoma of the

oropharynx, hypopharynx, larynx and oral cavity (DAHANCA 28) and will explore this approach also in hypoxic tumors. Of interest in that respect is the observation by DeSchuymer et al. [99] that by using the 15 gene hypoxia classifier in patients treated with accelerated CCRT, no significant outcome differences were observed between "more" and "less" hypoxic tumors.

Finally, the DAHANCA 29–EORTC 1219 study, tried to confirm the Danish data outside Denmark. The objectives of that trial were to demonstrate the benefit of nimorazole with accelerated CCRT and the predictive value of the hypoxic gene signature. Quality controlled accelerated RT was delivered using IMRT up to a dose of 70 Gy in 6 weeks. Cisplatin was delivered either weekly 40 mg/m^2 on weeks 1 to 6 or three-weekly 100 mg/m^2 on weeks 1 and 4. Nimorazole or placebo were delivered orally with a daily dose of 1.2 mg/m^2. The two co-primary endpoints were LRC for the entire population and the hypoxic-gene population (Fig. 10.6). Thirty-three percent of the tumors were hypoxic-gene positive. After two safety reviews, the Independent Data Monitoring Committee (IDMC) recommended to only use the weekly cisplatin regimen based on nephrotoxicity in the three-weekly arm, with more toxicity in the nimorazole arm (27 vs. 11.4% with the placebo arm). Overall, grade 3 or higher adverse events occurred in more than 90% of patients in both arms. Unfortunately, at the last review, the IDMC recommended early closure of the trial based on weak conditional power for the hypothesized treatment effect. At 2 years, the LRC probability was not clinically different between the two arms, neither in the entire population (63.8% with nimorazole and 72.1% with placebo) nor in the hypoxic-gene positive patients [100].
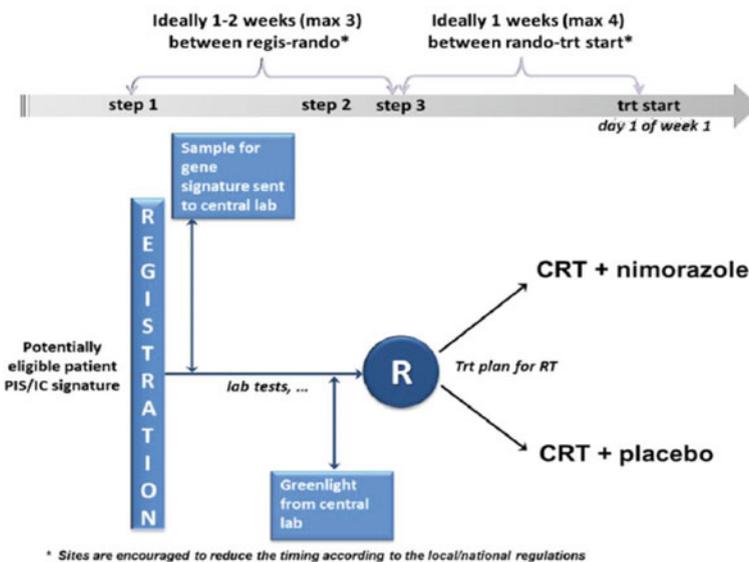
Fig. 10.6 DAHANCA 29–EORTC 1219 trial: study design. Gregoire V. et al. Radiother Oncol 2021 [100]

Hyperthermia

As mentioned in Elming's paper in Cancers (see also Table 10.7), HT induces many of the effects that are playing a role in handling the negative effects of tumor hypoxia and in that sense it has the potential to be one of the best agents for eliminating hypoxia [92]. Locoregional HT, at 40–44 °C, has been shown to be a potential radiosensitizer, a chemosensitizer and an immunomodulator with no significantly added side effects [101]. The thermodynamic changes are initiated at around 38 °C and result in a gradual increase in tumor blood flow and subsequent oxygenation, while the thermoradiobiological mechanisms lead to direct cell kill, thermal sensitization and inhibition of DNA repair between 39 °C and 45 °C [101, 102]. Thus, at the usual clinically achievable temperature of 40–42 °C, HT can lead to appreciable radiosensitization, chemosensitization and immunomodulation along with RT with or without chemotherapy. In a meta-analysis of six clinical trials comprising 451 cases of LA-HNSCC the combination of RT and HT improved the overall complete response rate by 25.5% over RT alone (p < 0.0001) without an excess of acute or late morbidity [103]. A narrative review of regional HT updating the period 2010–2019 reported data on three studies in NPC comparing CCRT versus CCRT with HT [104]. Two of the three studies showed improved complete response (CR), PFS and OS with the combined approach, while improved DFS was reported in the third study. There are no randomized trials of CCRT with HT versus CCRT alone in patients with LA-HNSCC. However, retrospective studies reported promising results, both in terms of efficacy and toxicity, when applying CCRT with weekly cisplatin and weekly HT in head and neck cancer patients [105, 106]. In particular, no enhanced mucosa and thermal toxicity was reported.

Contrary to the situation in head and neck cancer, randomized trials on CCRT with HT versus CCRT alone are available for patients with locally advanced cervical cancer (LACC), a cancer sharing similar histology with head and neck cancer, and showing a lot of similarities with LA-HNSCC in terms of treatment evolution. A first randomized trial in the Netherlands (the Dutch Deep Hyperthermia Trial), completed in 1996, showed significant benefit of adding HT to RT (3-year OS of 51 vs. 27%, p = 0.009) [107]. The results of this trial have led to the acceptance of RT plus HT as standard treatment for advanced cervical cancer in the Netherlands. However, the standard treatment of LACC nowadays, based on at least five randomized trials worldwide, consists of CCRT with weekly cisplatin (40 mg/m^2). In retrospect, the outcome data with RT plus HT were quite similar to those that can be obtained with CCRT (with cisplatin). A randomized trial (the RADCHOC trial), in which patients with LACC were randomized to RT plus HT or RT plus cisplatin, reported comparable outcome and comparable grade 3 or higher late radiation-related toxicity between the two treatment arms, suggesting HT might have a role to play as an alternative treatment if chemotherapy tolerance is an issue [92, 108]. It was therefore of interest to see whether HT to CCRT would further improve outcome and it did. In a systematic review and meta-analysis, the risk difference from three randomized clinical trials (total number of patients 738) for LCR and OS showed an advantage for CCRT plus HT over CCRT alone of 10.1% (p = 0.03) and 5.6% (p = 0.07), respectively

[109, 110]. This beneficial effect was also confirmed in a network meta-analysis, in which all 13 different therapeutic approaches for treating LACC from 49 clinical trials totalling 9894 patients were evaluated [111].

Crucial for such a set up for LA-HNSCC is having a proper HT unit for the head and neck region that would allow adequate heating and monitoring of HT during individual treatment sessions. In Rotterdam (The Netherlands), such a HT delivery system (The HYPERcollar: a novel applicator delivering heat at 433 MHz to the head and neck) has been further developed and is currently being validated in clinics for HT delivery in the head and neck region. Presently, a magnetic resonance (MR)-compatible version of this applicator is being used with a 1.5 T MR system, allowing an online monitoring of the temperature using non-invasive thermometry with the proton resonance frequency shift method [112, 113].

In conclusion, there is at present no proof that treatment intensification with the use of hypoxic sensitizers added to conventionally fractionated or accelerated cisplatin-based CCRT leads to better outcome in patients with LA-HNSCC. However, considering the strong background data, further studies guided by molecular markers of hypoxia seem appropriate. Since HT can effectively target hypoxia via a variety of different mechanisms and showed improved outcome when combined with RT in a number of solid tumor sites, among which NPC, there is potential that it may further improve outcome in LA-HNSCC over CCRT alone also in the non-NPC sites.

Adding Immunotherapy (Immune Checkpoint Inhibitors)

As mentioned by Machiels et al. in two THNO chapters (Chap. 13 in the 7th Critical Issues in Head and Neck Oncology, 2021 and Chap. 11 in the present issue) the integration of immune checkpoint inhibitors (ICIs) in the primary treatment of patients with LA-HNSCC so far has not reached the same success that has been seen when immune checkpoint inhibitors were used in the R/M disease setting. The reader is referred to these chapters for details. No improvement in outcome of patients treated with ICIs during chemoradiotherapy has been reported. The cause of this is not completely clear. One of the options mentioned as an explanation for the lack of benefit of anti-programmed death-1 (PD-1)/PD-Ligand-1 (PD-L1) mAbs in combination with (chemo)radiation is the large field of irradiation to regional lymph nodes that might neutralize immune competent cells. Unfortunately, some of these studies have more than one question at the same time, which complicates outcome data. So far, ICIs in the neoadjuvant setting, either alone or in combination with chemotherapy have shown promising results (high pathological response rates) in window of opportunity studies, but no data from randomized phase III trials exploring this option have been reported. Data on ICIs used as adjuvant therapy per se are eagerly awaited, because of its simplicity and purity.

Conclusions

Current treatment guidelines for patients with LA-HNSCC recommend multimodal treatment, including CCRT or surgery followed by RT, with/without CT. ICT followed by (chemo)-RT is an alternative approach for larynx preservation procedures in patients with locoregionally advanced laryngeal or hypopharyngeal cancer. The CT part of the CCRT consists of platinum-based chemotherapy, most often single agent cisplatin. Although for a long time high-dose cisplatin (100 mg/m^2) three-times every three weeks during RT has been the standard of care, recent prospective randomized studies have indicated that the weekly low-dose cisplatin (40 mg/m^2) is a good alternative with less toxicity, in particular in the postoperative (adjuvant) setting. For patients not eligible or not tolerating cisplatin there are other alternatives (such as carboplatin with or without 5-fluorouracil, taxanes or cetuximab). However, none of these have shown superior results over the use of cisplatin in randomized trials. Late toxicity is a major downside of CCRT, and this is most worrying for those with the highest chance of cure, i.e. low-risk HPV-positive OPSCC. De-escalation approaches have priority in these patients, but this needs to be done with the utmost caution. In the remaining patient populations (high-risk HPV-positive OPSCC, HPV-negative OPSCC and non-OPSCC patients) there is room for improvement in both locoregional control and in distant control. Recent strategies of potential interest above and beyond cisplatin-based CCRT are adding (1) more cytotoxic chemotherapy (both neoadjuvant and adjuvant), (2) targeted therapy (concomitant cetuximab, nimotuzumab, xevinapant) (3) hypoxic sensitizers (nimorazole), including hyperthermia, and, (4) immunological approaches (immune checkpoint inhibitors). However, these approaches are not applicable for all poor-risk LA-HNSCC patients and radiographic, proteomic and genomic biomarkers will play an increasing role in better defining prognostic groups and guide treatment selection with greater precision. Apart from hyperthermia, all these approaches beyond CCRT will be accompanied by an increase in toxicity. Therefore, taking into account the already existing toxicity profile of cisplatin-based CCRT, these treatment intensification options can only be considered in patients in a good general condition, with adequate organ functions and without prohibitive co-morbidities. Many of the above mentioned options are being investigated in prospective randomized trials and will hopefully lead to further improvement in outcome for these less favorable HNSCC patient categories.

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Chapter 11
Immune Checkpoint Inhibitors in the Curative Setting: Pre-clinical and Clinical Data

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Abbreviations

CCTG	Canadian Cancer Trials Group
DFS	Disease-Free Survival
ENE	Extra Nodal Extension
EORTC	European Organization for Research and Treatment of Cancer
Gy	Gray
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human Papillomavirus
ICI	Immune Checkpoint Inhibitor
INF	Interferon
LA	Locally Advanced
mAbs	Monoclonal Antibodies
MHC	Major Histocompatibility Complex
MOC	Mouse Oral Cancer
PD(L)-1	Programmed Cell Death Protein-(Ligand) 1
pTR	Pathologic Tumor Response
R/M	Recurrent and/or Metastatic
RT	Radiotherapy
TRAEs	Treatment Related Adverse Events

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Introduction

Pembrolizumab and nivolumab, two monoclonal antibodies (mAbs) targeting programmed cell death protein-1 (PD-1), improve the overall survival of patients with inoperable recurrent and/or metastatic (R/M) head and neck squamous cell carcinoma (HNSCC) [1].

For curable HNSCC, the role of immunotherapy is under investigation. Standard curative treatments are still based on unimodal or multimodal treatments consisting of surgery and/or (chemo)radiation depending on the stage and location of disease and the expected functional outcome. These treatments result in a survival rate of 80% and 50% at five years for early and advanced stages [2], respectively. In this chapter, we briefly review the potential role of anti-PD-1/PD-ligand (L) 1 inhibitors in the curative treatment of HNSCC, either in combination with curative-intent (chemo)radiation or surgical treatment.

Anti-PD-1/PD-L1 mAbs in Combination with Curative-Intent Primary Surgery

Surgery remains a treatment of choice for head and neck cancers. However, in locally advanced (LA) disease, more than half the patients will recur even after curative surgery with pathological disease-free margins. There are currently many studies investigating the role of neo-adjuvant or adjuvant immunotherapy in the context of surgery to reduce the risk of disease recurrence.

Mice bearing a mouse oral cancer and treated with pre-operative administration of anti-PD1 mAbs followed by surgery had a lower rate of new mouse oral cancer (MOC) cell engraftment after tumor re-challenge than controls or mice treated with surgery and adjuvant anti-PD-1 mAbs [3]. This means that pre-operative administration of anti-PD1 antibodies could promote the development of memory T cells. Furthermore, T cells recovered from tumor draining lymph nodes showed a significantly higher expression of interferon (INF)-gamma in response to the antigenic peptide in neoadjuvant treated mice compared to controls or adjuvant treated mice [3]. Similarly, Liu et al. [4] showed that triple-negative breast cancer mouse models treated with a neoadjuvant immune checkpoint inhibitor (ICI) (anti-PD1 and anti-CD137) plus surgery had better survival and higher levels of tumor-specific CD8+ cells in their blood and organs compared to mice treated with surgery and adjuvant immunotherapy. This provides a strong rationale for studying the efficacy of checkpoint inhibitors in head and neck cancers undergoing curative treatment, particularly before tumor surgery.

Anti-PD-1/PD(L)1 mAbs were investigated in several pre-operative window of opportunity studies. In a phase II study (NCT03021993), 12 patients with stage II-IVa oral squamous cell carcinoma (OSCC) were treated with three to four doses of nivolumab biweekly before curative surgery. The use of this checkpoint inhibitor

during the 30 pre-operative days was found to be safe, and there was no delay in surgical management. The objective response rate was 33%, and 10 patients were still alive after a median follow-up of 2.23 years [5]. The phase I/II CheckMate 358 study investigated the safety and efficacy of two pre-operative doses of nivolumab in 26 patients with human papillomavirus (HPV)-positive HNSCC and in 26 patients with HPV-negative HNSCC. Nivolumab was administered on days 1 and 15 and curative surgery was performed on day 29. Four patients (19.2%) in the HPV-positive cohort and three patients (11.5%) in the HPV-negative cohort experienced severe treatment related adverse events (TRAEs). No delays in surgical treatment were observed. Nivolumab induced radiographic tumor shrinkage ≥30% according to RECIST criteria v1.1 in 12.0% and 8.3% of patients in the HPV-positive and HPV-negative cohorts, respectively. In addition, pathological regression was observed in 23.5% of patients with HPV-positive HNSCC and in 5.9% patients with HPV-negative tumors [6]. Another window of opportunity phase II study [7] investigated pembrolizumab as a single dose administered two to three weeks prior to surgery in 36 patients with HPV-negative HNSCC. Pathologic tumor-response (pTR) was defined as the proportion of the resection bed with tumor necrosis, giant cells/histiocytes and keratinous debris: pTR-0 (<10%), pTR-1 (10–49%), and pTR-2 (≥50%). The endpoints were safety, pTR-2 and the relapse rate at one year of patients with high-risk pathological features identified on their surgical specimens (extra nodal extension (ENE) and/or positive margins). Patients having high-risk pathology findings after surgery received adjuvant pembrolizumab. The administration of pembrolizumab in the pre-operative period was safe, and no surgical delays or immune-related adverse events were observed. Twelve patients received adjuvant pembrolizumab in combination with postoperative chemoradiation without any complications. With a median follow-up of 22 months after surgery, the one-year relapse rate was 16.7% and 0% for patients with pathological high-risk and low/intermediate-risk features after surgery, respectively. After neoadjuvant pembrolizumab, eight patients had pTR-2, including two patients who experienced a major pTR (>90%), and eight patients had pTR-1. Most patients maintained stable disease, but 19% had a decrease in pathological staging compared to clinical staging. PD-L1 expression and CD8+ T-cell infiltration in baseline biopsies were positively correlated with pTR. Deconvolution analysis using RNA-sequencing showed a significant increase in immune infiltrate (M1 macrophages, CD8+ T cells, and CD4+ T-cells) in baseline biopsies for pTR-1/2 patients compared to biopsies from pTR-0 patients. In baseline biopsies, a higher expression of inflammatory and immune genes (e.g., *CXCL9, IFNG, CXCL10*, …) was found in patients with pTR-1/2 compared to those with pTR-0. Accordingly, enrichment analyses showed an increased expression of signatures involved in inflammation (e.g., Hallmark interferon gamma response, Hallmark inflammatory response) in baseline biopsies of patients with pTR versus no pTR. Increased expression of checkpoint molecules (e.g., *CTLA4, IDO1, PDCD1*) was demonstrated in post-treatment tumor biopsies in pTR-0 patients. Whole exome sequencing of baseline biopsies showed no correlation between tumor mutational burden (TMB), predicted neoantigen burden, and pTR. Furthermore, patients with pTR-1/2 showed increased T-cell receptor (TCR) diversity and clonality in the blood after neoadjuvant pembrolizumab. A larger phase

II study (NCT02641093) included 80 resectable p16-negative HNSCC patients with T3-T4 and/or two or more nodal metastases or clinical extra nodal extension (ENE). Patients were treated neoadjuvantly with pembrolizumab one to three weeks prior to surgery. After surgery, patients received pembrolizumab for a total of six doses with concurrent radiotherapy. Patients with high-risk HNSCC (positive margins and/or ENE) received concurrent cisplatin. Disease-free survival (DFS) after one year was the primary endpoint. One-year DFS was 97 and 66% in the intermediate and high-risk groups, respectively. Patients presenting a pathologic response had significantly improved DFS compared to patients without a pathologic response (93 vs. 72%) [8].

Currently, although anti-PD-1/PD-L1 therapy in combination with surgery with or without adjuvant (chemo)radiation gives promising signs of activity, the use of these drugs in combination with surgery for curative purposes is still not indicated in routine clinical practice.

Based on the results, several phase II/III studies are underway to better determine the role of anti-PD-1/PD(L)1 mAbs in curable HNSCC treated with primary surgery. These trials are described in Tables 11.1 and 11.2.

Anti-PD-1/PD-L1 mAbs in Combination with Primary Curative-Intent (Chemo)Radiation

Radiation therapy has the ability to enhance the immune response through several mechanisms. These include the release of neo-antigens during radiation-induced cell death [11], the activation of dendritic cells with enhanced expression of major histocompatibility (MHC) class I [12], and the release of cytokines [13], which all contribute to CD8+ T-cell activation. Radiation itself promotes the infiltration of immune cells into irradiated organs and increases PD-L1 expression [14]. However, radiation therapy can also induce cell damage to immune infiltrating cells. The complex interaction between immunotherapy, radiation therapy and optimal treatment sequencing still needs to be elucidated. The efficacy of radiotherapy combined with ICI depends on tumor models, dose, the irradiated site and type of irradiation, fractionation, and the timing/sequence of delivery. Similarly, Kanagavelu et al. [15] showed in different mouse models that the immune population in the tumor microenvironment is highly variable depending on the dose, irradiated site and tumor model. In addition, Marciscano et al. [16] found in some mouse models that irradiating the lymph nodes may be detrimental to the local control of the primary tumor and that immunocompetence was necessary to provoke a response to radiotherapy. Although conflicting, hypo-fractionated radiation is also of interest, and charged particles may be more immunogenic than photons [17]. However, there is no clinical evidence on which type of radiation therapy, combination and sequence is most appropriate for HNSCC patients, and there are conflicting results in several trials [18, 19].

Table 11.1 Studies including anti-PD1 therapy prior to curative surgery for HNSCC (non-exhaustive list)

Trial (Reference)	Phase	N	Agent	Time to surgery (days)	Primary endpoints	Adjuvant	Delay in surgery
Checkmate 358 NCT02488759 [6]	II	26 HPV-negative 26 HPV-positive	nivolumab day 1 and day 15	29	Safety and efficacy	No	No
NCT02296684 [7]	II	36 HPV-negative	pembrolizumab single dose	14–21	1) pTR-2 2) One-year relapse rate in patients with high-risk pathology	Yes in high-risk (ENE or R1)	No
NCT02641093 [8]	II	80 p16-negative T3-T4 N +	pembrolizumab single dose	7–21	1) One-year DFS 2) One-year OS & pathologic response	Yes with concurrent radiotherapy. Concurrent cisplatin for high-risk only (ENE or R1)	NA
Keynote 689 NCT03765918 [9]	III	704 Stage III-IVa	Randomization: – Arm A: neoadjuvant pembrolizumab (two cycles) + surgery + SOC + adjuvant pembrolizumab (15 cycles) – Arm B: surgical resection followed by adjuvant SOC	21	1) Pathologic response & event-free survival 2) OS & pathological complete response & safety	Yes if randomized in arm A with SOC	NA

Table 11.1 (continued)

Trial (Reference)	Phase	N	Agent	Time to surgery (days)	Primary endpoints	Adjuvant	Delay in surgery
NCT03700905 [10]	III	276 resectable locally advanced	Randomisation: – Arm A: nivolumab single dose + surgery + R(C)T + randomisation (nivolumab or nivolumab + ipilimumab) – Arm B: SOC	14	1) Three-year DFS 2) Locoregional control, distant metastasis-free survival, overall survival	Yes if randomized in arm A: SOC + nivolumab or nivolumab + ipilimumab	NA

DFS: disease-free survival; ENE: extra nodal extension; NA: not available; OS: overall survival; pTR: pathologic tumor response; R1: microscopically positive resection margin; SOC: standard of care

Table 11.2 Studies investigating anti-PD(L)1 therapy after curative surgery for HNSCC (non-exhaustive list)

Trial	Phase	N	Agent	Duration	Primary endpoints
NRG-HN003 NCT02775812	I	37 patients with high -risk, HPV-negative	Concomitant adjuvant radiochemotherapy and pembrolizumab (every 3 weeks)	Weeks 9 to 21	(1) To determine the recommended phase II dose of combined pembrolizumab and adjuvant radiochemotherapy based upon dose-limiting toxicity (2) 1-year DFS, OS, toxicity
NCT03715946	II	135 resectable SCC or undifferentiated carcinoma of the oropharynx	Transoral surgery + de-intensified adjuvant radiotherapy (50 Gy) and concomitant nivolumab (every 2 weeks) + nivolumab after radiotherapy [1 dose every 4 weeks for 6 weeks]	28 weeks	(1) Progression-free survival & PEG tube dependence
NCT03529422	II	33 intermediate risk	Adjuvant radiotherapy with concomitant durvalumab (6 cycles)	18 weeks	(1) 3-year DFS (2) Toxicity, OS, 5-year DFS
ADRISK NCT03480672	II	206 locally advanced intermediate and high-risk	Randomized trial between experimental arm (adjuvant pembrolizumab q3w one year + SOC) vs control arm (SOC)	One year	(1) Event-free survival (2) OS
GORTEC-NIVOPOSTOP NCT03576417	III	680 locally advanced with ENE and/or R1	Randomized trial of post-operative concomitant chemoradiotherapy ± nivolumab (3 doses)	43 days	(1) DFS (2) OS & toxicity
IMVoke010 NCT03452137	III	406 high-risk locally advanced	Randomized trial of adjuvant atezolizumab (16 cycles) vs placebo	16 cycles	(1) Event-free survival (2) OS, QoL

DFS: disease-free survival; ECE: extra nodal extension; HPV: human papillomavirus; NA: not available; OS: overall survival; PEG: percutaneous endoscopic gastrostomy; pTR: pathologic tumor response; QoL: quality of life; R1: microscopically positive resection margin; SOC: standard of care

Anti-PD1/PD(L)-1 mAbs in combination with (chemo)radiation have been investigated in several indications: (i) as a de-escalation strategy in good prognosis patients (e.g., stage I/II p16-positive oropharyngeal cancer), (ii) to replace chemotherapy in cisplatin-unfit patients, and (iii) as treatment intensification in combination with chemoradiation in poor prognosis patients (e.g., p16-negative LA HNSCC and stage 3 p16-positive oropharyngeal cancer) [20–22].

As anti-PD1/PD(L)-1 mAbs are generally well tolerated and have limited toxicities compared to standard chemotherapy, ICI are being investigated as a potential de-escalation strategy to avoid chemotherapy in good prognosis tumors [23]. HN005 (NCT03952585) is a three-arm randomized trial for non-smoking patients with T1-2 N1 or T3 N0-N1 p16-positive tumors that compares standard chemoradiation to reduced dose radiotherapy (60 Gy + nivolumab) versus reduced dose chemoradiation (60 Gy). Another trial (NCT03799445) combines nivolumab and ipilimumab with a reduced dose of radiotherapy for T1 N2, T2 N1-N2 or T3 N0-N2 HNSCC. The CCTG HN.9/EORTC1740, a randomized phase II trial, is currently investigating durvalumab plus radiation (concomitant and adjuvant) versus standard chemoradiation in p16-positive intermediate risk oropharyngeal cancer. All these trials involve highly selected populations with mainly low-risk oropharyngeal p16-positive cancers. A non-exhaustive list of the ongoing trials is described in Table 11.3.

ICI could also be used to replace chemotherapy in patients unfit for cisplatin. Patients unfit for cisplatin are generally defined as patients with either creatinin clearance <60 mL/min or neutrophils <1 500/μL or platelets <100 000/μL or serum albumin <35 g/L or peripheral neuropathy ≥ grade 2 or clinical hearing loss (confirmed by audiogram) or decreased left ventricular ejection fraction. In PembroRad (GORTEC 2015–01) [21], patients with locally advanced HNSCC unfit to receive high-dose cisplatin were randomized between radiotherapy (RT) + cetuximab and RT + pembrolizumab. Pembrolizumab was given only during RT. Loco-regional control (LRC), progression free survival (PFS) and overall survival (OS) were similar between the two groups. However, acute toxicity was lower in the pembrolizumab-RT arm than in the cetuximab-RT arm: 74% versus 92% patients with at least grade ≥3 acute adverse events (p = 0.006), mainly due to dermatitis in the radiation field, mucositis and cutaneous rash. In the REACH trial, patients unfit for cisplatin were randomized between avelumab + cetuximab + RT versus cetuximab + RT. In contrast to PembroRad, avelumab was not only administered concomitantly to RT but also for one year as adjuvant therapy. The primary endpoint was PFS. The avelumab-RT-based treatment did not significantly improve PFS compared to cetuximab-RT: the two-year PFS rates were 44% and 31%, respectively (p = 0.15) [24].

ICI therapy has also been investigated in combination with cisplatin-based chemoradiation in patients with LA HNSCC. As previously stated, LA HNSCC patients have a high recurrence rate [2], and 40–50% will relapse within two years despite multimodal treatment. JAVELIN 100 compared high dose cisplatin chemoradiation combined with avelumab or placebo. Avelumab was started one week before the initiation of chemoradiation, was continued every two weeks during chemoradiation, and then maintained as adjuvant treatment for one year. The trial did not

Table 11.3 Ongoing de-escalation trials with an ICI in HNSCC (non-exhaustive)

Trial	Phase	N population	Design	Primary endpoints
NCT03952585 (HN005)	Phase II/III	N = 711 T1-2, N1 or T3, N0-N1 <10 pack-year	Standard chemoradiation (70 Gy) versus Reduced dose radiotherapy (60 Gy) + nivolumab [6 cycles] versus Reduced dose radiotherapy (60 Gy) + chemotherapy	• PFS • QoL
NCT03799445	Phase II	N = 180 T1 N2, T2 N1-N2, T3 N0-N2	Nivolumab and ipilimumab with reduced-dose radiotherapy	• DLT • CRR • PFS
CCTG HN.9—EORTC1740 NCT03410615	Phase II	N = 180 T1-2 N1 (smokers) T3 N0-N1 (smokers) T1-3 N2 (any smoking status)	Cisplatin + radiotherapy vs Durvalumab + radiotherapy followed by Durvalumab	• EFS
CITHARE (NCT03623646)	Phase II	N = 11 p16-positive oropharynx T1 N1-N2 or T2-T3 N0 to N2 (AJCC 2018)	Standard chemoradiation Versus Radiotherapy + durvalumab	• PFS

PFS: progression free survival; QoL.: quality of life; DLT: dose limiting toxicity; CRR: complete response rate; EFS: event-free survival

meet its primary endpoint: median PFS was 16.9 months in the avelumab arm and not reached in the control arm (p = 0.92). Subgroup analysis showed that PD-L1 expressing tumors might benefit from the addition of avelumab, although this analysis was impaired by the low number of patients. In the REACH study, cisplatin fit patients were randomized between high-dose cisplatin chemoradiation and RT + avelumab + cetuximab. The trial was, however, closed prematurely for futility [24]. KEYNOTE 412 investigated concomitant and adjuvant pembrolizumab with cisplatin-based chemoradiation. After a median of almost 4 years of follow-up, median event-free survival was not reached with pembrolizumab plus chemoradiation and was 46.6 months with chemoradiation alone (hazard ratio [HR] = 0.83; P = .0429). This difference failed to meet the superiority threshold (efficacy boundary was P = .0242). The addition of pembrolizumab to chemoradiation appeared to result in greater event-free survival benefit compared with chemoradiation alone in PD-L1–positive patients, according to a post hoc analysis [25]. Other phase II and III studies are still ongoing in LA HNSCC and are described in Table 11.4. In particular, IMVoke010 is studying atezolizumab in the adjuvant setting only after concurrent chemoradiation and are described in Table 11.4.

Conclusion

Immunotherapy has the potential to improve the efficacy of treatment in patients with LA HNSCC. Although we await the outcome of several studies, the first reported trials have been discouraging.

Innovative approaches are needed to investigate the best way(s) to integrate ICI with multimodal curative treatment. For example, strategies such as better patient selection (PD-L1 expressing tumors), ICI after chemoradiation (similar to the PACIFIC trial in lung—IMVoke 100), or ICI in the neoadjuvant setting before surgery (KEYNOTE 689) are worthy of exploration. Another hypothesis to explain the non-benefit of anti-PD/PD(L)-1 mAbs in combination with (chemo)radiation is the large field of irradiation to regional lymph nodes that might neutralise immune competent cells. To circumvent that possibility, the REWRITE trial (NCT03726775) is investigating the activity of durvalumab in combination with less extensive nodal radiation therapy (irradiation of adjacent lymph nodes only). The primary endpoint of this trial is the rate of relapse in non-irradiated regional lymph nodes in a highly selected population (T1-T4 with clinical status N0-N1 or N2a-N2b non-palpable). Hopefully, the ongoing investigations and those of the future will guide how these agents can be best used in the curative setting.

Table 11.4 Other ongoing trials with an ICI in HNSCC (non-exhaustive)

Trial	Phase	N population	Design	Primary endpoints
DUCRO-HN (NCT03051906)	Phase I/II	N = 69 LA HPV-negative HNSCC	One arm: IMRT + cetuximab + durvalumab (6 months)	• PFS
NCT04831450	Phase II	N = 44 LA HPV-negative HNSCC	One arm: maintenance cemiplimab-rwlc after concurrent chemoradiation	• PFS
DEPEND trial (NCT03944915)	Phase II N = 36	N = 36 LA, non-metastatic, HPV-negative head and neck squamous cell carcinoma	Standard chemoradiation (70 Gy) *versus* Induction with carboplatin + paclitaxel + nivolumab followed by response-stratified therapy	• DRR

(continued)

Table 11.4 (continued)

Trial	Phase	N population	Design	Primary endpoints
IMVoke010 (NCT03452137)	Phase III	N = 400 LA HNSCC	Post definitive therapy + atezolizumab *versus* Post definitive therapy + placebo in adjuvant setting only	• EFS
TrilynX (NCT04459715)	Phase III	N = 700 LA HNSCC	Xevinapant concomitant and adjuvant to (chemo)radiation *versus* Placebo and (chemo)radiation	• EFS

LA: Locally advanced; HPV: Human Papilloma virus; HNSCC: head and neck squamous cell carcinoma; IMRT: Intensity modulated radiation therapy; PFS: progression free survival; DRR: deep response rate; EFS: event-free survival

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Chapter 12
Carcinoma of Unknown Primary: Diagnostics and the Potential of Transoral Surgery

Stijn van Weert, Jan-Jaap Hendrickx, and C. René Leemans

Introduction

Head and neck squamous cell carcinomas (HNSCC) of unknown primary site (CUP) have continued to intrigue head and neck surgeons. Historically, 1.5 to 9% of head and neck cancers were considered CUP [1–3]. The incidence of human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma (OPSCC) has dramatically increased during the past decades. This specific group of patients is relatively young and without classical risk behaviour. HPV driven OPSCC is recognized as often presenting with tumour burden in the neck accompanied by small and occult primary tumours. This has led to an increase in CUP [4–7]. Since identification of the primary tumour has implications for treatment and prognosis, the interest in improvement of diagnostics and surgery is growing. The eighth edition of both the UICC as well as the AJCC staging manuals have incorporated the specific entity of HPV-related HNSCC with its own clinical and pathological N classification, in which a HPV-positive CUP should be classified as HPV-positive OPSCC [8, 9].

The incentives for identifying the index tumour are clear. Firstly, it is important for patients and treating physicians to have a clear view and understanding of their disease. Secondly, a true treatment target can be identified which in turn may lead to possibilities for de-intensification of treatment by minimizing toxicity in terms of xerostomia, dysphagia and also atherosclerosis of the carotid arteries. This is specifically of importance in this increasing group of young patients suffering from HPV-positive OPSCC.

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History of Diagnostics in CUP

Over the previous decades, there was limited consensus in the literature with regard to diagnostic algorithms for CUP. Nevertheless, history has shown similarities and evolution in diagnostics in CUP [10–12]. Once an enlarged cervical lymph node has been identified as a metastasis of squamous cell carcinoma (SCC) by means of ultrasound guided fine needle aspiration (FNA) or core needle biopsy (CNB), a thorough history and physical examination combined with office-based endoscopy is a key step in initial diagnostics. CNB should be considered a reliable alternative in case of non-diagnostic FNA. The introduction of new CNB techniques may permit for more tissue yield in one needle pass and may further reduce the risk of seeding [13, 14]. When no abnormalities are found, a diagnostic contrast enhanced computer tomography (CT) or magnetic resonance imaging (MRI) of the head and neck is performed, often combined with positron emission tomography (PET). CT and MRI are not only useful in identifying the possible index tumour but can also delineate non-palpable nodes (e.g., retropharyngeal) thus pointing in a certain direction in search for the primary tumour [14, 15]. Endoscopy under general anaesthesia (EUA) with appropriate biopsies and palatine tonsillectomy (PTE) is consecutively performed. Historically, despite this proper work up, the identification rate has not exceeded 50% [16–18].

Currently, it is general practice to at least perform p16 immunohistochemistry (IHC) after FNA or CNB in case of proven cervical metastasis of SCC. When feasible, true HPV-positive disease needs to be confirmed by polymerase chain reaction (PCR) or in situ hybridization (ISH) testing to isolate HPV DNA. The surrogate marker p16 has led to false positive results leading to misjudgment of the potential primary tumour site. Especially in cytology specimens current literature advises to perform direct HPV testing [19–23]. Epstein Barr virus (EBV) testing is optional in case of suspicion of (mostly non-keratinizing) nasopharyngeal carcinoma (NPC) [24, 25]. Non-targeted biopsies are currently considered obsolete due to their low yield [26]. PTE is considered superior to biopsy of a clinically non-suspicious tonsil.

Recent and Current Improvements

With regard to morphologic and functional imaging, specifically MRI has improved regarding resolution as well as with optimization of diffusion weighted (DW) MRI. Adding DW-MRI to 18F−fluorodeoxyglucose (FDG) PET-CT does not seem to improve the sensitivity and specificity compared to PET-CT alone in detecting CUP. DW-MRI might be an alternative to PET-CT in detection of CUP in case PET-CT is unavailable. The costs of DW-MRI are lower. PET-CT on the other hand has the advantage of being a whole-body examination with synchronous screening for distant metastasis [27]. Besides PET-CT, PET-MRI seems to be a meaningful adjunct [28]. The usefulness of PET either combined with CT or MRI for identification of occult

tumours in the oropharynx is somewhat limited due to the physiological uptake in the mucosa-associated lymphoid tissue at this site. The Achilles heel of PET-CT remains the relatively low specificity [27, 29]. Nevertheless, reported identification rates of occult tumours with 18F- FDG PET-CT vary from 24.5 to 40.5% [30–34]. In a recent study by Stadler et al. PET-CT/MRI was superior in staging of the neck compared to ultrasonography. Discordance varied between 20 and 65% with the majority of cases being upstaged after PET-CT/MRI [35].

Image guided surgery (IGS) has become widely adopted in general. Narrow band imaging (NBI) has proven its benefit in head and neck cancer management and in CUP specifically. It can be used in office-based endoscopy. Its concept is based on the use of blue and green light with different wavelengths, optimizing visualization of changes in mucosal microvascular patterns suggestive for dysplasia and malignancy [36]. There have been reports on a 35% added identification percentage after a negative PET-CT and MRI [37]. Others have described high negative predictive values and high sensitivity [38, 39]. The use of high definition (HD) and ultra HD cameras with 3D and 4K technology has led to superior visualization of surface mucosa. Performing endoscopy assisted office-based biopsies is becoming more and more routinely applied due to this optimization of visualization. However, when no target for biopsy is found, EUA is evidently superior due to easier access and the possibility for meticulous palpation of the mucosa [14].

Algorithms for CUP over the past 5 years have shown increasing uniformity with a prominent role for tongue base mucosectomy (TBM), also called lingual tonsillectomy (LT) in HPV-positive CUP [1, 40–42]. Until recently, the role of NBI had not been specifically described in guidelines for CUP. The guideline by the American Society of Clinical Oncology (ASCO) was actually the first to recommend and incorporate NBI in the guideline for CUP in 2020 [40].

The main reason for debate remains what to do in case of a true CUP after full work up. Should treatment of the neck suffice either by neck dissection (ND) or RT (in case of single node without extranodal extension; ENE) or by chemoradiation for multiple nodes or in case of ENE? Should the oropharynx be irradiated in case of HPV-positive disease and to what extent?

Transoral Surgery—The Role of TLM and TORS

To improve the identification ratio in CUP, TBM has gained popularity in the diagnostic work up. Different guidelines generally advise to perform this in case of negative work up and negative PTE for (HPV) positive nodes in levels I, II, III and upper V [40–42]. Farooq et al. have reported on an identification ratio by TBM of the index tumour of 78% in case of initial negative radiology, EUA and PTE [43].

This can either be done by transoral laser microsurgery (TLM) or by means of transoral robotic surgery (TORS). The use of simple tonsillectomy instruments in performing TBM has also been described by Davies-Husband [44]. The limited line of sight and oropharyngeal accessibility of TLM makes it less useful in a subset

of patients. TORS allows for en bloc resections and a theoretically better margin assessment than TLM [45, 46]. The availability of TLM in most head and neck referral centers however allows for easy access to this method. In a recent study in patients with demonstrated oropharyngeal cancer by Parimbelli et al. the cost-effectiveness of TLM vs. TORS was analyzed. Their results suggest lower costs for TLM with the footnote that this depends on the number of re-interventions and the need for adjuvant treatment [47]. The most advocated method to perform TBM is through TORS, executed with the da Vinci surgical system by Intuitive Surgical© (Sunnyvale, California; US). A surgical robotic system is becoming more readily available throughout centers since other surgical specialties have increasing indications for robotic surgery such as urological surgery and gastro- intestinal surgery. Due to the use of the robotic arms and angled high-definition endoscopes as well as wristed instruments, the oropharynx is readily accessible in the majority of patients. Currently, the da Vinci Si and Xi surgical systems are the most widely used. The single port (SP) system might be beneficial in TORS. The first reports on its use in TORS are promising in terms of safety and outcome compared to the conventional multi- port systems used. Specifically for transoral surgery of the distal Upper Aero-Digestive Tract (UADT), the SP system seems superior in terms of accessibility [48–50].

TBM has earned its place in different international guidelines regarding CUP such as those from ASCO as well as the National Comprehensive Cancer Network (NCCN) [38, 40]. Several European authors have suggested modified CUP algorithms, which are comparable with overall minor differences between them [1, 51].

Where Does TBM Come In?

Although there is sufficient proof for the added value of TBM, the question remains as to when and how to perform TBM [16, 52, 53]. It is generally agreed that TBM comes into play in case of a negative office- based endoscopy (+NBI when available), a negative PET-CT (and MRI) and subsequent negative EUA and PTE. Several previous systematic reviews have shown that the pick- up rate of occult tumours is relatively high in the tongue base as compared to the palatine tonsils (Table 12.1) [16, 43, 54, 55]. The pooled identification rate of the index tumour is 72% (range 25–100%) as reported in the review by van Weert et al. (Table 12.2) [16, 52, 53, 56–65]. There is no consensus as to whether to perform staged (second procedure after negative EUA and PTE) TBM or not. Those in favor of a staged procedure would argue that patients have less pain and less feeding tube dependence and can avoid TBM in case of a positive PTE. Another reason might be a swifter recovery as not to further postpone possible radiotherapy (RT). Post-surgical pharyngeal stenoses have been described after a simultaneous procedure. A staged procedure seems cost- effective as reported by Byrd et al. [53].

Although positive TBM procedures have been described in HPV-negative disease, the wide majority are HPV-positive [16, 66]. This is due to the high prevalence of

Table 12.1 Occult tumour identification rates for TORS/ TLM. Note the relatively high high pick- up rate in the base of tongue. TORS; transoral robotic surgery, TLM; transoral laser microsurgery, TBM; tongue base mucosectomy, PP; pooled proportion

Author	Studies	Ident rate TORS/TLM	Ident rate TLM	Ident rate TORS%	Ident rate in PTE%	Ident rate by TBM%
Farooq et al. [43]	N=21 (556 cases)	78% (57 – 89%)	91% (PP)	74(25 – 100)	31(10 – 76.5)	53(28.5 – 100)
Mecciariello et al. [54]	N=12 (349 cases)			70.8(53.1 – 90)	28.7(13.5 – 70.8)	64(29 – 100)
Van Weert et al. [16]	N=12 (274 cases)			72(17 – 90)	28(14 – 100)	52(24 – 100)
Al-Lami et al. [55]	N=30 (777 cases)		80% (28.6 – 94%)	60(0 – 94)	32(10 – 76.5)	45(0 – 100)

Table 12.2 Identification rate for occult tumours with TORS. Range 25−100%

Author (year)		Proportion identified with TORS%
Abuzeid et al. [56]	Michigan, US	1/1 (100)
Blanco et al. [57]	Baltimore, US	1/4 (25)
Mehta et al. [58]	Pittsburgh, US	9/10 (90)
Patel et al. [59]	Seattle, US	34/47 (72)
Durmus et al. [60]	Columbus Ohio, US	17/22 (77)
Byrd et al. [53]	Pittsburgh, US, Toronto, Canada	19/22 (86)
Channir et al. [61]	Denmark	7/13 (54)
Geltzeiler et al. [52]	Pittsburgh, US	37/50 (74)
Krishnan et al. [62]	Adelaide, Australia	5/7 (71)
Hatten et al. [63]	Philadelphia, US	48/60 (80)
Winter et al. [64]	UK	17/32 (53)
Al-Mulki et al. [65]	Atlanta, US	23/29 (79)

CUP in the HPV- positive group as well as the strong correlation between HPV and OPSCC. There is no strict consensus regarding the usefulness of TBM in the HPV-negative population although recent literature supports the omission of TBM in HPV-negative disease [67].

How to Perform TBM?

The technique of TBM has been previously described [58, 61, 64]. Starting off with a midline incision from the level of the circumvallate papillae towards the vallecula, the incision is then carried through laterally at the level of the anterior base of tongue (BOT). Once the glossopharyngeal sulcus is reached, the incision is extended towards the vallecula. The tongue musculature marks the depth limit as to only remove the lymphoid tissue. After visualization of the pharyngoepiglottic folds, the first half of the BOT is removed (Fig. 12.1). There are modifications in this technique where for

example some authors do not use the midline incision. The general concept however remains the same.
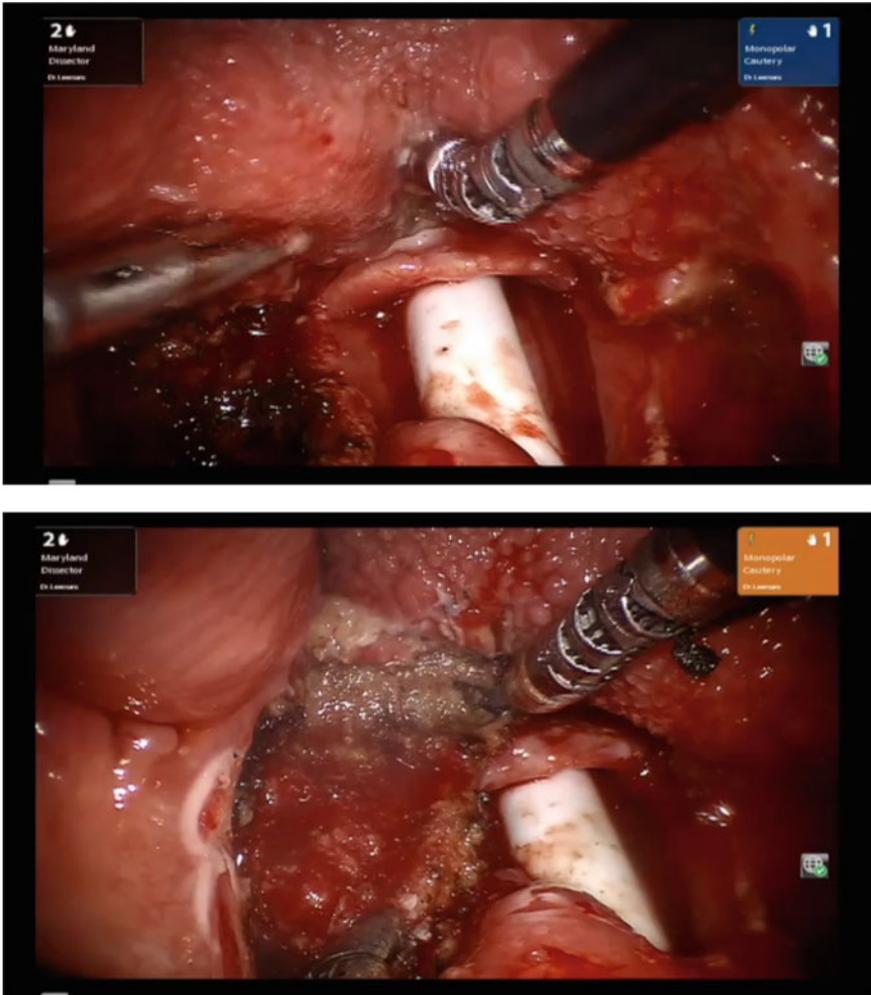
Fig. 12.1 Intraoperative images of a left-sided tongue base mucosectomy (TBM) performed with the da Vinci Si system. The first image shows the initial midline incision with the monopolar cautery. In the second image, the specimen is retracted backward with the Maryland dissector for the final incision in the vallecula
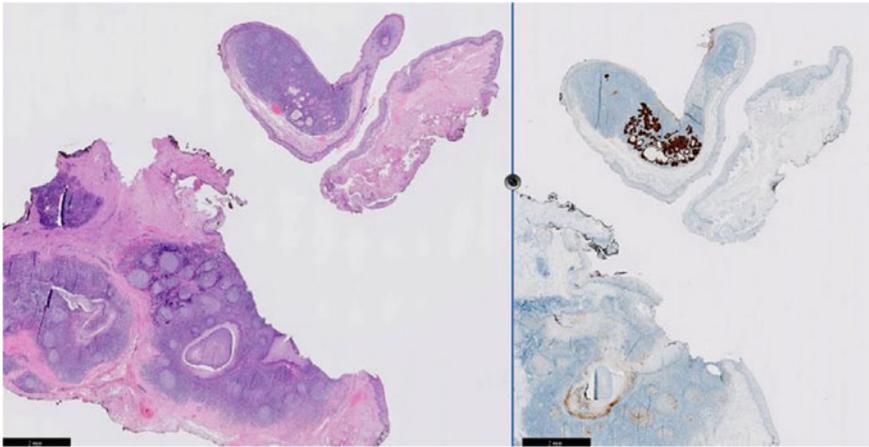
Fig. 12.2 Small (2.2 mm) partly cystic squamous cell carcinoma detected in the crypts of the lymphoid tissue in the base of tongue in a patient with multiple unilateral metastases in the neck. (left hematoxylin and eosin, right p16 immunostaining) [16]

How to Analyze the Resection Specimen?

After the surgeon has adequately orientated the specimen (preferably with needles on cork with the mucosa facing the cork in order to prevent desiccation [64]), it is sent to the pathology laboratory with a proper description of the clinical situation. In theory, ideally the pathologist would perform step serial sectioning (SSS). This entails a section every 0.5 mm through the entire specimen and is known from other head and neck procedures such as the sentinel lymph node biopsy. This is a very time-consuming procedure, which should harbor clear benefit in comparison to conventional sectioning. Recent preliminary data from the Royal Marsden in London through the clinical trial "Evaluation of Tongue Base MucOsectomy & Step sErial Sectioning (MOSES)" has shown that the actual added value of SSS seems relatively small. These data were presented at the ICHNO-ECHNO meeting in March 2022 in Brussels [68]. This poses the question whether the ends justify the means with regard to SSS in TORS for CUP. IHC for p16 on the specimen however may play an important role in identifying occult tumours of the tongue base (Fig. 12.2) [16].

Extent of Resection

Historically, there have been reports on occult tumours being found in the contralateral oropharynx with rates of up to 10% [69]. Geltzeiler et al. reported on an 80 versus

68% identification rate for bilateral vs. unilateral TBM, respectively [52]. Nevertheless, there has never been a clear consensus on this topic within the previous guidelines; these do not clearly state as to whether to perform uni-or bilateral procedures in which scenario. The ASCO guideline from 2020 has made several recommendations on this topic [40].

The wide majority of patients with CUP present with unilateral neck nodes. This of course implies a high chance of an ipsilateral oropharyngeal occult primary, specifically in case of level II nodes, which are most prevalent in CUP. The ASCO recommendation is to perform unilateral PTE in case there is no clinical suspicion of contralateral disease. In case a frozen section is negative, the surgeon may proceed to perform an ipsilateral TBM. In the event a frozen section is not done, the procedure should be performed in a staged fashion. Moreover, frozen sections are notably less reliable than definitive histopathology and require extra surgical time. In case a contralateral PTE is performed and a small focus of SCC is found, it should be assumed that this tumor is metachronous to the yet undiscovered ipsilateral primary actually causing the nodal tumour burden [69]. Paleri has recently argued to use the term "MALTectomy" (MALT: Mucosa Associated Lymphoid Tissue) -which means both PTE and TBM- and to perform this procedure unilaterally in case of a single HPV-positive neck node without signs of ENE. The identification rate in unilateral MALTectomy in the series reported by Paleri was as high as 85%. Specifically, identification of occult disease in the contralateral palatine tonsil was seldom seen. These findings are supported by the review of Farooq, in which 97% of the primary tumors were found ipsilaterally in 556 cases. The primary tumour was found contralaterally in 2% of cases and synchronous bilateral tumours were found in 1% [43].

The scenario of bilateral neck disease in CUP is far less prevalent and presumably caused by a BOT tumour (levels II-III) being considered a "midline structure". According to this rationale, it is recommended to perform ipsilateral TBM on the side of the largest nodal burden. This can then be extended to a bilateral TBM in case the initial hemi- TBM fails to identify the tumour. There is no true incentive to perform PTE in bilateral neck nodes given the rarity of bilateral neck metastases in SCC of the palatine tonsils [58, 70].

The Case of True CUP

A "true" case of CUP would be best defined as an ultimately unidentified primary tumour after full diagnostic work up including removal of mucosa associated lymphoid tissue. Several theories have been proposed addressing the issue of the non- appearing index tumour even after long term follow up following ND only without radiation therapy. One possibility could be that these tumours regress spontaneously, possibly due to immunological factors, as is known for other tumour types [71, 72]. Another scenario would be the speculation by Califano et al. which was also included in the review by Civantos et al.: in 56% of cases with random biopsies from possible primary tumour sites they found genetic alterations identical to those in the

neck nodes [14, 73]. These were described by Civantos as genetically malignant but phenotypically benign. In this case, these genetic alterations would be able to cause metastatic disease to the neck without inducing macroscopic disease at the primary site [14]. This intriguing issue will remain a topic of discussion in the years to come.

Is TBM Really Indispensable?

Identification of a primary lesion leads to appropriate staging and understanding of the disease. This is of importance for both the patient and the treating physician. Striving for single modality treatment whenever possible is important. In case the occult primary has not only been found but also adequately excised, the oropharynx can be left out of the field of radiation. This in turn will have a beneficial effect on costs, specifically in case the neck needs no radiation after ND. In case of an insufficient margin, re-excision should be contemplated. This will often be an option given the small volume of these occult tumours. Even if this would not be possible, the field of radiation may be minimized due to the successful localization of the primary tumour.

Previous studies have reported on superior outcome in case of discovering the primary tumour in the pre- HPV era [74, 75]. Karni et al. confirmed this finding with their report on TLM in CUP with a high identification rate in the oropharynx of up to 95%. HPV status was not analyzed in this study [76].

In 2014, Davis et al. reported on a significantly higher identification rate in case of HPV-positive disease which in turn was positively correlated with overall- and disease specific survival (Figs. 12.3 and 12.4). Of course, one can never be sure about the contributing factor of HPV-positivity on these outcomes regardless of identification of the occult tumour. Furthermore, this study also emphasizes that TBM in HPV-negative disease is debatable [66].

Definitive Treatment

In case the primary tumour is identified after thorough work up, the tumour should be staged and treated accordingly. The primary objective should be to avoid using three treatment modalities when possible to reduce treatment related long-term toxicity. Specifically in the current era of increasing incidence of HPV-associated OPSCC, there is a clear incentive for de-intensification of treatment in this mainly young patient population with their whole lives ahead of them. Early stage OPSCCs are treated either by primary radiation or by primary surgery.

The ideal indication for surgery alone should be a clinically single unilateral node without signs of ENE and a potentially resectable tumour that allows for proper margins, however these indications where the neck is concerned might be extended in the future. What a proper margin is in TORS for OPSCC has not been clearly defined.
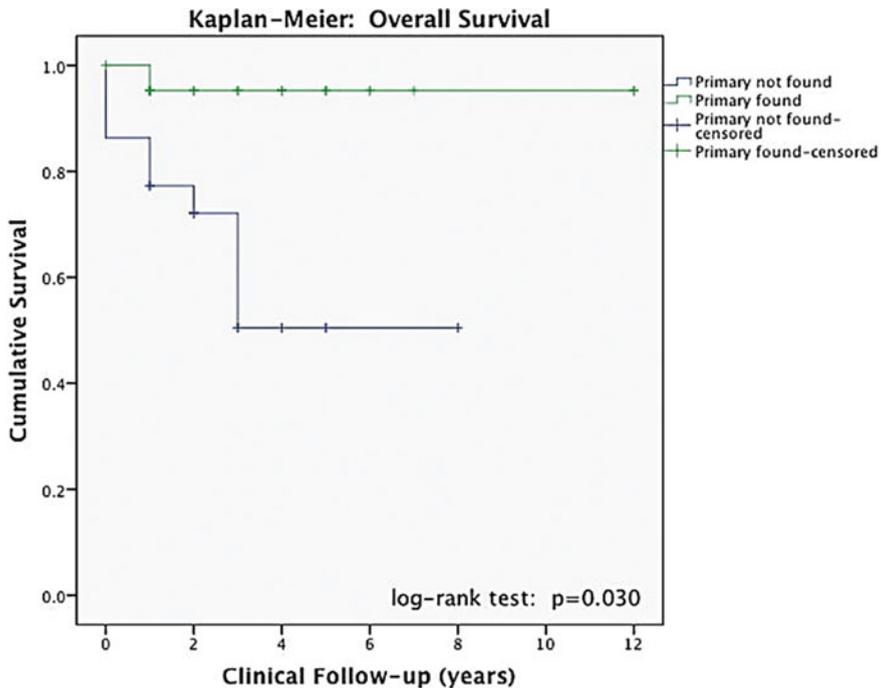
Fig. 12.3 Overall survival relative to identified and non- identified primary tumours [66]. Reprinted with permission ©

There have been recent reports supporting the theory that margins less than 1 mm (herein defined as close) might justify active surveillance at least in HPV-positive OPSCC. In this report by Holcomb et al., there was no significant correlation with close vs. clear margins (defined as >1 mm) relative to local control, disease-free and overall survival. Outcome did not improve with margins of 2 to 3 mm [77].

In case no tumour has been found in the CUP work up, ND alone might suffice with meticulous periodic office-based endoscopy surveillance. One should bear in mind that the eventual pathology report might up-stage the pN classification in up to 30% of cases when identifying additional positive nodes or ENE, which would necessitate postoperative (chemo)RT to the neck [17, 78]. It is important to consider this and discuss it with the patient in advance. A recent report by Grewal et al. confirmed the safe possibility of pharyngeal sparing RT (PSRT) as opposed to pharyngeal RT (PRT) in case of a pT0 TORS work up. PSRT is defined as RT to the neck at risk (with or without upfront ND) with omission of RT to the pharynx. PSRT did not compromise outcome and significantly reduced pharyngeal toxicity [79]. Other reports have described comparable outcomes in terms of locoregional control and survival in case of unilateral RT to the neck without irradiating the pharyngeal axis as opposed to the classical bilateral neck irradiation including the pharyngeal mucosa
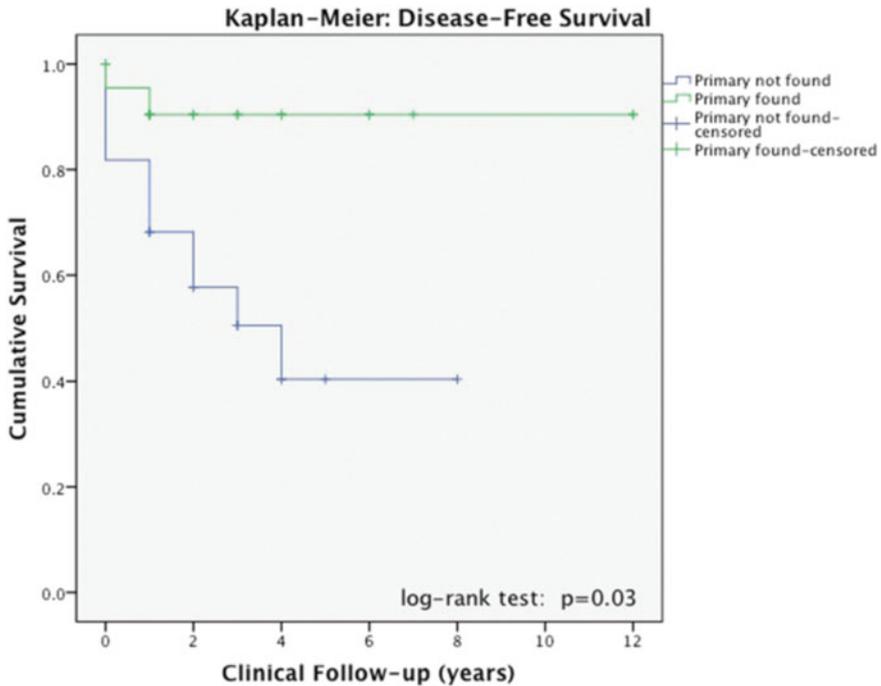
Fig. 12.4 Disease- free survival relative to identified and non- identified primary tumours [66]. Reprinted with permission ©

[80, 81]. Tiong et al. reported on good results on unilateral treatment for HPV-positive OPSCCs specifically, with no contralateral neck failure or primary emergence [82].

Since the entity of CUP within the spectrum of head and neck cancer remains relatively rare, most studies do not encompass large cohorts and most of the knowledge currently shared is based on systematic reviews, which have been published in quite a large amount over the last years. These reveal that treatment paradigms still differ internationally and even nationally [16, 40].

With the input of current literature on CUP, different scenarios can be suggested based on the outcome of TBM, HPV status, N-status and the presence or absence of ENE after negative EUA and PTE. A proposed algorithm based on current insights is shown in Fig. 12.5.

Conclusion

Although CUP in head and neck cancer is in general rare, the rising incidence of HPV-positive OPSCC has drawn new attention to the topic. Evolutions in radiology and endoscopy as well as the use of transoral surgical techniques have evidently
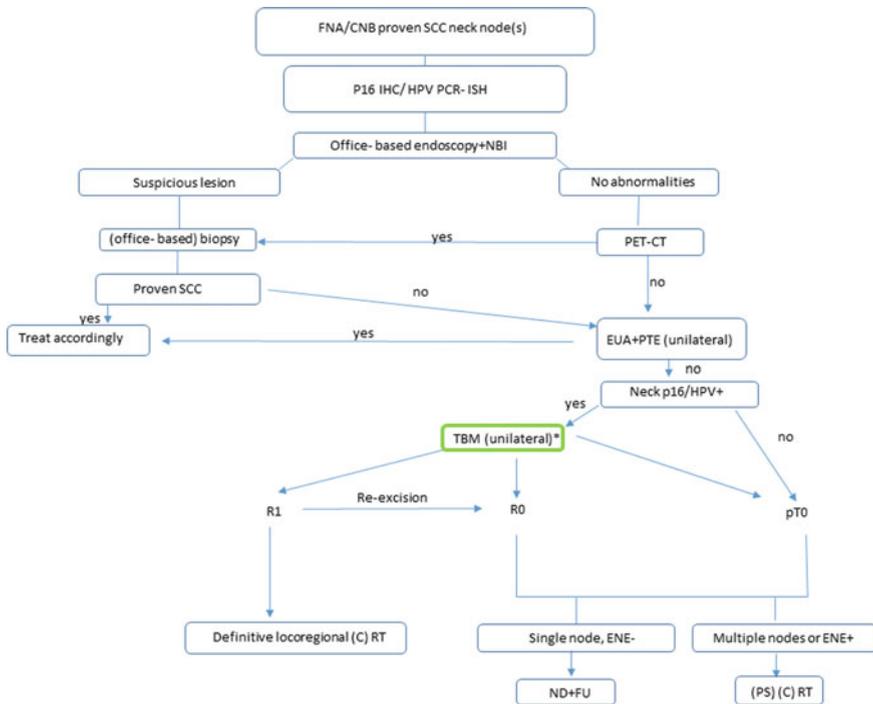
Fig. 12.5 Proposed algorithm for CUP and the role of TBM (tongue base mucosectomy). FNA; fine needle aspiration, CNB; core needle biopsy, IHC; immunohistochemistry, PCR; polymerase chain reaction, ISH; in situ hybridization, NBI; narrow band imaging, EUA; endoscopy under general anaesthesia, PTE; palatine tonsillectomy, CRT; chemoradiation, ENE; extranodal extension, ND; neck dissection, FU; follow up, PSRT; pharyngeal sparing radiotherapy. *; unilateral TBM suggested to be initially performed in selected cases (see ASCO guideline) [40]

contributed to a higher identification rate of these occult tumours. The historical discovery rate in the "naked eye" era with EUA and PTE with or without blind biopsies was approximately 50% at best and has now increased to approximately 80%. TBM has been adopted in every current guideline or algorithm, mainly in HPV-positive neck nodes in levels II, III and Va [1, 40, 83]. TBM has not only proven to be useful in discovering occult tumours but may also achieve an adequate resection of the index tumour, thus making PSRT possible. In the optimal case of a unilateral single neck node without ENE, TBM combined with ND can suffice as a single modality treatment; in case the primary tumour is excised or remains undetected (pT0), a periodic office based endoscopy of the pharynx seems a reasonable policy. This is even more reliable due to the HD features and possibilities of using NBI. Discovery of the primary lesion improves survival and allows for targeted and de-intensification of treatment. The use of TBM (and PTE) in HPV-negative CUP is questionable since the detection rate in this category has proven to be very low [16, 66, 84]. Treatment strategies still differ among centers with regard to for example

upfront ND or primary RT. The question is whether this is a true problem, as long as outcome in terms of survival and quality of life are comparable. Questions that have been raised recently mainly concern the extent of TBM and the necessity of performing bilateral PTE. Based on the data currently at hand, there seems to be an incentive for initial unilateral MALTectomy. Further research is mandatory to confirm this.

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Chapter 13
Systemic Treatment Sequencing and Prediction of First-line Therapy Outcomes in Recurrent or Metastatic Head and Neck Cancer

Petr Szturz and Jan B. Vermorken

Introduction

In squamous cell carcinoma of the head and neck (SCCHN), therapeutic decision making depends on several tumour-, patient-, and institution-related factors, the former being defined by a well-known categorization into localized (also known as early), locally (or locoregionally) advanced, and metastatic disease. According to the US Surveillance, Epidemiology, and End Results (SEER) Program data, about one third of newly diagnosed patients present with a localized tumour, almost half of them with locally advanced disease, and up to 20% may have distant dissemination. While cure rates of early disease surpass 80%, they almost halve in locally advanced SCCHN due to high rates of recurrences manifesting in about 60% of cases despite combined modality treatment instead of single-modality surgery or radiotherapy used in early disease [1, 2]. In oropharyngeal cancer, the decline in prognosis has been shown to be neutralized by human papillomavirus (HPV)-positivity [3]. On the other hand, even in developed countries, this favourable, viral-related subgroup represents only a minority of SCCHN [4]. Long-term survivorship is further halved in patients with distant metastases notwithstanding the introduction of targeted systemic agents and immunotherapy [5, 6]. The latter prognostic group consists of recurrent tumours as well, except for those that are salvaged with surgery or radiotherapy, particularly in the case of early larynx relapses and limited metastatic recurrences (oligorecurrences) of HPV-positive oropharyngeal cancer in the lungs [7, 8]. Relapses affect about 10-15% of patients with early disease, but they are up to 4 times more frequent in

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locally advanced disease [9–12]. Depending on primary tumour site, HPV-positivity in oropharyngeal cancer, primary treatment, and intensity of follow-up, the ratio between early, locoregional, and distant recurrences is roughly comparable with possibly a slight predominance of locoregional relapses [2, 13, 14]. According to disease stage at relapse, salvage surgery and/or (chemo)radiotherapy usually offered to the majority of patients without distant metastases yields five-year survival rates between 30 and 40% on average with even better outcomes after surgical resection [7, 14]. Importantly, these snapshot clinical scenarios need to be put in the context of gradual cancer progression towards more advanced stages, occurring at different rates in different individuals and both in the primary and recurrent disease settings. The respective treatment outcomes are summarized in Fig. 13.1.
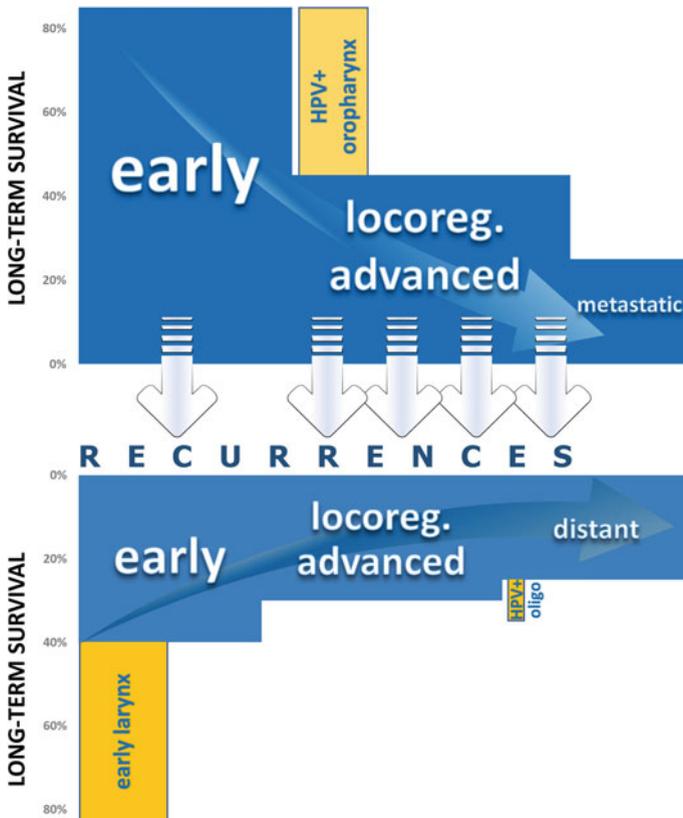
Fig. 13.1 In mucosal head and neck squamous cell carcinoma, survival outcomes differ according to clinical presentation. Disease progression is a continuous process, and recurrences are common. Apart from several exceptions, poorer prognosis correlates with more advanced stages both in the primary and recurrent settings. Long-term survival in local and regional stages corresponds to a period of at least 5 years, while in the metastatic setting we rely on the results of the Keynote-048 trial, as reported in 2019, with a median follow-up of 13 months in the immunochemotherapy arm and expect that mature data will probably show inferior outcomes as was the case of CheckMate-141

Similarly entangled are therapeutic strategies defining antitumoral management of each of these disease categories. In this chapter, we will put the first-line palliative treatment in broader context and try to decipher this entanglement. Figure 13.2 illustrates the development of anticancer modalities in two temporal axes. The horizontal axe signifies historical evolution covering the modern era since 1970s, when the current concept of multimodality approach was grounded. Being one of the typical examples of a patient's journey through the diagnosis and treatment of SCCHN as indicated above, the vertical axe demonstrates treatment sequencing models in a patient with initially locally advanced disease that later recurs and requires systemic therapy. Until the beginning of the 20th century, management of head and neck cancer was governed by surgery, albeit with generally very poor results and cure rates as low as 5%. Afterwards, radiotherapy began to develop, first independently and even replacing surgery as the mainstay between the two world wars but then gradually complementing resection and laying thus the foundation for treatment sequencing [15]. In the 1970s, adjuvant curative radiotherapy and low-dose methotrexate became the standard of care options in the primary and recurrent disease setting, respectively [16, 17]. At present, following 50 years of evolving multimodality management, median overall survival of these patients has significantly improved with a major impact of concurrent curative chemoradiation and advances in palliative therapy. The choice of the first-line systemic approach has thus a profound influence on patient outcomes, and we will discuss the role of patient- and disease-related factors in the context of the cancer care continuum.

Defining the First Line

First-Line Setting: Where it Begins and Ends

Candidates for first-line palliative treatment can be divided into two groups. The smaller one consists of those presenting with newly diagnosed SCCHN ineligible for locoregional treatment due to synchronous metastases, and the larger one of those with disease relapse after one or more previous locoregional interventions and with no further possibility of such therapy [18]. Recurrent SCCHN is not a homogenous entity but differs according to previous anticancer treatment and site of recurrence with important implications for the choice of first-line systemic regimens. Previous therapeutic attempts can be locoregional only, such as surgery and radiotherapy for early disease and surgery followed by radiotherapy for locally advanced disease, but can also involve systemic drugs. Since the first-generation of larynx preservation trials, platinum agents (cisplatin and carboplatin) are the most commonly used drugs in the locally advanced setting. They have become the cornerstone of induction chemotherapy and concurrent chemoradiotherapy [2, 19].

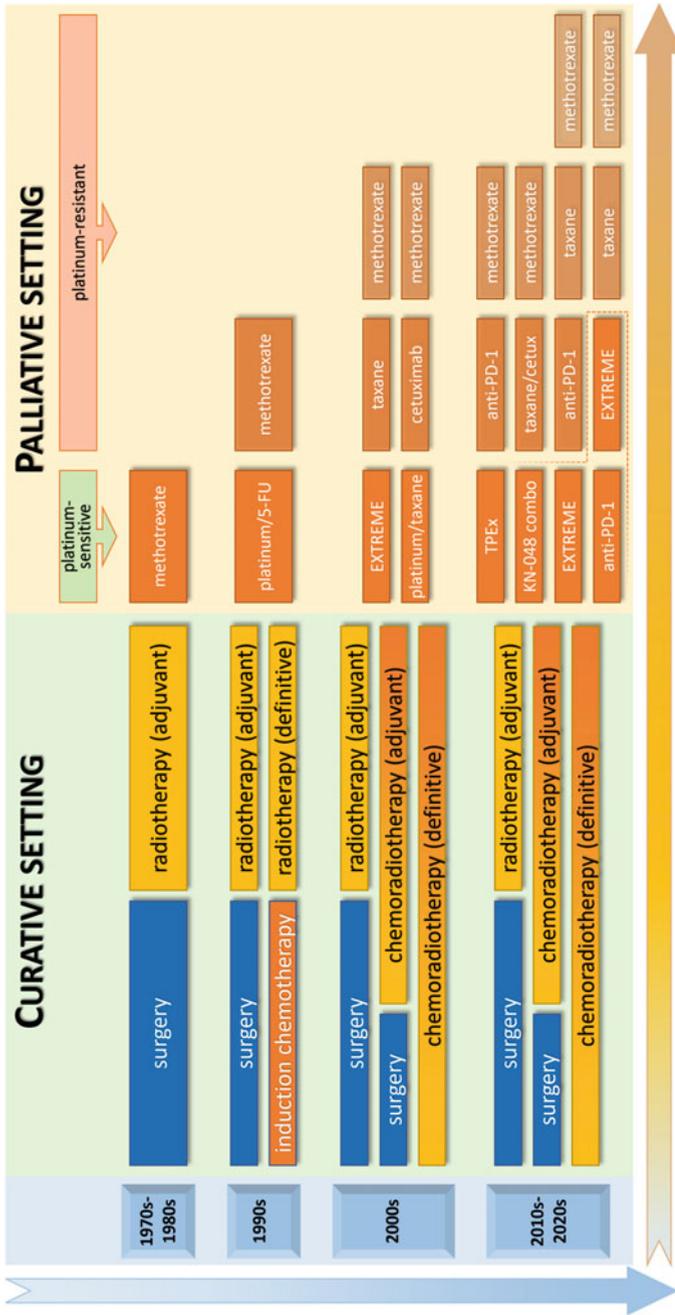**Fig. 13.2** Evolution of treatment sequencing in mucosal head and neck squamous cell carcinoma. In any phase of the disease course, participation in well-designed clinical trials is recommended. Abbreviations: 5-FU, 5-fluorouracil; EXTREME, platinum/5-fluorouracil/cetuximab; TPEx, cisplatin/docetaxel/cetuximab; KN-048 combo, platinum/5-fluorouracil/pembrolizumab; anti-PD-1, anti-programmed cell death-1 (nivolumab or pembrolizumab)

However, owing to cumulative toxicity of cisplatin, prior exposure can be considered a relative contraindication for its retreatment (i.e., after previous use in the primary setting) or rechallenge (i.e., after previous use in the recurrent and/or metastatic setting), especially if the total administered dose exceeds 300 mg/m^2 with up to 200 mg/m^2 being considered relatively safe [20–22]. Albeit less toxic, the use of carboplatin comes with lower efficacy [23]. Moreover, a short platinum-free interval portends poor prognosis. Time to progression or relapse of less than 6 months after termination of previous platinum-based regimen has been adopted in clinical trials and routine practice to identify cases resistant to platinum retreatment. Although longer periods of disease control are a prerequisite for the term "platinum-sensitivity", they do no guarantee a therapeutic success. Historically, one of the reasons for this categorization was the urgent need to allow a rapid access to reasonably effective drugs, which were very limited, to as many patients as possible. Nevertheless, this population is quite heterogenous comprising also patients who progress during platinum treatment, those who have a probably persisting locoregional disease after primary treatment that visibly progresses only 6 months later, and those who maintain remission of the primary tumour but present with new distant metastases. Here, we remind that in accordance with disease kinetics and tumour doubling time being usually in the order of several months, such new metastases must have already been subclinically present during the primary treatment, and contrary to induction chemotherapy, concomitant potentiation of radiotherapy by platinum agents does not diminish distant failure [2, 24]. Thus, there are differences in terms of the type of previous administration but also dose.

Unfortunately, the topic of platinum-resistance is still far from being fully understood, and for example addressing local and systemic platinum-resistance, if there is such a distinction, merits special attention. In addition, determining the actual disease-free interval may be challenging. Primary response assessment after chemoradiotherapy is recommended at 3 months with no further imaging being required in the majority of patients in case of complete remission. Thus, it is not that uncommon that imaging at 6 months is performed if suspicious findings are detected already at 3 months. Subsequently, if a recurrence is confirmed at 6 months, its attribution to the designation "platinum-sensitive" may be problematic because its inception was earlier than thought. This type of diagnostic pitfall and the fact that the armamentarium of systemic therapy has broadened during the past 15 years advocate the pertinence of increasing the time span of platinum-resistance or introducing the term of "partial platinum-sensitivity/resistance" as in ovarian cancer [25].

Taken together, cisplatin ineligibility may be either due to toxicity reasons or treatment resistance. The most common alternative regimens comprise a carboplatin/5-fluorouracil doublet, sharing the same platinum resistance issues, and cetuximab [22]. Justified by a hypothetically increasing number of sensitive cells in a growing tumour that recurred, cetuximab may in principle be subjected to retreatment or rechallenge after previous failure (e.g., first in concomitance with curative radiotherapy and then in the palliative setting), but data are still limited [26]. Even less evidence exists for retreatment or rechallenge with the same class of immune checkpoint inhibitors, which currently dominate the recurrent and/or metastatic setting and are increasingly

incorporated in ongoing clinical trials in the locally advanced setting. Here, some efficacy can be expected, but an off-immunotherapy period is obviously warranted [27, 28]. Disease-free interval prior to first-line palliative systemic treatment represents, therefore, a crucial indicator impacting on the drug choice. A short disease-free interval is a poor prognosticator, and the outcomes are almost uniformly worse than if the same treatment is given later. A cut-off of 6 months can still be reasonably used in clinical practice, but the relation is probably stochastic and not categorical.

As alluded to above, relapsing SCCHN differs according to the site of recurrence and can thus be classified into locoregional recurrence only, locoregional recurrence with metachronous (with respect to the primary tumour) metastases, and distant failure only. Intriguingly, locoregional relapse eligible neither for salvage surgery nor radiotherapy may not require the same systemic drugs as a widespread disseminated disease. In fact, the use of first-line immunotherapy, particularly as a single-agent regimen, is accompanied by an increased risk of progression in more than one third of patients and in some of them even in the form of hyperprogression, which is an abnormally accelerated tumour growth described in about one quarter of patients receiving immune checkpoint inhibitors and being more frequent in those presenting with locoregional recurrence relative to those with exclusively distant dissemination [29]. Keeping in mind the typical head and neck tumour location in a very sensitive area near vital structures, the increased risk of progression could explain a lack of survival benefit seen in subgroup analyses of the registration Keynote-048 trial in patients presenting with locoregional recurrence only [30]. On the other hand, this is exactly the group of patients in which a non-immunotherapy alternative for the first-line setting consisting of cetuximab/platinum/5-fluorouracil triplet seem to have a major effect (hazard ratio for death 0.65 [0.49, 0.87] in locoregional recurrence only versus 0.99 [0.72, 1.36] in metastatic tumours including also locoregional recurrences) [31]. Furthermore, a post-hoc pooled analysis of both arms of the TPExtreme trial (see below) showed a significantly improved progression-free survival in patients with a locoregional recurrence only [18].

About half of all patients starting with standard-of-care first-line treatment (see below) will also receive second-line therapy, where the drug choice is proportionally restricted. Importantly, the majority of these patients experience progression while on treatment, so there is no disease-free interval as could be the case after primary therapy. Although we might feel intuitively driven towards giving the most comprehensive therapy at the earliest possible opportunity, allowing thus the majority of patients to benefit from it, emerging evidence suggests that treatment sequencing may be the key of success. Illustrative to that are also the very recent results of three large randomized trials in first-line metastatic melanoma. Despite initial excitement and even FDA approval, they did not in the end confirm any significant clinical benefit of combining two of the most potent regimens, i.e., immune checkpoint inhibitors of programmed cell death-1 receptor (PD-1) or its ligand (PD-L1) with RAF and MEK inhibitors [32].

First-Line Treatment: Pros and Cons

Since 2008, the standard first-line treatment has been biochemotherapy according to the EXTREME trial combining the epidermal growth factor receptor inhibitor cetuximab with a platinum-doublet (cisplatin or carboplatin with 5-fluorouracil) in platinum-sensitive SCCHN patients. In comparison with the platinum-doublet alone, the EXTREME regimen significantly improved overall survival from 7.4 to 10.1 months, progression-free survival, and response rate, and all this without compromising quality of life [33, 34]. However, the regimen had several shortcomings including a high rate of severe acute adverse events observed in 82% of patients, poor long-term results with less than 5% of patients being alive at 5 years, absence of significant benefit in patients with distant dissemination according to a subgroup analysis, a lack of predictive biomarkers, and an inconvenient continuous administration of 5-fluorouracil [31, 33, 35]. Nonetheless, EXTREME dominated the first line for more than 10 years and withstood multiple challenges to be dethroned by other promising regimens.

Validating the first predictive molecular marker in SCCHN, the Keynote-048 trial introduced immunotherapy to the first line and demonstrated its superiority over EXTREME in patients with platinum-sensitive tumours marked positively for PD-L1 expressed as combined positive score (CPS). Immunochemotherapy (anti-PD-1 inhibitor pembrolizumab with a platinum doublet) significantly improved overall survival from 10.4 to 13.6 months and from 11 to 14.7 months in the in CPS $\geq$1 and CPS $\geq$20 subgroups, respectively. Immunotherapy alone proved such benefit only in the CPS $\geq$20 subgroup (10.7 versus 14.9 months) [36]. In the PD-L1 negative subgroup accounting for 15% of the study population, EXTREME defended its position. In the subgroup with low PD-L1 expression (CPS 1-19), immunotherapy should be combined with chemotherapy [37]. Furthermore, pembrolizumab prolonged median duration of response by more than 16 months, was substantially less toxic than EXTREME, and retained its efficacy in the elderly subgroup suggesting possible benefits in less fit patients as well [36, 38]. However, even this new schedule has its downsides. In comparison with EXTREME, both pembrolizumab alone and pembrolizumab with chemotherapy improved neither progression-free survival nor response rate, and progressions were more common, probably leading to a lack of overall survival benefit in locoregionally recurrent cases as mentioned above. In the immunochemotherapy arm, more treatment-related deaths than with EXTREME were noted, severe acute adverse events occurred in 74% of participants, and the inconvenient necessity of a continuous administration of 5-fluorouracil remained [36].

The third pivotal trial challenging EXTREME in patients with platinum-sensitive disease was TPExtreme (Fig. 13.3). Although the survival benefit of the better tolerated experimental TPEx arm (cetuximab/platinum/docetaxel) did not reach statistical significance, the trial provided us with valuable data that could improve the delivery of EXTREME, such as validation of biweekly administration of cetuximab in the maintenance phase, growth factor support to maximize dose intensity if tumour shrinkage
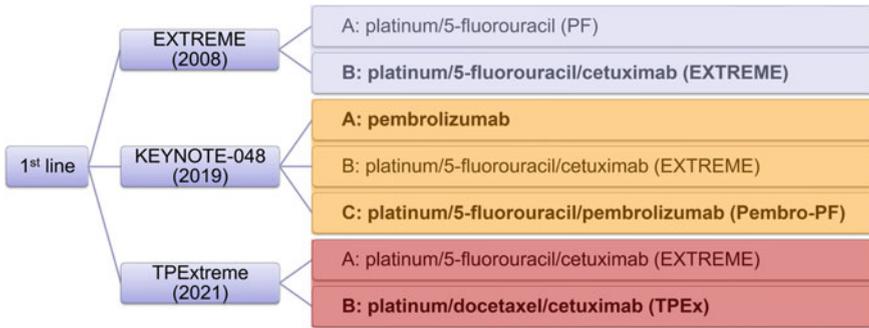
Fig. 13.3 Pivotal randomized trials in platinum-sensitive patients in the first-line palliative setting

is the main goal, or deintensification of cisplatin to decrease toxicity and subsequently enhance efficacy [18, 39]. Besides that, impressive outcomes were yielded in patients receiving second-line immunotherapy with overall survival reaching up to 21.9 and 19.4 months in the TPEx and EXTREME arms, respectively. Altogether, the TPEx regimen can be recommended as an alternative to EXTREME, but the eligibility criteria according to the study protocol are more restrictive (maximum age of 70 years, obligatory growth factor support and cisplatin use) [18].

In platinum-resistant disease, the current standard of care has been defined by two phase III trials, CheckMate-141 and Keynote-040, primarily focusing on the second-line setting but also including patients with confirmed progression within the first 6 months (22%) and between 3 and 6 months (15%) after primary treatment completion, respectively (Fig. 13.4). Both studies had a similar design exploring the anti-PD-1 inhibitors nivolumab and pembrolizumab, respectively, with almost the same comparator arms containing investigator's choice between single-agent cetuximab, methotrexate, and docetaxel [40, 41]. For Checkmate-141, a subgroup analysis of overall survival in platinum-refractory patients confirmed the benefit in this difficult-to-treat population even at 2-year follow-up (median of 7.7 versus 3.3 months) [42]. Interestingly, another subgroup analysis performed in both trials suggested that single-agent docetaxel is more effective than monotherapies with either methotrexate or cetuximab and that it may even be as effective as immunotherapy. Nevertheless, such conclusions are speculative and biased by small numbers of patients in the respective analyses [43].

Decision-Making Factors

Some of them have already been addressed. Here, we will provide a summary, and interested readers are advised to refer to our previous publication presenting a decision-making algorithm [44]. Except for the first two, all factors are continuous variables ranging from minimum to maximum values.
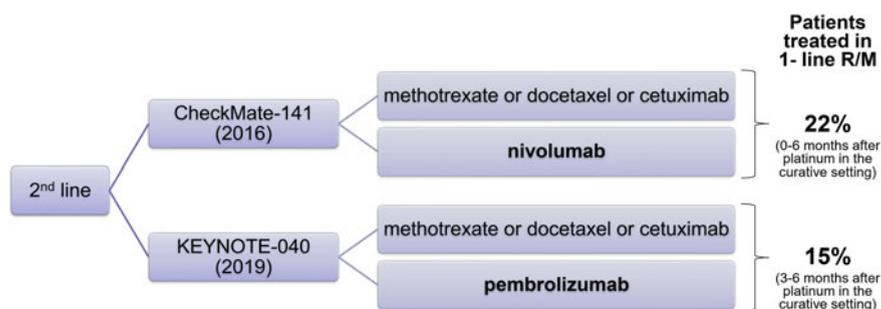
Fig. 13.4 Pivotal randomized trials in platinum-resistant patients in the first-line palliative setting

Categorical Variables

Platinum Eligibility

In patients with platinum-sensitive tumours, three treatment options are supported by randomized data: biochemotherapy (the preferred EXTREME regimen or alternatively TPEx), immunochemotherapy (Keynote-048 combination regimen), and immunotherapy alone (pembrolizumab according to Keynote-048). Patients with platinum-resistant tumours should preferentially receive immunotherapy (nivolumab or pembrolizumab) or chemotherapy. For the latter option, no standard-of-care has been defined but taxanes (paclitaxel or docetaxel) with or without cetuximab can be recommended if immune checkpoint inhibitors are not accessible.

Reflecting compromised organ functions, patient's general health status, previously administered dose, and some other specific situations, contraindications to cisplatin have been summarised elsewhere [22]. Contraindications to carboplatin are much less frequent, being mostly linked to impaired bone marrow capacity, hypersensitivity, first trimester of pregnancy, and lactation.

Disease Site

We have already indicated the caveat of locoregional recurrence in patients treated with immune checkpoint inhibitors and that distant dissemination may preclude efficacy of biochemotherapy [30, 31]. Another recent discovery points towards possibly restrained efficacy of immunotherapy in liver metastases owing to altered antitumour immunity and CD8+ T cell depletion [45].

Continuous Variables

Overall Health Status (From Fitness to Frailty)

In comparison to later stages of the disease course, treatment-naive patients are usually in a better overall condition, and their treatment tolerance and outcomes are superior. However, the majority of candidates for first-line systemic therapy present with a recurrence after multimodality management of locally advanced SCCHN and they may present with various adverse consequences thereof, especially if the disease relapses shortly after a platinum-based regimen.

Patient's health status can be appraised at two levels. The first is more general and corresponds to the well-known performance status, being one of the most commonly used measures in clinical practice to estimate overall survival and treatment toxicity. However, it has several downsides. The correlation between toxicity and performance status pertains to conventional chemotherapy based on data from the 1980s. Thus, extrapolation to the current setting is problematic, primarily due to advances in supportive care and introduction of new medicines because targeted therapies, particularly modern immunotherapy with immune checkpoint inhibitors, might fit less to this model [46, 47]. Another disadvantage is that performance status is not an equal replacement of functional status comprising patient ability to complete activities of daily living (ADLs like washing, dressing, feeding, mobility etc.) and instrumental ADLs (IADLs like housework, shopping, taking medicines etc.), and it is even a less suitable surrogate of comorbidity scales. This holds true mainly for the elderly population, in which comorbidities rank among the most common indispositions followed by impaired IADLs, nutritional compromise, depression, cognitive dysfunction, impaired ADLs, and deteriorated performance status (grade ≥2 according to the Eastern Cooperative Oncology Group scale), the latter of which is found only in about 20% [48, 49]. Although performance status continues to be a widely accepted stratification factor for clinical trials and has real-world applicability in many young patients, it does not unfortunately play this role in the elderly.

Not only account elderly patients for the majority of cancer patients, more than half of them are frail or vulnerable and only less than one third fit [1, 50]. Comprehensive geriatric assessment (CGA) addresses the multifaceted health characteristics of elderly people summarized as biological age. In routine practice, geriatric screening tests (e.g., G8) are less time-consuming but still appropriate to select those who are not fit and require a full CGA to conclude on their biological age. Fit elderly persons should receive full-dose standard therapy because they derive the same anticancer benefit as their younger counterparts, albeit still with a potentially higher risk of toxicity due to physiological changes in metabolism; vulnerable patients may need alternative regimens or dose reductions and frailty precludes conventional treatment [50, 51]. Nevertheless, immune checkpoint inhibitors may still be a good option in frail or poor performance patients irrespective of age [38, 47].

The second level of health appraisal focuses specifically on comorbidities that besides their impact on general well-being, can also imply distinct contraindications for some drugs such as renal insufficiency for cisplatin, coronary artery disease for 5-fluorouracil, solid organ transplantation for immune checkpoint inhibitors, and many more [22, 52, 53]. In these cases, alternative regimens are required. Cisplatin/5-fluorouracil doublet may be replaced by carboplatin/5-fluorouracil in the EXTREME and Keynote-048 regimens. The TPEx schedule substitutes 5-fluorouracil for docetaxel, and treating physicians can opt either for cisplatin/docetaxel or carboplatin/docetaxel. If no third agent is added, both cisplatin/paclitaxel or carboplatin/paclitaxel are viable options [18, 33, 36, 54]. Instead of immune checkpoint inhibitor monotherapy in the second line, patients may receive single-agent taxane, methotrexate, or cetuximab [40, 41]. Every deviation entails changes in toxicity profile with some of them being also linked to decreased efficacy as in the above-mentioned cases of carboplatin or single-agent substitutions for immunotherapy [23, 40, 41].

Tumour Burden (From High to Low)

Mounting evidence suggests that increasing tumour size negatively correlates with response to immune checkpoint inhibitors and other types of immunotherapy. The underlying mechanism relates to local and systemic changes induced by large tumours leading to formation of a more immunosuppressive microenvironment [55]. Another implication of tumour volume is the corresponding probability to elicit symptoms. Here, three treatment characteristics have a key relevance, involving objective response rate, rate of progressive disease, and time to response. They inform us about the potential of a given systemic therapy to counteract increasing tumour size menacing to cause symptoms [56]. While a chemotherapy component is crucial to assure high response rates both in biochemotherapy and immunochemotherapy regimens, the immunotherapy component, either given alone or with chemotherapy, may have deleterious effects in terms of higher rates of progressions as we discussed earlier and probably also on time to response when given as monotherapy. Theoretically, due to its indirect action through mobilisation of immune cells, more time is needed to obtain tumour shrinkage with immunotherapy. This has been only partially reproduced in clinical practice so far because median time to response was comparable between immunotherapy and chemotherapy arms in CheckMate-141 and Keynote-048 but was longer in the pembrolizumab arm in Keynote-040 (4.5 versus 2.2 months) [36, 40, 41]. Of note, administration of only two doses of nivolumab prior to curative resection of locally advanced SCCHN (i.e., already at one month from nivolumab initiation) yielded radiographic tumour reduction from baseline in about 50% of patients [57].

Disease Pace (From Fast to Slow)

The speed of tumour-cell proliferation measured as tumour doubling time and the speed of tumour-cell shedding leading to formation of new regional or distant metastases are two principal events defining tumour kinetics. It ranges from indolent cases over faster progressing cancers to cases of hyperprogression [29, 56]. A fast growing disease needs a similar approach as large tumours aiming at high response rates, low rates of progression, and a short time to response, whereas in a slowly growing disease we may prioritize less intensive regimens similarly to small tumours (e.g., local ablation, immunotherapy alone) or even periods of watchful waiting [44]. Recently, we introduced the term argometastases delineating slowly developing distant metastases which can be cured with local ablation [58].

PD-L1 Expression (From High to Low)

Tumours exposing this ligand on cell surfaces derive better outcomes from immune checkpoint inhibitors. At present, this statement holds true for a survival advantage shown with pembrolizumab in the first-line setting according to Keynote-048 where the expression was measured as CPS, i.e., including also non-tumoural cells, mostly lymphocytes and macrophages [36]. In Keynote-040, better survival and response rate were associated with a higher PD-L1 expression measured solely on tumour cells as tumour proportion score (TPS) of 50% or more. Importantly, CPS was not predictive in Keynote-040, and no correlation with either CPS or TPS was found in CheckMate-141 [40, 41]. Nevertheless, PD-L1 expression remains the only validated molecular marker in recurrent and/or metastatic SCCHN warranting further research to improve its predictive value.

Treatment Sequencing

Acknowledging the continuous process of malignant development from early to advanced stages, both in the primary and recurrent disease settings, as well as the inherent propensity of SCCHN to relapse (Fig. 13.1), therapeutic decision making should always look farther into the natural disease course and respond to questions as of what will be the next best step in case of failure. Treatment sequencing is therefore not a series of ad hoc decisions each time a new treatment is required but a comprehensive pre-planned individualized analysis of anticancer management divided into several consequential therapeutic blocks that are employed at disease progression or relapse (Fig. 13.2). In SCCHN, it represents an emerging new concept evolving along with the introduction of new treatment options or combinations.

Illustrative to this is a sequential administration of immune checkpoint inhibitors in the recurrent and/or metastatic setting as opposed to the standard concomitant approach proposed by Keynote-048. Median progression-free survival of

biochemotherapy is 5.6 months according to EXTREME [33]. If patients who progress receive single-agent nivolumab according to CheckMate-141, it takes about 3 months for the survival advantage of immunotherapy to manifest which is actually the time needed for a separation of survival curves between nivolumab and the comparator arm [40]. Taken together, patients starting biochemotherapy may be benefiting from immunotherapy after about 8 months. Interestingly, this seems to be the same period necessary for a separation of survival curves in the Keynote-048 trial. Moreover, median overall survival of concomitant immunochemotherapy is about 14 months [36]. In the TPExtreme trial, patients treated initially with biochemotherapy and then second-line immunotherapy had a median overall survival of almost 20 months [18]. There were such sequentially treated patients also in the standard arm of Keynote-048 but only about 25% [36]. In summary, these results suggest comparable or even better outcomes of a sequential versus concomitant approach. However, when translating them to clinical practice, further elements should be considered such as toxicity (not excluding immune-related adverse events), risk of progression (locoregional recurrence versus asymptomatic distant metastases or the presence of already symptomatic distant metastases), quality of life, and patient's perspective.

Conclusions

Growing knowledge from clinical trials and the real-world setting help us understand the natural course of head and neck cancer, predictors of its outcome, and principles of treatment sequencing. The above mentioned six factors should all be integrated in the decision-making algorithm but some of them may be prioritized while others brought to background, particularly if conflicting results are yielded (e.g., high disease burden with high PD-L1 expression or a locoregional recurrence with high PD-L1 expression).

Treatment sequencing with deferral of immunotherapy to the second line should also be part of the decision-making algorithm but may still be difficult to explain to patients confronted with the general assumption about a universal benefit of immunotherapy. In these situations, presenting the therapeutic model as a comprehensive approach with clearly defined turning points and available options may help pave the way towards an optimal solution for each patient.

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Chapter 14
Patterns of Response to Immune Oncology Drugs: How Relevant Are They in SCCHN?

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Introduction

According to the cancer immunoediting theory, the three "**E**s" of cancer immunoediting capture the immune system's role in protecting against tumor development. Initially and during the process of cancer immunosurveillance, the immune system successfully **e**liminates cancer cells through effective neoantigen processing and activation of effector T cells [1, 2]. Subsequently, the tumor evades the immune system and **e**quilibrium is attainable when the tumor is dormant but not eradicated. Eventually and through genetic instability that fosters the outgrowth of immunosuppressive cells that disrupt the immune system, cancer eludes the immune system and avoids elimination by effector T cells. During the **e**scape phase, cancer progresses and becomes clinically evident.

Tumors use complex, overlapping mechanisms to evade the immune system, such as inhibition of tumor antigen presentation, secretion of immunosuppressive factors and recruitment of immunosuppressive cells [3, 4]. In addition, cancer cells can dysregulate checkpoints (inhibitory) and activating signals that are responsible for the orchestration of immune response [5]. Targeting inhibitory immune checkpoints has emerged as an evolving strategy of active immunotherapy, aimed at promoting a sustainable immune response. The most studied and clinically relevant immune checkpoint pathway is the Programmed Cell Death-1 (PD-1)/Programmed Cell Death Ligand-1 (PD-L1) pathway. PD-1 is a T-cell co-stimulatory receptor commonly expressed on activated T cells, B cells and monocytes, whereas PD-L1 is expressed on tumor cells and several immune cells, such as activated T cells, B cells and Natural Killer (NK) cells [6]. Binding of PD-1 to its ligands PD-L1/PD-L2 leads to effector

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T cell exhaustion, repression of anti-tumor response, suppression of tumor immunity and subsequent tumor outgrowth. Anti-PD1-1 antibodies such as pembrolizumab and nivolumab successfully block PD-1/PD-L1/L2 interaction, triggering antitumor response through activation of effector T cells.

During the last 7 years, immune checkpoint inhibitors (ICIs) have been incorporated in everyday clinical practice and are now approved for a multitude of indications in numerous solid tumors, including squamous cell carcinoma of the head and neck (HNSCC). Despite great enthusiasm originating from the barrage of information and favorable results of clinical trials, it has become increasingly challenging to optimally assess the clinical benefit associated with ICIs. In addition, the use of these immunotherapeutic agents has brought to the forefront atypical patterns of response, which are distinct from those encountered with chemotherapy or targeted therapies.

In this chapter, we seek to illustrate available data on different types of responses to ICIs focusing on HNSCC.

Immunotherapy in Head and Neck Cancer

Cancer cells have the ability to generate an immunosuppressive tumor microenvironment (TME) to promote immune escape and tumor evolution [7]. In HNSCC, TME has an inflamed phenotype, characterized by an abundance of immune cells such as cytotoxic T cells (CTLs), immunosuppressive cells such as T regulatory cells (Tregs) and Myeloid-Derived Suppressor Cells (MDSCs) and production of Interferon gamma (IFN-γ) [8]. This phenotype is suggestive of a pre-existing anti-tumor immune response that was suddenly inhibited by the creation of an immunosuppressive TME by the tumor cells [9]. Thus, HNSCC represents a disease characterized by profound immunogenicity favoring a clinical response to immunotherapy.

Anti-PD-1 antibodies nivolumab and pembrolizumab have been approved in the platinum refractory recurrent/metastatic (R/M) setting since 2016. Checkmate 141 was a landmark, randomized phase III study that evaluated the clinical efficacy of nivolumab versus standard of care (physician's choice of either weekly docetaxel, methotrexate or cetuximab) in platinum refractory disease [10]. This study was the first to show an overall survival (OS) benefit in favor of an ICI (median OS 7.1 for nivolumab vs. 5.5 months for investigator's choice therapy, HR 0.70; 97.73% CI, 0.51–0.96, p = 0.01) in addition to a more favorable toxicity profile (13.1% of grade 3–4 events in the nivolumab arm vs. 35.1%) [10]. The benefit of nivolumab was prominent regardless of crossover that was allowed in the trial after protocol amendment and irrespectively of PD-L1 status [Tumor Positive Score (TPS)≤ 1% vs. >1%]. Notably, a trend for improved outcomes was demonstrated for PD-L1 positive and human papillomavirus (HPV)-associated disease; however, PD-L1 expression and p16 status were not required for enrollment and were therefore unknown for a large proportion of patients.

Pembrolizumab was granted accelerated approval for platinum-refractory R/M HNSCC based on the findings of the phase IB Keynote 012 clinical trial that showed

an overall response rate of 18% and durable responses that lasted more than 6 months in 85% of responders. OS at 12 months was 38% in the updated follow-up [11]. In the confirmatory phase III Keynote 040 trial, pembrolizumab induced a clinically meaningful prolongation of OS versus standard of care (SOC; methotrexate, 3-weekly docetaxel or cetuximab); (8.4 months with pembrolizumab vs. 6.9 months with SOC [hazard ratio 0. 80, $p = 0. 0161$]) [12]. Increased efficacy of pembrolizumab was observed in patients with either PD-L1 combined positive score (CPS) ≥1 or TPS ≥50% compared to their counterparts in a post-hoc exploratory analysis. Thus, European Medicine's Agency (EMA) approved pembrolizumab only for patients whose tumors express PD-L1 TPS ≥50%.

The phase III Keynote 048 clinical trial has resulted in the incorporation of pembrolizumab in the treatment algorithm of R/M HNSCC in the first-line setting. In this study, 882 patients with platinum-sensitive disease were randomized to either pembrolizumab monotherapy, pembrolizumab plus chemotherapy or the SOC (EXTREME regimen, platinum/fluorouracil plus cetuximab). PD-L1 CPS score, defined as the ratio of PD-L1-positive cells (tumor cells, lymphocytes, and macrophages) to the total number of tumor cells ×100 was used as a stratification factor. The study was not powered to detect differences in efficacy between the two immunotherapy arms that were both compared to SOC. Results were impressive, showing a significant prolongation of OS with pembrolizumab monotherapy in the PD-L1 CPS ≥20 (14.9 months vs 10.7 months for EXTREME) and CPS ≥1 groups (12.3 months vs 10.3 months) and non-inferior OS in the intention to treat (ITT) population (11.6 months in the pembrolizumab group vs 10.7 months in the EXTREME group, respectively). In addition, pembrolizumab in combination with chemotherapy demonstrated OS benefit vs the EXTREME regimen in the PD-L1 CPS ≥20 (14.7 months vs 11.0 months), PD-L1 CPS ≥1 (13.6 months vs 10.4 months), and ITT populations (13.0 months vs 10.7 months) [13]. In a post-hoc analysis that was recently published, in the CPS <1 subgroup, neither pembrolizumab nor pembrolizumab plus chemotherapy showed benefit in OS versus the EXTREME regimen, but the analysis was inconclusive due to the small number of patients in this population [14]. For the CPS 1–19 subgroup, pembrolizumab plus chemotherapy significantly improved OS compared to EXTREME (HR = 0.71, $p = 0.01$), whereas prolongation of OS with pembrolizumab monotherapy was non-significant versus EXTREME (HR = 0.86, $p = 0.25$) [14].

Combinations of anti-CTLA4 and anti-PD1/PD-L1 inhibitors have failed to show survival benefit compared to EXTREME in the first-line R/M setting. In the recent Checkmate 651 study that assessed the clinical efficacy of the combination of nivolumab plus the anti-CTLA-4 antibody ipilimumab versus the EXTREME regimen in the first-line setting of R/M HNSCC, no statistically significant increase of OS was observed either in the total population or the CPS ≥20 population [15]. However, 2-year rates of OS were very encouraging for patients receiving nivolumab and ipilimumab (41% and 34% in the CPS ≥20 and CPS ≥1 groups, respectively). Similarly, the phase III KESTREL study, a global phase III study randomizing patients with previously untreated R/M HNSCC to treatment with the anti-PD-L1 antibody durvalumab monotherapy versus the combination of durvalumab plus

the anti-CTL4 antibody tremelimumab versus the SOC EXTREME regimen [16]. KESTREL failed to reach the primary endpoint of OS in patients with PD-L1 TPS ≥50% or tumor-infiltrating immune cells (IC) ≥25% according to a press release from AstraZeneca in February 2021.

In the second-line setting, Checkmate 714 [17], a—randomized phase II study comparing nivolumab plus ipilimumab to nivolumab plus placebo failed to meet its primary endpoints of ORR and duration of response in platinum-refractory patients according to a press release from Bristol-Myers Squibb in January 2020. Moreover, the phase III EAGLE trial [18], designed to test the clinical activity of durvalumab or durvalumab plus tremelimumab versus SOC therapy (cetuximab, methotrexate, a taxane, or a fluoropyrimidine) in platinum-refractory disease, did not show any superiority of either durvalumab monotherapy or the combination with tremelimumab versus conventional chemotherapy.

In conclusion, ICIs such as nivolumab and pembrolizumab have shown remarkable efficacy in HNSCC and their use has been incorporated in the treatment algorithm in both first- and second-line settings, yielding improved clinical outcome both in terms of efficacy and toxicity.

Patterns of Response to Immune Checkpoint Inhibitors

Immunotherapy has revolutionized the field of oncology and has changed the way physicians evaluate the clinical benefit of therapy and treatment sequelae. Compared to chemotherapy, ICIs display pharmacokinetic and pharmacodynamic differences. For example, for the majority of chemotherapy drugs, the biological effect increases as the plasma concentration of the drug rises and the intensity of adverse events is dose-dependent [19]. In the context of chemotherapy efficacy, the term therapeutic window refers to a range of doses which optimize between efficacy and toxicity, reaching the greatest therapeutic benefit without causing unacceptable adverse events. On the contrary, efficacy of immunotherapy is not characterized by a dose-dependent treatment effect, but rather a more delayed effect with a variable proportion of long-term survivors (plateau of the curve) [20]. In addition, immune-related adverse events (irAEs) can have a delayed onset and prolonged duration compared to chemotherapy side effects [19].

Most importantly, the use of ICIs has been linked to the development of several atypical patterns of treatment response. For example, ICIs can cause tumor shrinkage that is maintained over time despite treatment discontinuation (prolonged response), minimal or non-existent modifications in target lesions (stable disease), initial tumor progression followed by shrinkage on subsequent imaging (pseudoprogression) or unexpectedly rapid tumor expansion with clinical patient deterioration (hyperprogression) [21].

Pseudoprogression

The phenomenon of pseudoprogression was first described in a phase II trial evaluating the efficacy and toxicity of ipilimumab in 155 patients with previously treated advanced melanoma. Di Giacomo et al. described the case of one patient that experienced an initial increase in tumor size of lung metastases followed by remarkable tumor regression for a prolonged duration [22]. Pseudoprogression has been since then defined as a temporary increase of tumor size or burden followed by a subsequent tumor reduction on following imaging.

Pseudoprogression does not represent actual tumor growth. It has been hypothesized that it biologically originates from the infiltration of the tumor by activated T cells that are prompted to the tumor site following treatment with ICIs, edema and necrosis [23]. Indeed, in the original report by Di Giacomo et al., a biopsy performed at the time of initial progression showed infiltration by CTLs and granzyme B+ indicating a functional T cell population [22].

Using classical radiographic criteria for assessment of response to immunotherapy, pseudoprogression might lead to early discontinuation of treatment in clinical trials in patients that may have had a later response to immunotherapy. The development of immune-specific related response criteria (irRC) that were introduced based on data from patients treated with ipilimumab in melanoma trials, mirrors this exact need of being able to continue immunotherapy in patients with atypical patterns of response, such as pseudoprogression or mixed response, without the false alarm of disease progression (PD) [24]. These criteria differed from classical RECIST in the definition of partial response (immune-related partial response, irPR; decrease in ≥50% in disease burden), and immune-related PD (irPD; an increase in tumor burden by ≥25% relative to nadir).

More recently, immune-related RECIST criteria (irRECIST) have been established as a result of a further refinement of radiologic criteria to better evaluate responses to immunotherapy. These criteria were formulated as a mixture of iRC and RECIST criteria and the main differences compared to iRC are (a) the use of unidimensional measurements; while in iRC criteria lesions are measured in two dimensions (longest diameter and longest perpendicular diameter), in irRECIST, lesions are measured using only the longest diameter, (b) irPR is defined as ≥30% from baseline and (c) irPD must be confirmed with a second imaging study at least 4 weeks later to enable development of delayed immune response. Thus, irPD is confirmed only if the second scan demonstrates new findings or new unequivocal progression compared to the first one. In irRECIST, repeat assessment is allowed up to 12 weeks [23, 25]. Last, iRECIST criteria were developed in March 2017 following an expert consensus. These modified criteria introduce new nomenclature, such as an immune unconfirmed progressive disease (iUPD) or immune confirmed PD (iCPD). More specifically, when PD based on RECIST 1.1 criteria is observed, this is termed iUPD and it needs confirmation with subsequent imaging to be regarded as true progression (iCPD), which is defined as an increase of at least 5 mm of the

total measurements of target lesions from iUPD. Criteria iRECIST suggest subsequent imaging to be performed between 4 and 8 weeks post iUPD, compared to 4–12 weeks in irRECIST [26].

In head and neck cancer, the incidence of pseudoprogression has been reported as below 3% in several studies. Of note, responses were assessed by RECIST criteria in the majority of the studies. Seiwert et al. reported the results of the PD-L1 positive cohort of the phase Ib Keynote 012 study, in which patients with R/M HNSCC received pembrolizumab as second- or later-line of treatment [27]. In this study, among 45 patients assessed by central review for response, one patient experienced an atypical response before a confirmed complete response, probably a pseudoprogression, although it is not clear whether the increase in tumor size was observed clinically or on imaging. In the expansion cohort of the same study, no pseudoprogression was noted [28]. In Keynote 040, responses to pembrolizumab were also assessed based on RECIST criteria [12]. As shown in the swimmer's plot in the appendix of the Keynote 040 publication, nine patients were continued on pembrolizumab beyond progression and 2 of them were still on treatment 8 weeks post unconfirmed progression (based on iRECIST), indicating a 0.8% (2 out of 247 patients that received pembrolizumab) of pseudoprogression. On the other hand, Haddad et al. reported the results of treatment beyond progression with nivolumab in patients with R/M-HNSCC who participated in the Checkmate 141 trial [29]. Among 146 patients with disease progression, 62 patients continued treatment with nivolumab; in this study, continuation of immunotherapy was allowed if the drug had clinical benefit as assessed by the investigator. Among 62 patients, 15 experienced a decrease in target lesions and 3 had a confirmed PR, indicating a 1.3% incidence of pseudoprogression [29]. Last, in a retrospective report by Sridharan et al. aiming to evaluate predictors of response to immunotherapy in 100 patients with R/M-HNSCC, the rate of pseudoprogression was 1% (1 out of 100 patients) [30].

Based on iRECIST and given the rarity of pseudoprogression across tumor types (<10%) [25, 29, 31, 32], it is critical to appropriately select patients who are candidates to continue immunotherapy beyond progression by striking a balance between premature cessation and delay of a subsequent potentially efficacious treatment strategy. Key points include (a) the identification of early signs of clinical deterioration that imply a true disease progression; indeed, although there may appear to be tumor growth on initial imaging and physical examination, pseudoprogression should not be accompanied by clinical deterioration and (b) a biopsy at the time of progression that shows infiltration by immune cells might be helpful and (c) the patient should not have experienced severe adverse events. Further studies are warranted to identify potential biomarkers for immune response.

Hyperprogressive Disease (HPD)

A meticulous study of survival curves in several immunotherapy clinical trials reveals an early crossing of the curves during the first 6 months of treatment that indicates a subgroup of patients with deleterious effects induced by immunotherapy [10, 13, 33, 34]. For example, in Keynote 048, the survival curves clearly favor the EXTREME arm compared to pembrolizumab monotherapy arm during the first few months [13]. This phenomenon might be partly explained by the concept of hyperprogression, which represents immunotherapy-induced rapid acceleration of tumor growth kinetics [23].

HPD was first reported by Champiat et al., who conducted a retrospective study that included 218 patients with different solid tumors treated with anti-PD-1/PD-L1 inhibitors in phase I studies [35]. The authors compared the tumor growth rate (TGR-an estimation of increase of tumor volume over time) prior the initiation and post treatment with ICIs. HPD was defined as PD by RECIST criteria combined with twofold increase of the TGR. The incidence of HPD was found to be 9% in this study, and no association with tumor burden or specific tumor type was noted. However, HPD correlated with worse outcomes [35].

Following this publication, several groups, including ours, have described their experience with HPD [36–39]. The reported incidence of HPD ranges from 4 to 29% largely depending on the tumor type and the relevant definition. In HNSCC, the incidence of HPD ranges from 6 to 29%. More specific, a second group of authors from San Diego added time to treatment failure (TTF) to the definition of HPD, which was defined as TTF <2 months, >50% increase in tumor burden compared with the size prior to treatment (notably, the first assessment of treatment response was performed within two months after immunotherapy initiation), and twofold increase in progression pace. They conducted a retrospective analysis and Next-Generation Sequencing (NGS) genomic analysis in a cohort of 155 patients with various solid tumors, among which 11 had HNSCC. One out of 11 patients with HNSCC had HPD (incidence 1%). Interestingly, this study found a correlation of MDM2/4 amplification and EGFR alterations with TTF <2 months and a correlation of MDM2 amplification with increased incidence of HPD [37].

Subsequently, Saâda-Bouzid et al. retrospectively analyzed and compared the TGR before immunotherapy and at first imaging in a cohort of 34 patients with HNSCC [38]. HPD was defined as TGR ratio ≥2. In this study, the incidence rate of HPD was found to be 29%. In addition, HPD significantly correlated with a regional recurrence, but not with local or distant recurrence and was associated with a shorter PFS (2.9 vs. 5.1 months, P = 0.02) [38].

Our group reported data regarding HPD in a cohort of 117 patients with R/M HNSCC. Tumor growth kinetics were assessed and were available in 49 patients [39]. Using the same definition as the French group [38], HPD was documented in 15.4% of the whole cohort. Occurrence of HPD was associated with worse PFS (1.8 months in patients with HPD vs. 6.1 months in patients with non-HPD, p = 0.0001) and OS (6.53 months in patients with HPD vs. 15 months in patients with

non-HPD, $p = 0.0018$) and clinical parameters such as primary site in the oral cavity and administration of immunotherapy in the second/third-line setting. In addition, EGFR amplification was solely documented in patients with HPD, although not statistically significant [39].

Kanjanapan et al. reviewed the records of 352 patients with various solid tumors that participated in early clinical trials [40]. Among 32 patients with HNSCC that were included in the study, two had HPD (incidence 6%). No association with clinical characteristics (except female sex) or survival was found in this study. Most recently, Park et al. reported the incidence and clinical effect of HPD in 125 patients with R/M-HNSCC treated with immunotherapy in 11 Korean centers [41]. HPD was observed in 14.4% of patients, and a correlation was found with younger age, primary site in the oral cavity (similar to the findings of our group) and prior irradiation. Interestingly, no correlation with survival was reported and a clinical benefit with subsequent treatment was shown.

In a recently published systematic review and meta-analysis of 24 studies with 3109 patients, the authors sought to summarize the definitions and incidence of HPD across different tumor types [42]. A pooled incidence of HPD equal to 13.4% was found, with a wide range due to the various definitions of HPD. The authors concluded that the incidence of HPD might be miscalculated if only TG kinetics are used, because no clinical criteria are taken into consideration [42]. In addition, TG kinetics are complex to estimate and require pre-baseline CT scans, which are not easily available particularly in treatment-naïve patients where immunotherapy is being administered as first-line treatment. The timeframe for a second imaging assessment is also a point of discussion, as it should be less than two months to enable capture of hyperprogressors at an early timepoint. Nevertheless, clinical criteria to evaluate clinical deterioration, such as TTF or increase in tumor burden, might not be sufficient to accurately estimate clinical status.

Several potential mechanisms have been implicated in the development of HPD. First, blockade of the PD-1/PD-L1 immune checkpoint might result in the recruitment of Tregs that create an immunosuppressive TME. Second, immune blockade might be counteracted by the activation of several alternative checkpoints contributing to T cell exhaustion. In addition, several immunosuppressive cells such as MDSCs and macrophages might lead to the production of cytokines and other factors, such as IL-10 and IFN-γ. Furthermore, it can be hypothesized that PD-1/PD-L1 blockade can trigger the activation of tumorigenic signaling pathways [43, 44].

Given retrospectivity of data and the lack of validated biomarkers, many researchers question the originality of HPD and claim that it could be the natural evolution of a tumor unresponsive to immunotherapy [21]. Although scarce genomic and clinical associations have been reported, these might be random findings. In addition, hyperprogression has been less frequently reported in patients receiving chemotherapy and targeted therapy and might not be uniquely observed in immunotherapy-treated patients. Randomized trials comparing immunotherapy with no treatment, albeit ethically rather unacceptable, would help elucidate the phenomenon of HPD. This could be possibly feasible at later lines of therapy, where no standard treatment exists; for example, nivolumab has been compared to placebo

in the third line setting of gastric/gastroesophageal junction cancer [45]. Neverthe-less, patients do experience rapid tumor growth following immunotherapy, and we must try to exploit clinical experience and molecular characterization originating from expert centers.

Conclusions

In conclusion, it becomes increasingly clear that patterns of response and progression to immunotherapy differ from those observed with chemotherapy and targeted agents. Although it has been rarely reported in head and neck cancer, pseudoprogression is a well-described phenomenon that has led to the development of immune-specific related response criteria that enable continuation of treatment beyond progression. On the other hand, HPD on anti-PD1 agents has been reported in 9–29% of head and neck cancer patients. The heterogeneity of HPD definitions and the retrospec-tivity of reported data have created a controversy between real hyperprogression and natural history of disease progression. To this direction, there is an urgent need to identify biomarkers for prediction of progression or hyperprogression with the view to optimize treatment outcomes.

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Chapter 15
Stereotactic Body Radiation Therapy in the Management of Recurrent and/or Oligometastatic Head and Neck Cancer

Daan Nevens and Petr Szturz

Introduction

In head and neck cancer (HNC) patients, locoregional recurrences occur in up to 50% in those initially presenting with locoregionally advanced disease [1]. Furthermore, 15% of patients present with a second primary tumor during their follow-up [2]. For these patients with recurrent or second primary HNC, surgery provides the greatest chance of long-term survival [3]. Unfortunately, only a minority of HNC patients with a locoregional recurrence or a second primary tumor is diagnosed with resectable disease. Therefore, these patients often end up receiving systemic therapy with a palliative intent [4]. To improve local control in this patient group, reirradiation has been proposed [5]. However, reirradiation, typically up to doses of 66–70 Gy in fractions of 2 Gy, comes with several challenges due to a high risk of severe toxicity [6]. Stereotactic body radiation therapy (SBRT) could represent a suitable RT technique in this situation [7]. Derived from intracranial stereotactic radiosurgery, the methodology was introduced to clinical practice by Lax and Blomgren at the Karolinska Hospital in Sweden in September 1991. Based on delivering precisely targeted high doses of radiation in one or several fractions, the concept of SBRT has rapidly gained acceptance and progressively spread around the world. SBRT has practical benefits in the reirradiation setting, but on the other hand, the high dose per fraction can be considered a risk factor associated with severe toxicity [8].

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Besides the reirradiation setting, there is an increasing interest in the use of SBRT in the setting where HNC patients present with few metastases (oligometastatic disease). Apart from new systemic modalities leveraging the immune cells to target cancer, focus has been drawn towards local ablative approaches against metastases, in particular in oligometastatic patients [9]. Over the past few years, efforts have been undertaken to make a firm definition of oligometastatic disease and its different states [10, 11]. According to a recently published European Society for Radiotherapy and Oncology (ESTRO) and American.

Society for Radiation Oncology (ASTRO) consensus, the following two conditions must be met: a maximum of five metastases and all of them must be safely treatable [11]. Metastasectomy has been traditionally considered the gold standard in the oligometastatic disease setting when a local ablative approach was considered. However, in patients who are unwilling or unable to undergo an invasive procedure or deemed to be at high risk of postoperative complications due to underlying comorbidities, SBRT has emerged as a valid alternative to surgery.

This chapter aims to summarize the available literature regarding the use of SBRT in recurrent and second primary HNC as well as in oligometastatic HNC.

Reirradiation Using SBRT in Recurrent or Second Primary Head and Neck Cancer

Several studies have been published showing safety and efficacy of SBRT-based reirradiation. However, most of them included small patient groups with different inclusion and exclusion criteria and diverse fractionation schedules, making firm conclusions impossible and hampering the introduction of this technique in clinical practice in this setting. Recently, a meta-analysis was published comprising 10 papers [11–20] published between 2006 and 2016 [21]. The number of patients included in these studies ranged from 22 to 107. The majority had squamous HNC. The dose of SBRT-based reirradiation ranged from 24 to 44 Gy with a median value of 30 Gy, mostly delivered in 3–6 fractions. Median gross tumor volume (GTV) ranged from 19.1 to 103 cm^3. Concerning the efficacy outcomes, median overall survival (OS) ranged from 8.6 to 16.2 months with a pooled median of 11.9 months. The pooled overall response rate was 61.7% (95% CI: 51.1–71.3). The 2-year local control (LC) rates ranged from 26 to 64%, and the pooled rate was 47.3% (95% CI: 33.1–62.1). The pooled grade ≥3 late toxicity rate was 9.6% (95% CI: 5.0–17.6) and grade 5 toxicity rate was 4.6% (95% CI: 2.4–8.6).

This meta-analysis demonstrated that, for patients with inoperable recurrent HNC or a second primary tumor in the HN region, SBRT-based reirradiation is a feasible therapeutic option with an acceptable severe toxicity rate below 10% and a good, pooled response rate of about 62%. Following SBRT-based reirradiation, the pooled 2-year OS rate was disappointingly only 30%. This is, however, in line with or even slightly better than standard reirradiation using longer schedules where a 2-year OS

rate of 15–26% was observed [22] with a higher burden for the patient in terms of a treatment period reaching up to 7 weeks and severe late toxicity rates of more than 30%. On the other hand, SBRT is a much shorter treatment, typically administered over a period of 14 days or less.

There are several factors that might have influenced the observed OS rates. Some papers demonstrated that radiation dose and tumor volumes can affect OS following SBRT-based reirradiation [15, 20]. It is believed that high-dose SBRT is essential to achieve prolonged OS especially for recurrent tumors since they might harbour radioresistant tumor cells that were not eradicated by previous chemoradiation [23]. Furthermore, Vargo et al. reported that gross tumor volume of less than 25 cm^3 was associated with increasing OS in comparison to larger tumors [22].

In the above-mentioned meta-analysis [21], reirradiation with SBRT appears to be safe with a pooled event rate of grade ≥ 3 complications of 9.6% and only three trials reporting rates of 10% or higher. Among the included studies, Vargo et al. reported grade 3 toxicity in 6% of patients and no grade ≥ 4 toxicities following 8 fractions of 5–5.5 Gy, Furthermore, Lartigau et al. found that 30% of patients experienced grade 3 toxicities following 6×6 Gy [11, 16].

Many studies in this field used SBRT-based reirradiation together with systemic therapy, hoping for a synergic effect and a better OS, however the used schemes were very heterogeneous [21]. Vargo et al. concluded that combining cetuximab with SBRT resulted in a 1-year OS of 40% [11]. Lartigau demonstrated that SBRT-based reirradiation with cetuximab is a valuable alternative to salvage surgery with a 1-year OS of 48% [16]. Recently, immune-checkpoint inhibitors pembrolizumab and nivolumab demonstrated durable antitumor activity for recurrent and metastatic HNC ineligible for RT or surgery both in the first line (Keynote-048 [4]) and second line (Checkmate-141, Keynote-012, and Keynote-040 [24–26]). Therefore, also for cases of recurrent HNC, it is necessary to continue investigating the combined therapeutic efficacy of systemic agents and local modalities such as SBRT.

SBRT in HNC Patients with Oligometastatic Disease

Numerous retrospective and prospective studies showed that SBRT can improve disease-free and OS in the oligometastatic setting while maintaining good treatment tolerance [27–29]. However, randomized data comparing SBRT with metastasectomy in operable patients are lacking. Moreover, covering different primary tumor types and organ sites, mostly outside the head and neck region, the available evidence remains difficult to interpret.

In HNC oligometastatic disease, we will discuss retrospective and prospective studies separately. Until present, the former group has provided quantitatively more data albeit with several important limitations inherent for this study type. The definition of oligometastatic disease has not been uniformly defined yet. This comes forward especially in retrospective analyses where different author groups may use different diagnostic criteria; and sometimes the designation "oligometastatic" may

even be attributed to a given case only after a retrospective review of his or her medical records. Hence, the results should be interpreted with caution, particularly if comparing different publications.

Pasalic et al. evaluated 82 patients with head and neck cancers of different histological types presenting with either synchronous or metachronous lung metastases. Forty-three of the 82 patients had oligometastatic squamous cell carcinomas (1–3 lesions). One and 2-year local control was 96% and 90%, respectively, and 1- and 2-year overall survival 74% and 66%, respectively [30]. In their primary analysis, Bates et al. focused solely on oligometastatic disease, reporting 1- and 2-year OS of 78% and 43%, respectively, in 27 squamous HNC patients (3 had nasopharyngeal cancer and 3 unknown primary) with up to five synchronous and metachronous metastases mostly affecting the lungs but also other organs encompassing the bones, liver, lymph nodes, and soft tissues. Local control of treated lung nodules was 74% and 52% at 1- and 2-years, respectively [31]. A similarly large cohort was described by Bonomo et al. who evaluated 27 squamous HNC patients with solitary lesions in the lungs. The investigators achieved an objective response rate at 3 months of 75% with 1- and 2-year time to progression of 56% and 35%, respectively [32]. Finally, Franzese et al. collected data of 48 consecutive HNC patients with a maximum of 5 oligometastases in up to 2 organs. Forty percent of primary tumors were salivary gland cancers and nasopharyngeal carcinomas. Efficacy results were available for the whole cohort with 1- and 2-year local control rates of 83% and 70%, respectively [33].

In addition, we have learnt from retrospective studies that human papillomavirus (HPV)-positive oropharyngeal cancer patients are probably better candidates for local ablation, including SBRT, compared with viral-unrelated HNC. This holds true particularly for cases presenting with slowly and late developing lung oligometastases. Although such clinical presentation is rare, it has been associated with long-term survival after metastasis-directed therapy and has implications for postprimary treatment follow-up. The latter does not usually comprise imaging methods. However, HPV-related oropharyngeal carcinoma might be one of the exceptions requiring a more comprehensive surveillance [9].

Prospective evidence on SBRT in oligometastatic HNC is scarce. Only a few randomized phase II trials were conducted, and most reports are single arm studies with marginal number of HNC patients [34, 35]. Sutera et al. recruited 147 patients with up to five metachronous, biopsy-proven metastases visualized on FDG-PET/CT in at most three organs, comprising the lungs (>50%), lymph nodes, bones, and other sites. There was a large variety of primary tumors with more than half of them compromised of lung cancer (22%), colorectal cancer (21%), and HNC (11%). Because of an excess of early deaths, median OS of 17.6 months in 16 patients with HNC, out of which only 11 had squamous HNC, was inferior to that observed in other primary tumor subgroups. However, the 42% 5-year OS yielded in this cohort of 16 patients compares favorably to outcomes in surgical studies but can be biased by the very small patient number [36].

The only randomized trial exploring the addition of SBRT to a standard systemic palliative treatment according to primary cancer was the SABR-COMET phase II study. It was the first trial in oligometastatic disease to explore OS as the primary

endpoint while previous randomized trials aimed at proving benefit in PFS. In SABR-COMET, oligometastatic state was defined by a maximum of five metachronous lesions with not more than three of them per organ (meaning that a maximum of 3 organs could be involved). Patients included were not considered candidates for metastasectomy. The three most frequently included primary tumors were breast, colorectal, and lung cancers (each about 18%), which were not balanced between the two study arms. The number of HNC patients was not specified except for a short comment in the supplementary materials on a case of oropharyngeal cancer treated for a lung metastasis. In the whole cohort of 99 patients, SBRT improved 5-year overall survival from 18 to 42%, however, at the cost of increased grade 2 or worse treatment-related toxicity (9% versus 29%) including grade 5 adverse events (0% versus 5%). Another noteworthy observation was the similar proportion of patients presenting with distant failure at untreated sites in the two study arms [37]. Therefore, despite the overall promising outcomes, the results of SABR-COMET testify the need for a proper patient selection to limit unnecessary toxicities and prevent indiscriminate use of local ablation in patients with few metastases, as some of them may in fact present with a widespread microscopic dissemination. A novel approach to this issue is to consider the speed of cancer development in the first place. It is characterized by rates of tumor-cell shedding and proliferation. Distant lesions with a slow speed of development, dubbed argometastases, may comply better to the need of identifying suitable patients for local ablation than if we rely solely on a low number of lesions [38].

Conclusion and Recommendations

SBRT reirradiation seems a promising modality for patients with inoperable recurrent HNC or second primary HNC providing acceptable safety along with short overall treatment times. OS following SBRT reirradiation remains moderate, which might be due to insufficient doses used in published studies. There is a need for well-designed trials of SBRT-based reirradiation in terms of dose escalation and combined treatment strategies with systemic agents. Currently, the largest body of evidence supporting SBRT-based reirradiation is furnished by the meta-analysis by Lee et al. evaluating this treatment in local and regional recurrences and second primary tumors [21]. Further studies in well-defined patient groups are necessary.

Based on the available literature, we can make the following recommendations for the use of SBRT in the reirradiation setting:

- Only patients with small local or regional recurrences are good candidates; ideally the GTV should be below 25 cm^3 [11].
- 5×7 Gy or 6×6 Gy are commonly used schemes [21].
- Following an R1 resection, 5×6 Gy should be prioritized [21].
- Three fractions per week with the time interval between fractions of 48 h is recommended [16].
- Overall treatment time should not exceed 14 days [11].

Another emerging role of SBRT is in the management of patients with oligometastases. Here, we need to understand which patients benefit the most, when the right moment is to intervene, how SBRT compares with surgery in operable patients and with other modalities (e.g. radiofrequency ablation), and what the impact of combination strategies is (e.g. with immune checkpoint inhibitors). Therefore, we advocate conducting dedicated studies for oligometastatic squamous HNC patients. One of the steps forward is the ongoing EORTC 1945 OligoRARE trial (NCT04498767) that investigates SBRT in addition to standard of care treatment in patients with oligometastatic rare cancers including HNC.

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Chapter 16
Precision Medicine in the Treatment of Malignancies Involving the Ventral Skull Base: Present and Future

Marco Ferrari, Stefano Taboni, Giacomo Contro, and Piero Nicolai

List of Abbreviations (Excluding Those Explained in the Text)

AFF2	AF4/FMR2 family member 2 gene
AKT1	RAC(Rho family)-alpha serine/threonine-protein kinase gene 1
ALDH1A3	Aldehyde dehydrogenase 1 family member A3
ALK	Anaplastic lymphoma kinase
ALPL	Alkaline phosphatase tissue-nonspecific isozyme gene

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ANXA2	Annexin A2 gene
BET	Bromodomain and extraterminal
BRAF	v-Raf murine sarcoma viral oncogene homolog B
BRCA1	Breast cancer gene 1
BUB1	Budding uninhibited by benzimidazoles 1 gene
CA9	Carbonic anhydrase 9
CAR-T	Chimeric antigen receptor T (cells)
CCL1	C–C motif chemokine ligand 1 gene
CCL15	C–C motif chemokine ligand 15 gene
CCND1	Gene encoding cyclin D1
CD	Cluster of differentiation
CDC34	Cell division cycle 34 ubiquitin conjugating enzyme gene
CDK	Cyclin-dependent kinase
CDKN2A	Cyclin-dependent kinase inhibitor 2A gene
CENPF	Centromere protein F gene
CpG	5′-C-phosphate-G-3′
CRTC1	CREB regulated transcription coactivator 1
DCC	Deleted in colorectal cancer gene
DEK	DEK proto-oncogene
DMD	Duchenne muscular dystrophy gene
DNAJB8	dnaJ heat shock protein family (Hsp40) member B8 gene
DNTT	DNA nucleotidylexotransferase gene
DOTATATE	Tetraxetan-(Tyr3)-octreotate
DTIC	Dimethyl traizeno imidazole carboxamide
E2F	E2 transcription factor
EFNA2	Ephrin A2 gene
EGFR	Epidermal growth factor receptor
EIF2S1	Eukaryotic translation initiation factor 2 subunit alpha gene
EIF6	Eukaryotic translation initiation factor 6 gene
ERBB2	Erb-B2 receptor tyrosine kinase 2 gene
ERCC1	Excision repair cross-complementation group 1 gene
ERK1	Mitogen-activated protein kinase 3
EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit gene
FBXO5	F-Box protein 5 gene
FDA	Food and drug administration
FER	Proto-oncogene tyrosine-protein kinase FER
FES	Feline sarcoma oncogene
FGF20	Fibroblast growth factor 20 gene
FGFR3	Fibroblast growth factor receptor 3

GNA11	Guanine nucleotide-binding protein subunit alpha-11 gene
HER2	Human epidermal growth factor receptor 2
HLA	Human leukocyte antigens gene
HPGD	15-Hydroxyprostaglandin dehydrogenase gene
HSPB1	Heat shock protein beta-1 gene
HSPB7	Heat shock protein family B (small) member 7
IDH	Isocitrate dehydrogenase gene (1 and 2)
IDO1	Indoleamine-pyrrole 2,3-dioxygenase 1
IFN	Interferon
IL	Interleukin
ITGB4	Integrin subunit beta 4 gene
JAK	Janus kinase
JNK	c-Jun N-terminal kinases
KIT	KIT proto-oncogene receptor tyrosine kinase
KRT14	Keratin 14 gene
LAMB4	Laminin subunit beta 4
LAMC2	Laminin subunit gamma 2 gene
MAML2	Mastermind like transcriptional coactivator 2
MAPK	Mitogen-activated protein kinase
MET	Tyrosine-protein kinase Met gene (also known as hepatocyte growth factor receptor)
MRP	Multidrug resistance-associated protein
mTOR	Mammalian target of rapamycin kinase
MUSES	Multi-institutional collaborative study on endoscopically treated sinonasal cancers
MVA-BN-brachyury-TRICOM	Modified vaccinia Ankara virus, Bavarian Nordic Brachyury triad of costimulatory molecules
MYB	MYB proto-oncogene
MYBL1	MYB proto-oncogene like 1
MYC	MYC proto-oncogene
NF1	Neurofibromin 1 gene
NFIB	Nuclear factor I B gene
NK	Natural killer
NOTCH	Neurogenic locus notch homolog protein
NR4A3	Nuclear receptor subfamily 4 group A member 3 gene
NUT	Nuclear protein in testis
PARP1	Poly [ADP-ribose] polymerase 1
PD-L1	Programmed death-ligand 1
PDGFR-β	Platelet-derived growth factor receptor beta
PFOU5F1B	POU domain class 5, transcription factor 1B pseudogene 1
PI3K	Phosphoinositide 3-kinases

PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PKC	Protein kinase C
PRAME	Preferentially expressed antigen in melanoma
PTEN	Phosphatase and tensin homolog gene
PTP4A3	Protein tyrosine phosphatase 4A3 gene
PTPN1	Protein tyrosine phosphatase non-receptor type 1 gene
RAS	Rat sarcoma virus gene (including KRAS, HRAS, and NRAS)
ROCK	Rho-associated, coiled-coil-containing protein kinase 1
SATB2	Special AT-rich sequence-binding protein 2
SIX1	SIX homeobox 1 gene
SMARCA4	Switch/sucrose non-fermentable-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4
SMARCB1	Switch/sucrose non-fermentable-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1
SOX4	SRY-box transcription factor 4
SRC	Proto-oncogene tyrosine-protein kinase Src
SSTR	Somatostatin receptor gene (2 and 5)
STAT	Signal transducer and activator of transcription
SWI/SNF	Switch/sucrose non-fermentable (complex)
TGF	Transforming growth factor
TIMP2	Tissue inhibitor of metalloproteinases 2 gene
TNFRSF25	Tumor necrosis factor receptor superfamily member 25 gene
TOPO1	Topoisomerase I gene
TUBB3	Tubulin beta 3 class III gene
TUBB3	Tubulin beta 3 class III gene
UV	Ultraviolet
VEGFR1/2	Vascular endothelial growth factor receptor 1/2

Introduction

Management of malignant tumors arising or encroaching on the ventral skull base poses a significant challenge to physicians. The density in neurovascular structures with essential functions represents a considerable part of the challenge and leads specialists to deliver the locoregional treatment (i.e., surgery and radiation therapy)

with high precision, by combining an adequate control of the disease with preservation of relevant, uninvolved structures. Another factor contributing to the challenge is the wide range of histologies that can involve the skull base. While some tumors have high sensitivity to non-surgical treatment (e.g., lymphomas), others are associated with dismal prognosis if treated non-surgically (e.g., adenocarcinoma). Thus, treatment based on a reliable diagnosis is paramount to adequate management. On the other hand, a different response to treatment can also be found within the same histology (e.g., sinonasal undifferentiated carcinoma), implying the need for establishing guidance for precision treatment even beyond conventional histopathological diagnosis.

Research in the field of skull base tumors is very active and has identified several pathological features that might serve as prognostic indicators and assist in predicting the response to treatment. The present chapter aims to provide an overview of actual and potential "treatment-driving tumor characteristics" (TDTC) of ventral skull base malignancies, focusing on sinonasal, nasopharyngeal, and bony-cartilaginous tumors. TDTC denoting a more aggressive behavior of the lesion can lead to escalation of locoregional treatment and/or indicate systemic therapy. For instance, surgeons may adjust the extent of intervention by obtaining a wider margin of resection, adopting a less conservative approach towards critical structures (e.g., the orbital cavity), or performing an elective treatment of the neck. Radiation oncologists may tailor the target volume contour and dose delivery based on certain TDTC associated with aggressive local behavior. Moreover, a rational use of systemic agents might be of help even in the curative setting in high-grade malignancies of the sinonasal tract and adjacent areas. On the other hand, when TDTC suggest a more indolent behavior, treatment can be de-escalated to avoid unnecessary morbidity.

Sinonasal Tumors

The sinonasal tract harbors the widest variety of tumor histologies in the human body. Among the malignant lesions, intestinal-type adenocarcinoma (ITAC), squamous cell carcinoma (SCC), olfactory neuroblastoma, mucosal melanoma (MM), adenoid cystic carcinoma (ACC), neuroendocrine carcinomas, and sinonasal undifferentiated carcinoma (SNUC) are the most frequently encountered.

Intestinal-Type Adenocarcinoma

ITAC is a malignant epithelial tumor that mostly takes origin from the olfactory cleft (Fig. 16.1) [1]. Its etiopathogenesis is intimately associated with exposure to dust arising from hardwood, leather or cork working [2, 3]. From a clinicopathological standpoint, cancer-specific prognosis of ITAC is associated with histopathological subtype, stage, and margin status [4]. Surgery followed by adjuvant radiotherapy is

Fig. 16.1 Solid-subtype, sinonasal intestinal-type adenocarcinoma with intracranial extension. **a**, **b** Coronal and sagittal magnetic resonance images depicting the extension of the tumor. **c** Intraoperative image showing the dissection of the intracranial aspect of the tumor (T) and dura mater (D) off the brain surface (B)
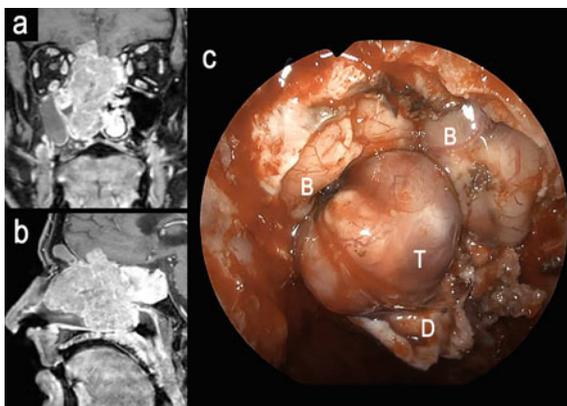
the mainstay of treatment [5, 6]. Unimodal treatment with surgery alone is currently deemed adequate for early-stage, non-high-grade, completely excised ITAC [7]. Five- and 10-year rates of overall survival are 72.7 and 58.0%, respectively [4].

From a pathological-morphological standpoint, grade and subtype are the most relevant features that serve as TDTC for ITAC. Barnes described five types of ITAC: papillary, colonic, solid, mucinous, and mixed [8]. Kleinsasser and Schroeder reported four variants: papillary-tubular cylinder cell, graded from I to III, alveolar goblet, signet-ring cell, and transitional [9]. Overall, papillary and colonic variants (roughly corresponding to papillary-tubular cylinder cell grade I and II) are associated with more favorable prognosis, whereas solid and mucinous subtypes (papillary-tubular cylinder cell ITAC grade III, alveolar goblet, and signet-ring cell) are associated with worse outcomes [4, 10–13]. However, results on the association between ITAC grade/subtype and prognosis are not univocal and this information is frequently not available prior to treatment. Hence, it is unlikely that these morphological classifications can be efficiently used as TDTC unless more reliable subtype markers are discovered. Another morphological feature associated with worse prognosis is tumor budding, a finding described in colorectal oncologic pathology as the presence of isolated single tumor cells or small clusters of up to 5 cells in the tumor stroma. Maffeis et al. and Meerwein et al. found a substantial association between tumor budding and prognosis [14, 15]. Thus, tumor budding might act as TDTC, but data on pre-treatment detectability are currently lacking.

Hermsen et al. found that the total amount of chromosomal alterations is associated with ITAC subtype, with the papillary morphology bearing a significantly lower amount of copy number alterations [16]. This might represent a more reproducible and accessible way to measure tumor aggressiveness prior to treatment. Another interesting finding has been reported by Lopez-Hernandez et al., who clustered a series of ITAC into five groups with different prognoses based on chromosomal gains and losses detected by microarray comparative genomic hybridization [17]. In particular, clusters 1 and 5 had the best and worst prognosis, respectively, while clusters 2, 3, and 4 were associated with an intermediate outcome. Moreover, Rampinelli

et al. reappraised genetic alterations associated with more aggressive behavior, all of which could be used to create a more reproducible signature that stresses the need for intensified treatment [6]. More recently, Re et al. reported that miR-205 and miR-449 overexpression is associated with a higher rate of recurrence in ITAC [18].

Several molecular features of ITAC have been previously highlighted in an attempt to guide systemic treatment. Functional p53 has been demonstrated to predict the response to chemotherapy with cisplatin, 5-fluorouracil, and leucovorin by the group of the "Istituto Nazionale dei Tumori" in Milan, Italy [19–21]. However, this finding has not been confirmed by other authors. In view of its mutational profile, which is associated with a low rate of EGFR, HER2, KRAS, and BRAF mutations and a high rate of EGFR copy number gain, ITAC is theoretically a good candidate for anti-EGFR therapy [22–24]. However, real-life experience is scarce and less encouraging in this regard [25]. Since the gene MET is frequently mutated (64%), MET inhibitors potentially represent an attractive solution [26, 27]. Moreover, a small subgroup of patients with a HRAS mutation (16%) may benefit from the administration of RAS or MAPK/ERK pathway inhibitors [27]. More recently, Schatz et al. reported that EIF2S1 and EIF6, which are potentially targetable markers, are upregulated in ITAC, thus representing a putative TDTC [28]. Finally, Sánchez-Fernández et al. reported that 8 actionable somatic mutations with a respective FDA-approved agent available were found in a series of 48 ITAC [29]. Overall, the role of predictive molecular biomarkers in ITAC is still underexplored and poorly understood, thus making them a potential TDTC although further research is needed.

There are few data on the ITAC-immune system interaction. Specifically, PD-L1 expression in the tumor and infiltrating immune cells have been reported in 17 and 33% of cases, respectively [30]. García-Marín et al. found that the density in CD8+ lymphocytes is associated with prognosis and concluded that, overall, ITAC is a poorly immunogenic tumor with some potential for immune checkpoint inhibitors (ICI) in well differentiated subtypes [31]. Thus, there are no sufficient data to determine a TDTC that suggests immunotherapy should be used in ITAC (Table 16.1).

Squamous Cell Carcinoma

The term SCC of the sinonasal tract groups together different epithelial cancers exhibiting squamous differentiation (Fig. 16.2) [33, 34]. From a pathological standpoint, they are classified into a classical variant, which is further divided into keratinizing and nonkeratinizing, and non-classical subtypes including the adenosquamous, spindle-cell, basaloid, papillary, and verrucous SCC. Surgery combined with (neo)adjuvant therapies is the mainstay of treatment [33]. A large majority of cases are treated with surgery followed by radiation therapy. Unimodal treatment is rarely indicated for well selected early-stage sinonasal SCC [4]. The role of neoadjuvant chemotherapy prior to definitive surgical and/or non-surgical treatment is debated, but there is some evidence on its beneficial role in specific clinical circumstances such

Table 16.1 Potential treatment-driving tumor characteristics (TDTC) of sinonasal intestinal-type adenocarcinoma

TDTC	Putative treatment customization
High-grade, involved margins, and/or high-stage tumor [7]	Indication to adjuvant radiotherapy
Solid, papillary-tubular cylinder cell grade III, alveolar goblet, and signet-ring cell variants [11, 12, 32]	More aggressive locoregional treatment
Tumor budding [14, 15]	
High copy number alterations [16, 17]	
Aneuploidy, 4q32-ter, ANXA2, DCC, H-RAS, MET, MYC, PFOU5F1B, PTP4A3, PTPN1, TIMP2, TIMP3, TP53 [6]	
miR-205 and miR-449 overexpression [18]	
Functional p53 [19–21]	Neoadjuvant chemotherapy with cisplatin, 5-fluorouracile, and leucovorin
MET mutation [26, 27]	MET inhibitors
HRAS mutation [27]	RAS or MAPK/ERK pathway inhibitors
EIF2S1 and/or EIF6 upregulation [28]	Anti-eukaryotic translation initiation factor agents
Mutation of PIK3CA, BRCA1, IDH1, ERBB2, BRAF, KRAS, CDKN2A, NF1 [29]	Use of respective targeted therapies

as orbital encroachment [33, 35–40]. Recently, short-term prognosis was found to be improved in patients with sinonasal SCC undergoing neoadjuvant chemotherapy compared with the standard of care [41]. Five-year survival in SCC suitable for endoscopic surgery-including treatment is 66.2% [4], whereas it decreases to 39.7–44% when considering sinonasal SCC regardless of the type of surgery employed [42, 43].

From a pathological standpoint, there are several features that have been shown to have a prognostic effect. Adenosquamous and spindle-cell SCC are associated with worse outcome, whereas the papillary variant shows better survival [34, 44, 45]. Degree of differentiation was also found to have an impact on prognosis, with well differentiated tumors behaving more indolently [4]. Consistently, inverted papilloma-related SCC is associated with a higher degree of differentiation and better prognosis compared with de novo SCC [46, 47]. NUT carcinoma, which is characterized by monotonous tumor cells and "abrupt" keratinization, is considered by some authors as the most dedifferentiated variant of SCC and, as such, is associated with dismal outcome regardless of treatment intensity (Fig. 16.2) [48–50]. However, BET inhibitors, histone deacetylase inhibitor, and small molecules are currently emerging as novel therapies that may potentially improve the prognosis of this aggressive cancer [51]. Nonkeratinizing and multiphenotypic variants associated with human papillomavirus (HPV) show a more favorable behavior, with the latter displaying
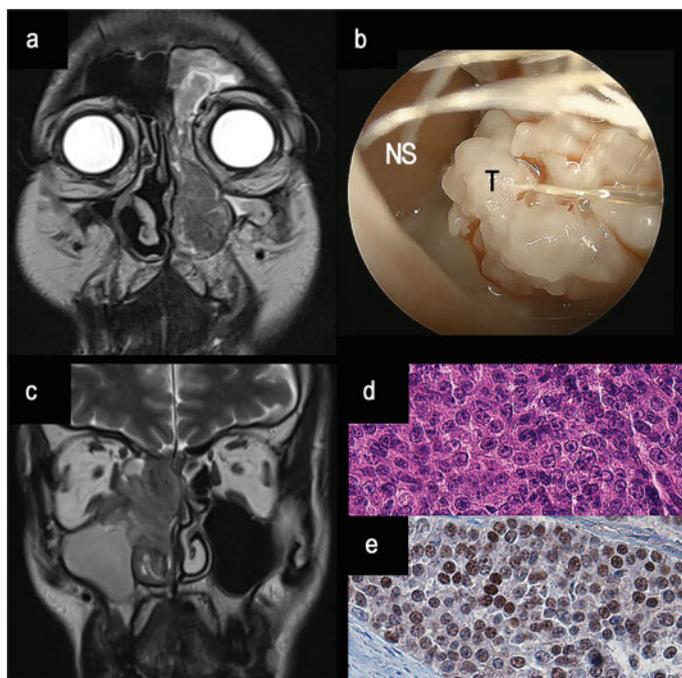
Fig. 16.2 Squamous cell carcinoma of the sinonasal tract. **a**, **b** Preoperative magnetic resonance and intraoperative endoscopic appearance of a moderately differentiated squamous cell carcinoma. **c–e** Pre-treatment magnetic resonance imaging, hematoxylin–eosin-stained slice, and NUT immunohistochemistry of a nasoethmoidal NUT carcinoma with orbital encroachment

noticeable local aggressiveness with limited propensity to distant metastasis [52–57]. Factors associated with nodal metastasis have also been analyzed. High-stage, involvement of the hard palate or superior alveolar ridge, microscopic lymphovascular invasion, and detection of Epstein–Barr virus (EBV) have been associated with a higher risk of nodal metastasis [58–60].

The fact that sinonasal SCC comprises a variety of different tumors has been previously emphasized [34]. This observation has led some authors to suggest a step forward in the way of defining sinonasal SCC, which consists of a molecular classification. Haas et al. classified sinonasal SCC in 4 types: (1) carcinogen-driven; (2) HPV-associated; (3) gene fusion-SCC (i.e., DEK-AFF2 fusion-related SCC); (4) EGFR-altered SCC [61]. Taverna et al. proposed a 6-type classification by (1) dividing HPV-related SCC in monotypic (mainly associated with HPV-16) and multiphenotypic (mainly associated with HPV-33); (2) distinguishing EGFR-altered SCC in lesions with an EGFR gene mutation (in exon 19 or 20) and those with EGFR gene amplification; and (3) reporting SCC with KRAS mutation (mostly arising from oncocytic papillomas) [62]. Besides deepening the current understanding of sinonasal SCC, these molecular classifications might partially serve as TDTC. For

instance, HPV-related sinonasal SCC has been associated with favorable prognosis by several groups [52–57]. Moreover, EGFR-mutated SCC might be targeted with some tyrosine kinase inhibitors [61]. Interestingly, DEK-AFF2 fusion-related SCC shows a relatively aggressive behavior, with high propensity to metastasize in the nodal basin and at distant site, thus suggesting the need for treatment intensification [63, 64].

Several molecular features of SCC have been associated with prognosis, thus representing potential TDTC. For instance, deregulation of microRNAs showed an impact on prognosis: miR-9-5p upregulation was associated with improved survival, and let-7d downregulation and miR-137, miR-21, and/or miR-34a upregulation with decreased survival [65–67]. Overexpression of pS6, CA9, podoplanin and/or TrkB have also been associated with worse outcomes [68–71]. Takahashi et al. revised the most relevant prognostic biomarkers described for head and neck SCC and found that only expression of EGFR was associated with prognosis of sinonasal SCC, with EGFR-positive tumors showing worse outcomes [72].

The SCC-immune system interaction has been analyzed by some authors [73, 74]. PD-L1 expression in more than 5% tumor cells has been found in 30.2% of sinonasal SCC [75]. Similarly, Riobello et al. reported a 34% rate of membranous expression [30]. Despite being based on limited data, the overall response rate and median progression-free survival after ICI treatment were 27.2% and 4.2 months, respectively, which compares favorably with non-sinonasal SCC of the head and neck as reported by Park et al. [76]. The same authors highlighted a trend towards a better response in SCC highly expressing PD-L1, which can thus represent a TDTC (Table 16.2).

Olfactory Neuroblastoma

Olfactory neuroblastoma (ONB) arises from the olfactory neuroepithelium. Hence, it is most frequently located in the olfactory cleft, even if rare cases of ectopic ONB have been reported (Fig. 16.3) [77]. The grade of ONB is classified according to Hyams and substantially affects prognosis [78–80]. Moreover, prognosis of ONB is negatively impacted by advanced age, male gender, locally advanced stage, nodal involvement, and positive margins [4, 81–86]. Classification of local extension is more controversial in contrast to the previously mentioned cancers. Originally described by Kadish et al. in 1976, the classification of ONB local extension has been thoroughly studied and refined [86–89]. Treatment of ONB is based on surgery and adjuvant radiotherapy [4, 86, 90]. Overall, ONB is associated with better prognosis than other sinonasal cancers [4]. This fact substantially affects the type of surgery performed for ONB, which is currently performed with an endoscopic technique in most cases: in early-stage tumors, surgical strategies including unilateral nasoethmoidal resection and/or dura-sparing ablation have been developed [91, 92]; in advanced-stage diseases the threshold to define the tumor as suitable for surgical resection has been pushed to include brain invasion [93, 94]. Adjuvant RT has a positive effect on prognosis in

Table 16.2 Potential treatment-driving tumor characteristics (TDTC) of sinonasal squamous cell carcinoma

TDTC	Putative treatment customization
Adenosquamous or spindle-cell variant [34, 44]	More aggressive locoregional treatment
Moderate or poor differentiation [4]	
De novo SCC (i.e., non-inverted papilloma-related) [46, 47]	
DEK-AFF2 fusion [63, 64]	
Downregulation of let-7d and/or upregulation of miR-137, miR-21, and/or miR-34a [65–67]	
Expression/overexpression of EGFR, pS6, CA9, podoplanin, and/or TrkB [68–72]	
High-stage, involvement of the hard palate or superior alveolar ridge, microscopic lymphovascular invasion, EBV detection, DEK-AFF2 fusion [58–60, 63, 64]	More aggressive regional treatment
EGFR exon 19 or 20 mutation [61]	Use of tyrosine kinase inhibitors
NUT expression [48–51]	Special escalation of locoregional treatment More stringent threshold to define resectable disease Use of BET inhibitors, histone deacetylase inhibitor or small molecules
HPV detection [52–56, 61, 62]	More propensity to indicate curative-intended treatment in borderline cases
PD-L1 expression [76]	Use of immune checkpoint inhibitors

most published series [4, 81, 82, 86]. Intensity-modulated particle beam radiotherapy (IMPT), either alone or as an adjuvant treatment, is being used in several centers for ONB, but no long-term follow-up data are currently available [95–97]. Meerwein et al. performed an individual patient data meta-analysis on 128 patients treated with surgery alone and concluded that carefully selected low-grade, early-stage, and completely excised ONB could be managed unimodally [98]. The indication to elective neck irradiation is still debated, with a remarkable rate of regional failure (including the retropharyngeal site) as the main argument in favor and the absence of positive prognostic effect in terms of overall survival and high percentage of salvageable recurrent cases as main arguments against [86, 90, 99, 100]. The role of chemotherapy is controversial. On the one hand, there is evidence that high-grade ONB display a more frequent response to neoadjuvant chemotherapy [101], while on the other hand recent publications report hints of a potential null-to-negative therapeutic effect of chemotherapy in patients receiving surgery-including treatment [102–104]. Interestingly, Topcagic et al. found some immunohistochemical biomarkers of sensitivity or resistance to chemotherapeutic agents [105]. In particular, ERCC1 underexpression was associated with sensitivity to cisplatin, TOPO1 overexpression

Fig. 16.3 Olfactory neuroblastoma. **a**, **b** Coronal and sagittal magnetic resonance images depicting the extension of a Hyams grade 2 olfactory neuroblastoma. **c** Intraoperative image showing the dissection of the intracranial aspect of the tumor (T) and dura mater (D) off the brain surface (B)
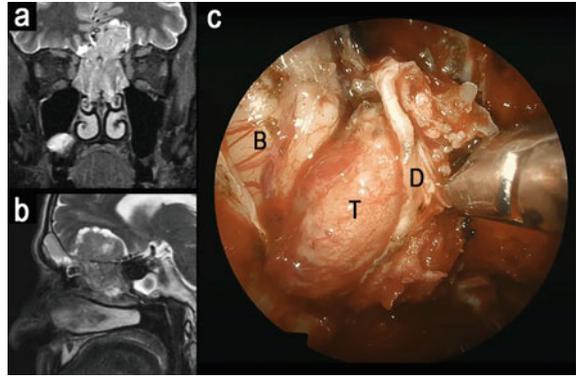
with sensitivity to irinotecan, TUBB3 overexpression with resistance to vincristine, and MRP1 overexpression with multidrug resistance. Of note, the recent evidence that ONB frequently expresses the somatostatin receptor (SSTR-2 in 75–99% and SSTR-5 in 7.5%) [106–109] serves as a rationale to include radioactive somatostatin-analogues (i.e., peptide receptor radionuclide therapy) in the spectrum of treatment options for ONB, which has been shown to be effective in the recurrent/metastatic setting [86, 110].

A commendable step forward in the understanding of ONB was recently published by Classe et al., who presented a thorough, multi-omic analysis of 59 cases [111]. Their study integrated information on exome sequencing, transcriptomics, protein expression-based clustering, methylomics, and immune environment analysis. By comprehensively assessing these data, Classe et al. proposed to subdivide ONB into the "neural" and "basal" types. Neural ONB are not associated with recurrent mutations and are usually well differentiated with a low proliferation index, hypomethylation of neural enhancers, and low density of tumor-infiltrating lymphocytes. Basal ONB can bear TP53 and IDH2 gene mutations, which is associated with the CpG island methylator phenotype, cytokeratin (i.e., cytokeratin AE1/AE3) expression, high proliferation index, DNA hypermethylation, and a high density of tumor-infiltrating lymphocytes. Most importantly, the prognosis of basal ONB is significantly worse than neural ONB. Thus, reliable markers of basal versus neural subtype could serve as TDTC to tailor treatment of ONB. For instance, Wu et al. confirmed that IDH2 mutation is associated with worse prognosis and can be reliably detected with either immunohistochemistry or real-time polymerase chain reaction [112]. Romani et al. profiled the gene expression of 32 ONB and found that some deregulated pathways (i.e., TGF-beta binding, epithelial-mesenchymal transition, UV response, allograft rejection, IFN-alpha response, angiogenesis, IL-2-STAT5, and IL-6-JAK-STAT3 signaling) were associated with reduced disease-free survival [113]. Moreover, they found that ONB with expression of cytokeratin, which could be considered a surrogate of the basal subtype, were associated with E2F targets, MYC targets, and KRAS hallmark pathways alongside with BUB1 gene upregulation, all of which potentially represent a rationale for targeted therapy. Turri-Zanoni

et al. reviewed the prognostic biomarkers of ONB and reported that alteration in the PI3K/mTOR signaling pathway, CDK-dependent cell cycle regulation, CCND1 amplification, FGFR3 amplifications, and DMD gene deletions may have a role in the pathogenesis of ONB [106]. In this regard, Spengler et al. recently reviewed the use of biological agents in ONB and found that sunitinib, cetuximab, bevacizumab, imatinib, everolimus, and pazopanib were all active in adequately selected cases [114].

As for other sinonasal cancers, a number of molecular features of ONB were found to be associated with tumor grade, which is established based on histomorphological features, and thus have prognostic implications. Since grading is subjectively determined and hence flawed by potentially low inter-rater agreement, there is a strong need for prognostic biomarkers associated with grade that would be usable as TDTC. Ki67 proliferation index $\geq 25\%$ and low microvascular density are associated with high grade and worse survival [115–117]. SATB2 was found to reliably segregate grade 4 versus grade 1–3 ONB according to Hyams [118].

The interaction between ONB and the immune system is still poorly understood. Friedman et al. reported that ONB is associated with a low tumor mutational burden, hence suggesting a limited utility of immunotherapy [119]. However, there is evidence that both primary and metastatic ONB tissue express PD-L1 and display an associated tumor and stromal infiltrate of PD-1-positive and CD8-positive lymphocytes [120]. Interestingly, the higher the density in tumor-infiltrating lymphocytes the worse the prognosis [111], which places ONB in the minority of tumors, such as renal cell carcinoma, with an inverse relationship between immune infiltrate and prognosis [121]. To date, there is still insufficient evidence to support the use of immunotherapy in ONB (Table 16.3).

Mucosal Melanoma

Sinonasal MM is one of the most aggressive tumors in the wide spectrum of sinonasal malignancies (Fig. 16.4). This is clearly witnessed by the experience of the Memorial Sloan Kettering Cancer Center (New York, US): Flukes et al. recently showed that treatment outcomes remained stable over the last 2 decades despite the increasing use of immunotherapy [122]. T staging of this tumor reflects its intrinsic aggressiveness, with T3 representing the minimum category attributable to any MM of the head and neck. Of note, even an in situ (i.e., intraepithelial) MM is classified as T3 according to the latest TNM criteria. The detrimental prognostic effect of paranasal sinus involvement is well known, and Lechner et al. proposed a revision of T classification, with tumors involving the epithelium or submucosa of paranasal sinuses with no bony-cartilaginous, deep soft tissue or skin invasion to be classified as T4a instead of T3 [123]. Similarly, Moya-Plana et al. suggested that anatomical criteria applied to stage non-melanoma sinonasal cancers could be useful for MM [124]. Surgery is considered the mainstay of treatment and there is evidence that can be safely performed through an endoscopic transnasal approach if adequately indicated and as long as

Table 16.3 Potential treatment-driving tumor characteristics (TDTC) of olfactory neuroblastoma

TDTC	Putative treatment customization
Grade 3–4 according to Hyams [78–80, 101]	More aggressive local treatment Indication to neoadjuvant chemotherapy[a]
Ki67 proliferation index ≥25%, low microvascular density, SATB2 expression, high tumor-infiltrating lymphocytes density [111, 115–118]	More aggressive local treatment
ERCC1 underexpression [105]	Cisplatin-containing chemotherapeutic regimen[a]
TOPO1 overexpression [105]	Irinotecan-containing chemotherapeutical regimen[a]
TUBB3 overexpression [105]	Chemotherapeutic regimen without vincristine[a]
MRP1 overexpression [105]	Avoidance of chemotherapy should be considered owing to multidrug resistance[a]
SSTR-2 and/or SSTR-5 expression [86, 107–110]	Use of ^{177}Lu-DOTATATE or other radioactive somatostatin analogues[b]
Direct or surrogate evidence of basal ONB (e.g., cytokeratin AE1/AE3 expression, Ki67 proliferation index ≥25%, and/or evidence of mutated IDH2) [111–113]	More aggressive local treatment

[a] Indication to chemotherapy is still debated as there is no recent evidence supporting its use other than the fact that high-grade olfactory neuroblastomas are more likely to respond
[b] Recent evidence suggests nearly constant expression of SSTR-2, implying it can be potentially removed from the list of TDTC

negative margins can be achieved [4, 125–131]. The role of adjuvant radiotherapy is debated. While some studies have demonstrated an increased local control in patients treated with surgery and adjuvant radiotherapy, no clear evidence of benefit on overall survival is currently available [132–138]. Grant-Freemantle et al. recently published a meta-analysis on 2489 patients with MM and found that adjuvant radiotherapy had a significant positive effect on local control and overall survival when considering head and neck sites together, whereas this effect was lost when the analysis focused on sinonasal localizations [139]. Neoadjuvant treatment with hyperfractionated radiotherapy and concomitant chemotherapy with weekly cisplatin followed by surgery has been proposed by Hafström et al. [140]. They reported a 5-year overall survival rate of 70% in patients with MM stage IVA. However, this represents a single-center experience and further research is required to establish whether this treatment sequence confirms to have an advantage on survival. Interestingly, the Japan Carbon-Ion Radiation Oncology Study Group reported a 2-year overall survival of 69.4% in a series of 260 patients affected by head and neck MM treated with definitive carbon-ion therapy [141], which compares favorably with other large series on patients who underwent curative treatment including surgery [4, 122, 137]. Of note, in the Japanese study a multivariable-confirmed positive prognostic effect of
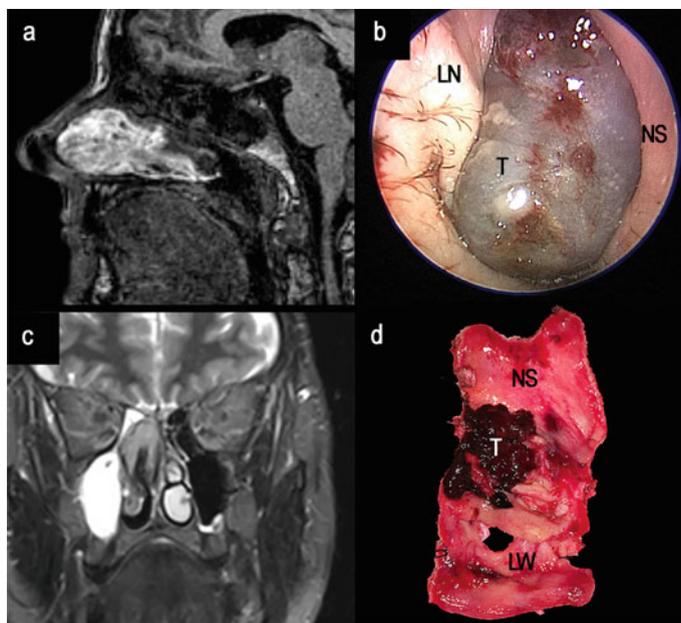
Fig. 16.4 Sinonasal mucosal melanoma. **a** T1-weighted magnetic resonance image demonstrating the spontaneous hyperintensity displayed by certain melanotic mucosal melanomas. **b** Endoscopic appearance of a melanotic mucosal melanoma of the right nasal cavity. **c, d** Preoperative magnetic resonance imaging and main surgical specimen of a case of mucosal melanoma of the right nasal cavity. LN, *limen nasi*; LW, lateral wall of the nasal cavity; NS, nasal septum; T, tumor

concomitant chemotherapy with DTIC, with/without nimustine hydrochloride and vincristine, was demonstrated, with 2-year overall survival increasing to 75.8% (of note, these MM either were deemed unresectable or patients refused surgery) [141]. This benefit came at the cost of an increased mucosal toxicity, though no patient interrupted radiotherapy because of mucositis.

The remarkable rate of distant metastasis regardless of the stage at presentation [4, 142] makes MM similar to a systemic tumor, leading several authors to conclude that systemic therapy should be part of the future standard of care even in the curative setting [143]. Since tumor volume has been demonstrated to predict the risk of distant metastases and death, then it could be employed as a TDTC to implement systemic therapy in initial treatment [141, 144]. Currently, chemotherapy has a limited role in the management of MM, especially in the curative setting [145]. However, in a phase II randomized clinical trial by Lian et al., the temozolomide and cisplatin adjuvant chemotherapy outperformed surgery alone and surgery followed by adjuvant IFN therapy, with 2-year recurrence-free survival of 41.0, 0.0, and 11.7%, respectively [146]. This study corroborated the concept that systemic therapy has a substantial role in determining the prognosis of MM patients, as emphasized by other authors [147]. Several biotherapeutics and immunotherapies are emerging as

potential systemic agents for MM [148]. Among these, imatinib [149] and binimetinib (although evidence exists for skin melanoma) [150] in KIT-mutated and NRAS-mutated MM, respectively, are the most promising ones. In terms of frequency, NRAS is more often mutated compared to KIT and BRAF (22–30% versus 5–12.5% and 0–8%, respectively) and is associated with poorer survival [131, 151–153]. A recent study found a higher rate of BRAF mutation (32%) [154]. The use of immunotherapy is controversial. The majority of data on immunotherapy for sinonasal MM are gathered from recurrent/metastatic cases. Recently, Ganti et al. published a National Cancer Database study demonstrating that immunotherapy had a positive prognostic effect in patients with metastatic disease [155]. In their study, they included all patients registered in the National Cancer Database between 2004 and 2015. Since ICI were approved for solid tumors by the FDA in 2012, a considerable proportion of patients included in their study received a non-ICI-based immunotherapy, which is most likely represented by IFN therapy. Subsequently, Klebaner et al. published a National Cancer Database study that narrowed the inclusion period to 2012–2015, so that most patients who received immunotherapy were treated with ICI [156]. Surprisingly, they did not demonstrate a positive prognostic effect of immunotherapy on metastatic sinonasal MM patients. Therefore, one could hypothesize that the beneficial role of IFN therapy outweighs that of ICI in MM patients. Consistently, Sun et al. demonstrated increased survival in patients treated with subdermal injection of either the bacillus Calmette–Guérin, or IL-2 or IFN-α-2b [157]. However, Lechner et al. recently published a multi-institutional study on 505 sinonasal MM patients and demonstrated that therapies including ICI conferred the highest survival rate in the recurrent/metastatic setting [123]. When focusing on specific agents and their combination, the most relevant data come from the pooled analysis by D'Angelo et al., who reported an objective response rate to nivolumab, ipilimumab, and their combination of 23.3, 8.3, and 37.1%, respectively (versus 40.9, 21.2, and 60.4% in skin melanoma, respectively) [158]. However, response can be dissociated, as described by Chao et al., who reported on 4 patients with distant metastases at presentation treated with immunotherapy, out of which 2 had a response and 1 stability of the distant metastases but all had progression of disease at the primary site [159]. On the other hand, Philipp et al. reported a case of initial pseudoprogression with subsequent complete response to combined ipilimumab and nivolumab in a patient affected by an inoperable sinonasal MM [160]. Intrinsic or acquired resistance to immunotherapy is poorly understood in MM but a recent publication reported 3 cases with switch of oncogenic driver (i.e., from KRAS, KIT, or no driver to NRAS) as the mechanisms determining acquisition of resistance to ICI [161]. Thus, the role of ICI remains controversial, and more studies are needed to determine whether they can provide a prognostic advantage.

Elective treatment of the neck is another controversial aspect of MM. A recent publication from the MD Anderson Cancer Center (Houston, Texas, US) on 198 patients treated over a 31 year timespan demonstrated that the rate of nodal recurrence in initially node-negative patients was 17 and 18% in patients receiving elective neck adjuvant radiotherapy and in those who did not, respectively [162]. In a meta-analysis

on 939 patients, De Virgilio et al. found a similar 17.0% regional recurrence rate in patients with clinically negative, untreated neck [163].

Despite the prognosis of MM is overall poor, some pathologic and molecular prognostic markers have been identified. Ki67 proliferation index >30% and lymphovascular invasion have been associated with decreased survival [164]. Overexpression of PARP1 and IDO1 were found to have a negative effect on overall survival, which lead to hypothesize the use of PARP1- and/or IDO1-inhibitors in this subset of MMs [165]. Similarly, overexpression of phosphorylated Akt1 has been associated with increased cancer-specific mortality [166]. In turn, the Akt pathway was found to be inhibited by the miR-4633-5p molecule, whereof loss of expression was associated with an increased risk of metastasis [167]. Mutations in the NF1 gene (found in 33% of MM) have also been associated with decreased survival [168].

Recent studies have contributed to increase the understanding of the crosstalk between immune system and sinonasal MM. Yin et al. performed a comprehensive analysis of 44 MM and found that immunotype was substantially associated with prognosis [147]. They classified tumors in terms of tumor-infiltrating immune cell density, according to Erdag et al. [169], and found that cases with complete depletion of immune cells (32% of their series) had dismal prognosis, those with diffuse immune cell infiltration (18% of their series) were all progression-free, and those with immune cells mainly concentrated in the stroma and perivascular tissue (50% of their series) had an intermediate prognosis, which is consistent with the fact that "brisk" tumor-infiltrating lymphocytes have been associated with improved outcome by other authors [164, 170]. They also found that CD8$^+$ T cells and NK cells were positively associated with prognosis, and Th2 T cells and M2 macrophages with disease progression [147]. Interestingly, expression of PD-L1 was not associated with prognosis [171].

As a final remark, despite some TDTC can be hypothesized for MM, the dismal outcomes associated with this tumor should orient research towards the identification of therapeutic strategies that are capable of improving the overall prognosis, whereas a precision medicine-approach has to be more realistically postponed until a more effective standard of care is available (Table 16.4).

Sinonasal Undifferentiated Carcinoma and SWI-SNF-Deficient Sinonasal Carcinomas

SNUC was first described by Frierson et al. in 1986, and represents one of the most fascinating entities in the field of sinonasal pathologies (Fig. 16.5) [172]. Being a diagnosis of exclusion, SNUC initially served as a basket entity so that several tumors that were difficult to define by histopathology were misdiagnosed as SNUC [173]. Treatment outcomes of patients affected by SNUC have been historically poor, but reached a first turning point with the evidence that multimodal therapy was key in improving prognosis [174–182]. More recently, the seminal paper by Amit et al.

Table 16.4 Potential treatment-driving tumor characteristics (TDTC) of mucosal melanoma

TDTC	Putative treatment customization
Tumor volume ≥5 [144] or ≥25.4 [141] cm^3 at pretreatment imaging (2 different cutoffs have been proposed)	Indication to adjuvant chemotherapy
Ki67 proliferation index >30%, lymphovascular invasion, overexpression of phosphorylated Akt1, downregulation of miR-4633-5p, mutation of NF1 [164, 166–168]	More aggressive treatment (i.e., more aggressive surgery, indication to adjuvant radio- and/or chemotherapy)
KIT mutation [149]	Use of imatinib
NRAS mutation [150, 153, 161]	Use of binimetinib More aggressive treatment (i.e., more aggressive surgery, indication to adjuvant radio- and/or chemotherapy) Avoidance of immune checkpoint inhibitors
Overexpression of PARP1 and/or IDO1 [165]	Use of PARP1- and/or IDO1-inhibitors More aggressive treatment (i.e., more aggressive surgery, indication to adjuvant radio- and/or chemotherapy)
Poorly represented immune cell infiltrate, low density of CD8$^+$ T cells, low density of NK cells, high density of Th2 T cells, high density of M2 macrophages [147, 164, 170]	More aggressive treatment (i.e., more aggressive surgery, indication to adjuvant radio- and/or chemotherapy)

reported that response to neoadjuvant chemotherapy is the most reliable factor in selecting the definitive treatment strategy [183]. In their study, the authors from MD Anderson Cancer Center (Houston, Texas, US) included 95 treatment-naïve patients affected by SNUC, all treated with neoadjuvant chemotherapy followed by either definitive chemoradiation or surgery and adjuvant (chemo)radiotherapy. They found that in the group of responders to chemotherapy (n = 64, 67%), 5-year disease-specific survival was 81% in patients treated with definitive chemoradiation and 54% in those treated with surgery and adjuvant (chemo)radiotherapy. Thus, definitive non-surgical treatment is associated with significantly better outcomes in responders. In contrast, in the group of non-responders to chemotherapy (n = 31, 33%), 5-year disease-specific survival was 0 and 39% in patients treated with definitive chemoradiation and those treated with surgery and adjuvant chemoradiation, respectively. Hence, the majority of patients benefit most from induction chemotherapy followed by chemoradiation, but 1 out of 3 patients should be treated with upfront surgery and subsequent chemoradiotherapy to confer the highest chance of survival. In this sense, response to neoadjuvant chemotherapy is the most relevant TDTC in SNUC and several other oncologic centers have conformed with a neoadjuvant chemotherapy-driven approach [4, 184]. Takahashi et al. discovered that a 24-gene signature is able to predict response to chemotherapy with cisplatin and etoposide in SNUC [185]. The potential practical implications of this signature would be of
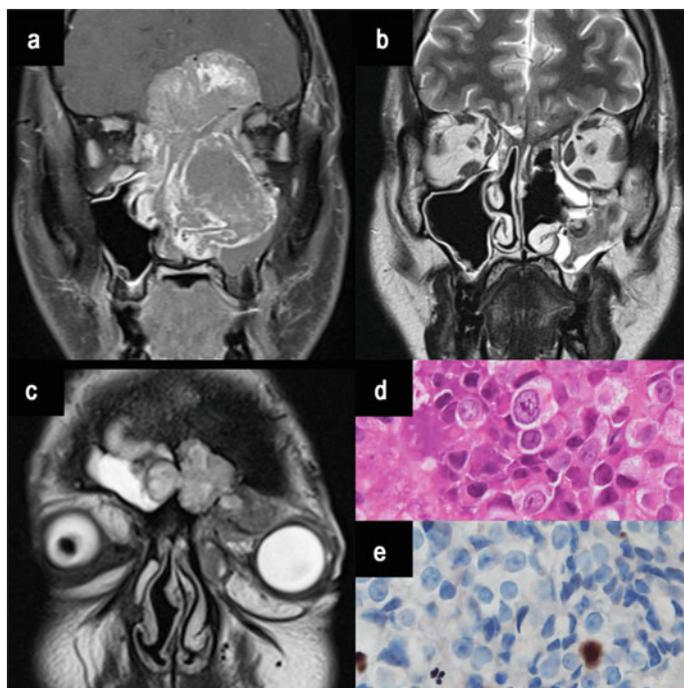
Fig. 16.5 Sinonasal undifferentiated carcinoma and SWI-SNF-deficient carcinomas. **a**, **b** Pre-treatment and post-neoadjuvant chemotherapy magnetic resonance images showing a partial response in a case of sinonasal undifferentiated carcinoma with no association to specific molecular identifier. The patient was treated with definitive chemoradiation with complete response. **c–e** Pre-treatment magnetic resonance imaging, hematoxylin–eosin-stained slice, and SMARCB1 immunohistochemistry of a fronto-orbital SMARCB1-deficient carcinoma

high interest in non-responders (i.e., patients to be treated with surgery and adjuvant therapy according to the response-driven paradigm), who could avoid the non-negligible toxicity of neoadjuvant chemotherapy and directly undergo locoregional treatment. Whether responders could skip neoadjuvant chemotherapy and be sent directly to chemoradiation remains doubtful, as this would mean de-escalating the treatment schedule that led to the aforesaid outcomes [183]. Of note, Lehrich et al. published a National Cancer Database study on 440 SNUC patients demonstrating that neoadjuvant chemotherapy does not have an impact on survival, which could suggest that if chemotherapy does not lead to response-driven selection of definitive treatment, then the benefit of its employment is lost [186]. On the other hand, a French multi-institutional study demonstrated that neoadjuvant chemotherapy is an independent protective factor in terms of recurrence-free survival [187]. Regarding neck management, there is evidence that the elective treatment of the nodal basin significantly reduces the rate of regional recurrence from 26.4 to 3.7% [188].

A subset of SNUC, accounting for 20–47% of tumors, is associated with HPV-16 and shows increased survival in one study [189, 190]. Several authors found mutations in the IDH1/2 genes in SNUC, with a prevalence ranging between 35 and 82% [191]. This genetic characteristic, particularly for the IDH2 gene, has been associated by some authors with a more favorable outcome compared to IDH2-wild type SNUC [192–194]. Libera et al., however, found that IDH2-mutation was associated with decreased survival in a series of 53 poorly differentiated sinonasal carcinomas including 6 SNUC [195]. SWI/SNF-deficiency has been discovered as the genetic hallmark of a very aggressive group of tumors originally considered as a subset of SNUC (Fig. 16.5) [196–200]. In particular, SMARCB1-deficient and SMARCA4-deficient carcinomas are two malignancies that were initially described under the umbrella of SNUC but displaying an exquisitely aggressive behavior, which might lead them to be considered as separate entities with respect to "true SNUC" [201, 202]. Interestingly, targeting EZH2 and CDK4/6 proved effective in preclinical models of SWI/SNF-deficient ovarian and lung cancer [203, 204]. Moreover, there is evidence that SWI/SNF-deficiency is associated with remarkably increased response to ICI in patients with colorectal cancer [205]. These findings could drive future research and lead to the discovery of systemic agents to effectively target SWI/SNF-deficient sinonasal carcinomas.

Takahashi et al. demonstrated that the ERBB2 gene is amplified and HER2 over-expressed and phosphorylated in SNUC [206]. They also showed that lapatinib efficiently inhibits HER2 signaling pathway in a SNUC cell line. Bell et al. reported that BRCA1 is overexpressed in SNUC, thus demonstrating a biological rationale for the use of PARP inhibitors in this cancer [207].

There are no substantial data on the use of immunotherapy for SNUC. However, a case of metastatic SNUC with complete response to nivolumab has been reported [208]. Interestingly, PRAME, which is a candidate target for immunotherapy, was found to be overexpressed in SNUC (Table 16.5) [207].

Sinonasal Neuroendocrine Carcinomas

Neuroendocrine neoplasms of the sinonasal tract are rare and poorly understood tumors. Their rarity alongside with the fact that several other sinonasal malignancies can display neuroendocrine features has contributed to the heterogeneity in different series [209–211]. The nomenclature of sinonasal tumors will be revised in the upcoming 5th Edition of the World Health Organization Classification of Head and Neck Tumors [212]. Specifically, neuroendocrine neoplasms of the upper aerodigestive tract and salivary glands will be classified into well differentiated (referred to as "neuroendocrine tumors" and further classified in grade 1–3 according to mitotic activity) and poorly differentiated (referred to as "neuroendocrine carcinomas" and further classified in "small cell" and "large cell"). A proportion of neuroendocrine neoplasms, which can be as high as 44.4% in areas characterized by endemic nasopharyngeal carcinoma, is represented by post-irradiation cancers [213]. Anecdotally,

Table 16.5 Potential treatment-driving tumor characteristics (TDTC) of sinonasal undifferentiated carcinoma

TDTC	Putative treatment customization
Partial or complete response to neoadjuvant chemotherapy [183]	Indication to definitive chemoradiation
Stable disease or progression of disease after neoadjuvant chemotherapy, possibly inferred based on deregulation of expression of the following genes: HSPB1, ALDH1A3, LAMB4, IL20, ALPL, NR4A3, FBXO5, KRT14, DNTT, CCL15, ITGB4, LAMC2, CD19, CDC34, DNAJB8, EFNA2, FGF20, SIX1, IL9, CCL1, HSPB7, CENPF, GNA11, TNFRSF25 [183, 185]	Indication to surgery and adjuvant chemoradiotherapy
HPV-negative SNUC [189]	More aggressive treatment
IDH2-wild type SNUC [192–194]	
SMARCB1-deficient and SMARCA4-deficient carcinoma [196–200, 205]	More aggressive treatment More stringent threshold to define resectable disease Use of immune checkpoint inhibitors
HER2 overexpression [206]	Use of anti-HER2 agents
BRCA1 overexpression [207]	Use of PARP1-inhibitors

Fig. 16.6 Sinonasal small cell neuroendocrine carcinoma. **a–c** Pre-treatment magnetic resonance imaging, hematoxylin–eosin-stained slice, and chromogranin immunohistochemistry of a nasoethmoidal small cell neuroendocrine carcinoma
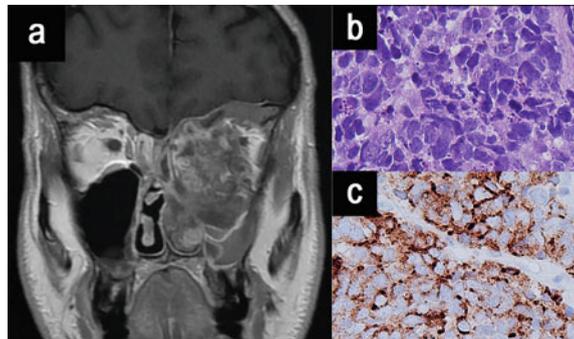
neuroendocrine carcinoma has been reported as part of a collision tumor including either SCC or exocrine adenocarcinoma [214, 215].

Prognosis of sinonasal neuroendocrine neoplasms is poor, with 3-, 5-, and 10-year overall survival of 42.4, 38.9, and 34.0%, respectively, according to the MUSES study [4]. Van der Laan et al., however, showed that well and moderately differentiated neuroendocrine carcinomas are associated with a 5-year disease-specific survival of 70.2%, in contrast to 46.1% in the small cell variant, which is consistent with what reported by other authors (Fig. 16.6) [210, 216]. Moreover, small cell neuroendocrine carcinoma of the sinonasal tract showed better survival outcomes compared with other sites of the head and neck [217]. Large cell neuroendocrine carcinoma

of the sinonasal tract is exceedingly rare and a possible relation with HPV infection has been reported [218]. Interestingly, Dogan et al. recently surmised that large cell neuroendocrine carcinoma and IDH2-mutated SNUC constitute a phenotypic spectrum of the same tumor entity [193]. There is consensus on the fact that treatment of sinonasal neuroendocrine neoplasms should be multimodal [219–223]. However, the best sequence of treatment and the indication for (neo)adjuvant non-surgical therapies are debated. Response to neoadjuvant chemotherapy is a strong positive prognostic factor [220]. Turri-Zanoni et al. showed that patients treated with neoadjuvant chemotherapy, which was indicated in poorly differentiated neuroendocrine carcinomas, had a 5-year overall survival rate of 88.8% compared to 9.3% in those who were not [210]. On the other hand, van der Laan et al. did not find a benefit of chemotherapy in the treatment of sinonasal small cell neuroendocrine carcinomas [216]. As for other tumors with neuroendocrine phenotype, use of ^{68}Ga-DOTATATE PET-CT and ^{177}Lu-DOTATATE has been reported for staging and treatment purposes, respectively [224, 225].

Overall, neuroendocrine neoplasms of the sinonasal tract are poorly understood and the best treatment strategy is still matter of research. Thus, no TDTC can be currently determined for this histology.

Adenoid Cystic Carcinoma

ACC is a rare and capricious cancer that arises from major and minor salivary glands. For its almost invariable tendency to perineural spread, management of ACC represents a challenge in a nerve-dense area such as the skull base (Fig. 16.7). Grade is intimately associated with prognosis and depends upon the presence and proportion of a solid histological architecture within tumor tissue [226–229]. Whenever feasible, surgical resection followed by adjuvant radiotherapy is considered the standard of care [5]. A variant with squamous differentiation features and predilection for involvement of the sinonasal tract and skull base, called "metatypical ACC", has been recently described, with diagnosis of ACC being corroborated by the identification of the fusion between MYB/MYBL1 and NFIB, which is typical of this cancer [230, 231]. Even if only 3 cases have been described, this variant seems to behave more aggressively. ACC with "high-grade transformation" is another distinct entity, characterized by faster progression and propensity to metastasize to the neck [232]. The sinonasal tract is among the most frequent sites of origin of this variant [232].

The University of Pittsburgh (Pittsburgh, Pennsylvania, US) group judiciously stated that the realistic aim of surgery in sinonasal/nasopharyngeal ACC is gross total resection rather than microscopically clear margin resection, which can be rarely achieved in this histology [233]. This approach is consistent with the evidence that margin status does not appear to be an independent factor associated with survival in ACC of the minor salivary glands of the head and neck and sinonasal tract [234, 235]. On the other hand, this aspect is debated and the philosophy of the University of Pittsburgh group should not be misinterpreted: whenever negative margins can be
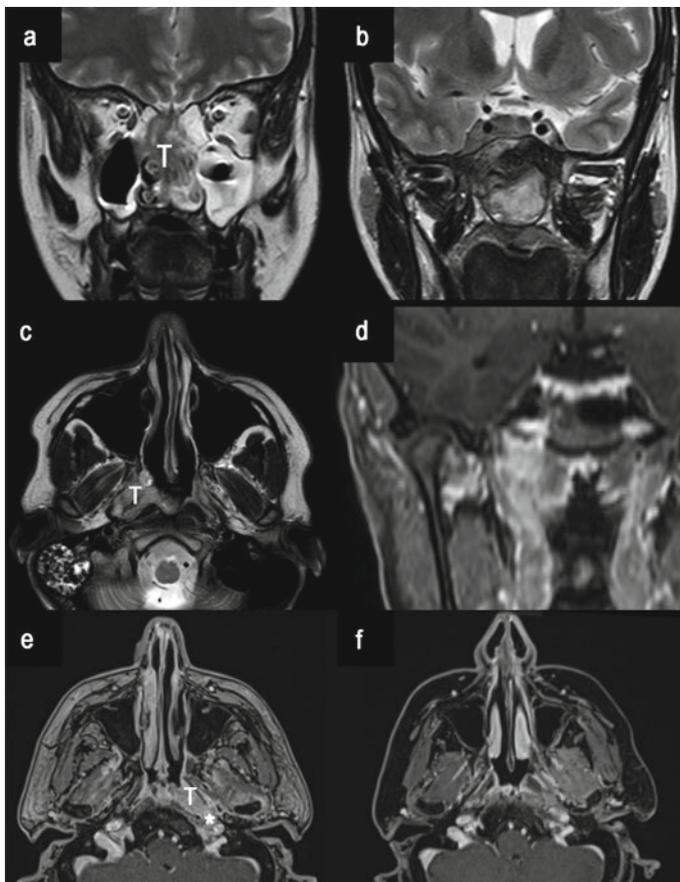
Fig. 16.7 Sinonasal and nasopharyngeal adenoid cystic carcinoma. **a**, **b** Preoperative magnetic resonance imaging of a nasoethmoidal solid-type adenoid cystic carcinoma with involvement of the sphenoid sinuses and right cavernous sinus. **c, d** Preoperative magnetic resonance imaging of a nasopharyngeal cribriform-type adenoid cystic carcinoma with abutment of the petrous internal carotid artery. **e, f** Pre- and post-treatment magnetic resonance imaging of a cribriform-type nasopharyngeal adenoid cystic carcinoma with involvement of the external carotid and jugular foramina (asterisk). The patient was treated with proton beam radiotherapy. T, tumor

realistically achieved while avoiding unreasonable morbidity they should be pursued as for any other cancer deemed suitable to curative surgery [236, 237].

The role of adjuvant radiotherapy is debated. Unsal et al. published a study on 694 patients collected from the National Cancer Database, reporting that patients treated with surgery alone had better prognosis compared to those treated with surgery and adjuvant radiotherapy [238]. However, this result was not assessed with a multivariable model, and is potentially biased by the fact that patients for whom adjuvant radiotherapy was indicated could bear risk factors associated with worse prognosis,

such as advanced stage [239]. Overall, surgery followed by adjuvant radiotherapy is the most frequent and effective treatment strategy, at least in terms of local control, according to a systematic review with meta-analysis and single-center series [240–242]. While intensity-modulated particle therapy has shown encouraging results, the duration of follow-up of published series is not sufficient to draw clear conclusions on its added value in ACC treatment [243–247].

Song et al. showed that elective irradiation of the neck conferred no benefit in terms of overall and progression-free survival in a cohort of 166 sinonasal ACC [248]. Wang et al. reported a similar finding with a propensity score matching approach, similar to the International Head and Neck Scientific Group review of 774 patients [249, 250]. Likewise, elective neck dissection did not show to provide a prognostic advantage [251]. On the contrary, elective neck treatment has been suggested for ACC with "high-grade transformation" [252].

Given the remarkably high recurrence rate, disease-specific mortality, and occurrence of distant metastases, several efforts have been performed to identify effective systemic treatments. Atallah et al. reviewed the relevant literature and reported that the following targeted agents have been tested to date: axitinib, bortezomib, cetuximab, dovitinib, everolimus, gefitinib, imatinib, lapatinib, sorafenib, sunitinib, vorinostat, crenigacestat, and lenvatinib [253]. Although a considerable rate of stable disease has been observed with some of these drugs (up to 85–90% with sunitinib, vorinostat, or cetuximab), the pooled rate of patients with partial response was only 18/438 (4.1%). In the same review, the pooled rate of responders to chemotherapy was 32/222 (14.4%). The NOTCH pathway was found to be frequently altered in ACC, particularly in those not bearing a MYB-involving fusion [254]. The genes NOTCH1-3 and SPEN are the most frequently mutated [255]. From a genetic standpoint, Ho et al. classified ACC in 4 clusters: (1) ACC with both MYB and NOTCH1 mutated, (2) ACC with MYB mutated and NOTCH1 wild type, (3) ACC with MYB wild type and TERT mutated, and (4) ACC with MYB wild type and NOTCH1 mutated [256]. Wang et al. demonstrated that the solid component of ACC, which predicts poor prognosis [226–229, 257], is intimately associated with NOTCH pathway deregulation, which could represent the key mechanism of aggressive clone selection in this cancer [255]. Consistently, Xie et al. showed that the NOTCH1-HEY1 pathway is associated with epithelial–mesenchymal transition of ACC [258]. Thus, one could hypothesize that using NOTCH inhibitors is critical in improving outcomes of ACC patients. However, objective response has been observed in only 0–17% patients treated with drugs targeting the NOTCH pathway [259, 260]. Moreover, with an immune-excluded microenvironment, M2-polarized macrophages, high density of myeloid-derived suppressor cells, and low mutational load, ACC is also an unpromising candidate to immunotherapy [261]. Indeed, unsatisfactory response rates to ICI have been reported [262]. Overall, there is currently no effective systemic therapy for ACC, since the large majority of molecular alterations found in this cancer are not actionable and the proportion of patients eligible for effective targeted therapy is currently low [256, 263].

Table 16.6 Potential treatment-driving tumor characteristics (TDTC) of sinonasal and nasopharyngeal adenoid cystic carcinoma

TDTC	Putative treatment customization
High grade (i.e., presence and amount of solid component) [226–229, 257]	More aggressive treatment
Metatypical variant [230]	
NOTCH mutation/pathway deregulation [254–256]	
High-grade transformation variant [232, 252]	More aggressive treatment Indication to elective neck treatment

In conclusion, ACC of the sinonasal tract represents a distinct challenge and the current potential TDTC are limited to those characteristics associated with more aggressive behavior or an unusual propensity to nodal metastasis (Table 16.6).

Nasopharyngeal Tumors

Nasopharyngeal carcinoma (NPC), with its 3 variants according to the World Health Classification (nonkeratinizing SCC, keratinizing SCC, basaloid SCC), is by far the most common malignancy involving this area. Other histologies, such as carcinomas originating from minor salivary glands and the entity called "low-grade nasopharyngeal papillary adenocarcinoma" (LGNPPA) are only rarely observed [264].

It is well established that the treatment of choice for NPC is radiotherapy alone, preferably in the form of intensity-modulated radiotherapy (IMRT), in stage I–II disease and chemo-radiotherapy in stage III–IVA. The selection of patients to receive chemotherapy as induction or adjuvant treatment is a therapeutic area that is currently being explored [265]. Surgery can have a role in the treatment of selected persistent/recurrent lesions as an alternative to re-irradiation. A recent multicenter, randomized, phase 3 trial including 200 patients with recurrent NPC confined to the nasopharyngeal cavity, post-naris or nasal septum, superficial parapharyngeal space, or the base wall of the sphenoid sinus, has shown that endoscopic surgery significantly improved overall survival compared with IMRT [266]. In the present chapter, the discussion will focus on those tumors whose treatment is less standardized compared to NPC, in view of their rarity and the variable response to (chemo)radiation.

According to the largest single-institution study on 28 patients, LGNPPA affects subjects with an average age of 41.5 years, with a preference for females [267]. A consistent number of LGNPPA is pathologically characterized by a papillary growth, and may mimic papillary thyroid carcinoma, thus being called "thyroid-like" LGNPPA. Evidence of a transition from the mucosal surface to the tumor, predominance of stratified nuclei, negativity for thyroglobulin, and absence of thyroid lesions at imaging studies are all criteria favoring a diagnosis of thyroid-like LGNPPA [268].

The continuity of LGNPPA tumor cells with positive cytokeratin staining indicates that the lesion arises from the surface mucosal epithelium rather than from submucosal seromucinous glands [269]. In view of its tendency to present as a polypoid lesion with superficial growth, and no spread to regional lymph nodes and distant sites, LGNPPA is ideally amenable to surgery, and many of the cases reported in the literature have been successfully treated with transnasal endoscopic surgery [267]. The role of radiotherapy is questionable. Mutations in KRAS, NRAS, BRAF, EGFR, and ALK have been so far excluded [267].

Among minor salivary gland cancers, ACC, adenocarcinoma not otherwise specified, and mucoepidermoid carcinoma (MEC) are the most frequent histologies [270].

Similar to sinonasal localizations, ACC of the nasopharynx invariably presents at an advanced local stage, with frequent radiologic signs of perineural spread, bony permeation of the clivus and temporal bone, and critical relationships with the internal carotid artery (Fig. 16.7). In view of these features, radical resection can be rarely achieved so that surgery, if technically feasible, generally leaves behind microscopic or macroscopic disease. However, a population-based analysis of 383 patients with minor salivary gland cancers extracted from the Surveillance, Epidemiology, and End Results Program (165 of which were ACC) demonstrated that any form of treatment schedule including surgery provided high 5-year disease-specific survival [270]. Of note, as in any retrospective large dataset analysis, the bias of treatment selection in relation to tumor extent should not be neglected. In a recent retrospective review on the role of endoscopic transnasal surgery in treatment of 30 patients with ACC of the sinonasal tract and nasopharynx, the University of Pittsburgh (Pittsburgh, Pennsylvania, US) group concluded that tumor grade has a significant impact on prognosis [233]. They recommended endoscopic resection followed by radiotherapy for low-grade tumors and suggested that intermediate/high grade tumors might benefit from novel treatment strategies. Regarding radiotherapy, there is a need to demonstrate the superiority of particle therapy over IMRT, in terms of local control of the disease as well as morbidity, with a high level of evidence. For medical therapy, the limited effect of chemotherapeutic agents is well known, while there is still a lack of drugs effectively targeting the numerous molecular alterations, as described in the section on sinonasal tumors (Table 16.6).

MEC is classically divided into low-grade, intermediate-grade, and high-grade, with grading having a significant impact on prognosis. The tumor is frequently associated with a t(11;19)(q14–21;p12–13) translocation that creates a CRTC1-MAML2 fusion gene. This feature is known to have a favorable prognostic effect, and not unexpectedly is found more frequently in low/intermediate grade lesions than in high-grade tumors [271]. However, many other alterations have been identified in MEC. Morita et al. recently looked for CRTC1/3-MAML2 fusions and gene alterations in EGFR, RAS family (KRAS, HRAS and NRAS), PIK3CA, BRAF, and AKT1 in 101 MEC cases [272]. They also searched for mutations in TP53. CRTC1/3-MAML2 fusions were found in 62.4% of cases. KRAS, HRAS and PIK3CA mutations were detected in 6.9, 2.0, and 6.9%, respectively, but other EGFR pathway genes were not mutated. In total, gene mutations (RAS/PIK3CA) in the EGFR

pathway were detected in 14.9% of cases, and TP53 mutations in 20.8%. CRTC1/3-MAML2 fusions were associated with better prognosis and RAS/PIK3CA mutations with worse prognosis, and both were selected as independent prognostic factors for the overall survival. TP53 mutations had no prognostic impact. CRTC1/3-MAML2 fusion-positive rates were inversely associated with the patient age and fusions were found in 82% of patients aged <30 years. There has been no phase II study exclusively evaluating MEC. Targeted therapies investigating MEC together with other histologies included cetuximab [273], nintedanib [274], and sorafenib [275], but no subgroup analysis was performed. Isolated cases of favorable response of high-grade MEC to pembrolizumab have been reported [276, 277]. In a patient with an extensive high-grade lesion of the parotid gland, pembrolizumab was used as "first-line" therapy and complete pathologic response was achieved [276]. In another two patients with distant metastasis from parotid high-grade MEC, prolonged partial response to pembrolizumab was observed [277].

In conclusion, the treatment of choice for glandular malignancies of the nasopharynx is still based on the combination of surgery and radiotherapy. The role of chemotherapy, biotherapy, and immunotherapy need to be further elucidated, in light of improved understanding of their molecular profile.

Non-epithelial Skull Base Cancers

A variety of non-epithelial cancers can arise from mesenchymal tissues of the skull base, among which chondrosarcoma and chordoma are the histologies that have raised the greatest interest over the last decades. Of note, diagnosis of these cancers is frequently based on imaging findings and histological information is not always available prior to treating the patient. This fact should be taken into account when considering pathological and biological prognostic factors as potential TDTC.

Chondrosarcoma

Chondrosarcoma of the skull base is a rare tumor that arises from cartilaginous areas dispersed throughout the cranial base, mostly represented by synchondrosis (Fig. 16.8). It can be either sporadic or associated with hereditary enchondromatosis such as Maffucci syndrome and Ollier disease [278, 279]. Raza et al. reported that the petroclival synchondrosis is the most frequently affected site (49%), followed by the sphenoethmoidal (18.4%) and intersphenoidal (12.2%) synchondroses [280]. From a histological standpoint, chondrosarcomas can be conventional (further classified in grades I–III), mesenchymal, clear cell, myxoid, and dedifferentiated [281]. Surgery consisting of "maximum safe resection" is the mainstay of treatment and can be performed through an endoscopic transnasal approach in adequately selected cases [282–290].
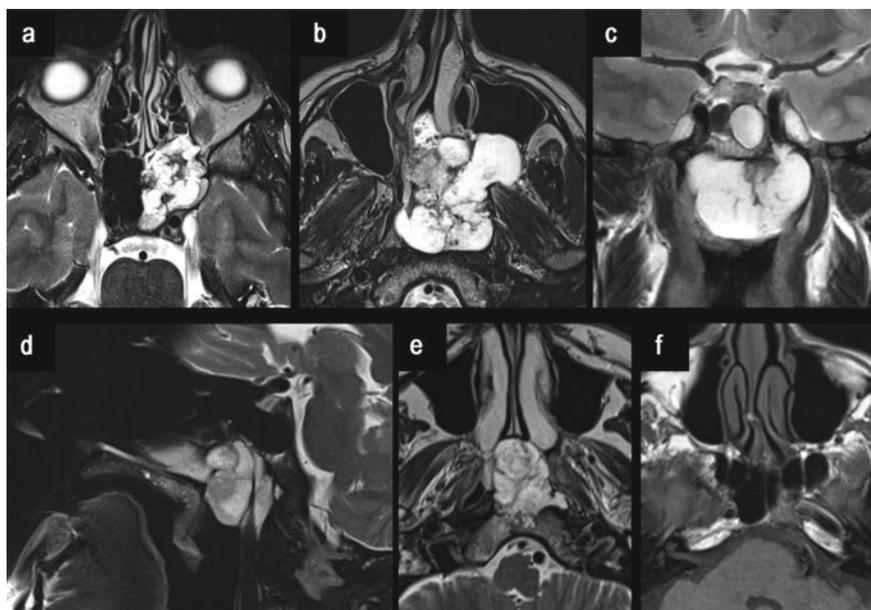
Fig. 16.8 Chondrosarcoma and chordoma of the ventral skull base. **a–c** Preoperative magnetic resonance imaging of a left sphenoethmoidal grade 2 chondrosarcoma. **d–f** Preoperative magnetic resonance imaging of a chondroid chordoma involving the lower and middle clivus, prevertebral muscles, and left petroclival junction

Adjuvant radiotherapy is thought to provide advantages in terms of local control and the possibility to avoid it in selected cases is a matter of discussion. In fact, some authors suggest to reserve radiotherapy as a potential salvage option in grade I–II chondrosarcomas initially treated with "maximum safe resection" [291–293]. When progression-free survival is stratified by grade, only grade II and III are associated with a prognostic benefit when treated with adjuvant radiotherapy [290]. Given the strict adjacency to vital skull base structures, the dose distribution provided by intensity-modulated radiation therapy and particle radiotherapy is particularly effective in chondrosarcoma [294–299]. For instance, Holtzman et al. reported that proton beam radiotherapy on primary or residual post-surgical chondrosarcoma resulted in 4-year overall survival and local control of 95 and 89%, respectively [300]. However, compared to surgery only, surgery followed by proton beam radiotherapy increased 10-year progression-free survival from 58.2 to 87.5%, but did not improve disease-specific survival [301]. Overall, proton beam radiotherapy was more effective compared with photon radiotherapy in a National Cancer Database study on 736 sinonasal and skull base chondrosarcomas [302]. A systematic review with meta-analysis including 243 patients treated with surgery and postoperative carbon ion radiotherapy reported local control in 88% of cases with an overall survival of 79% at 10 years after treatment and grade ≥3 toxicity seen in 0–4% of patients [303].

Preclinical experiments on grade III chondrosarcoma have shown that the PARP inhibitor olaparib may serve as a radiosensitizer, particularly for particle therapy [304].

Mesenchymal and dedifferentiated chondrosarcomas are thought to have increased chemosensitivity [305]. Indeed, Raza et al. reported that the use of neoadjuvant (4 cases) or adjuvant (1 case) chemotherapy with vincristine, adriamycin, and ifosfamide increased progression-free and distant recurrence-free survival in mesenchymal and dedifferentiated chondrosarcomas [290].

Age >35/40 years and encasement of >2 major arteries (defined as ≥25% contact with the carotid, basilar or vertebral artery wall) were found to significantly predict progression of disease [306, 307]. The mesenchymal, clear cell, and dedifferentiated subtypes were demonstrated to predict progression of disease, as well as grade II and III [290, 302, 308–311]. SOX4 overexpression, which is related to miR-30a and miR-335 downregulation, was found to be associated with worse prognosis [312–314].

Tatman et al. demonstrated that phosphorylation of the following kinases is associated with recurrence: FES, FER, SRC family kinases, PKC, and ROCK, along with members of the mitogen-activated protein kinase pathway (JNK, ERK1, p38) [315]. The authors highlighted that several of these enzymes can be targeted with FDA-approved agents such as bosutinib, sunitinib, dasatinib, and nilotinib. In turn, even if the Hedgehog pathway was found to be upregulated in chondrosarcoma, Hedgehog pathway inhibitors do not provide significant clinical benefit [316, 317]. PD-L1 is not expressed in conventional chondrosarcoma, whereas it was detected in certain cases of dedifferentiated subtype, which also showed increased tumor-infiltrating lymphocytes and HLA class I expression [318]. Indeed, partial response to ICI has been reported in a case of dedifferentiated chondrosarcoma [319].

While the paucity and heterogeneity of data prevent drawing clear indications to customize treatment of chondrosarcoma, some potential TDTC alongside with patient-related factors can be hypothesized based on the available evidence (Table 16.7).

Chordoma

Chordoma is a malignant tumor taking origin from remnants of the notochord and is classified in conventional, chondroid, sarcomatoid, or dedifferentiated types [320]. Recently, a SMARCB1-deficient poorly differentiated chordoma has been identified and considered as a further subtype [321]. Cranial chordomas are most frequently centered along the uppermost portion of the embryonal position of the notochord, from the craniocervical junction to the sella turcica (Fig. 16.8). Surgery consisting of gross total resection is the mainstay of treatment and less-than-total resection is independently associated with worse outcome [322, 323]. Given their central, midline position in the cranial base, chordomas are most frequently addressed through an endoscopic transnasal approach [324, 325]. Of note, surgery performed outside of

Table 16.7 Potential treatment-driving tumor characteristics (TDTC) of ventral skull base chondrosarcoma

TDTC	Putative treatment customization
Grade II–III conventional chondrosarcoma [290, 302, 304, 311]	Indication to adjuvant radiotherapy[a] Use of PARP inhibitor as radiosensitizer[b]
Mesenchymal subtype [290, 305]	More aggressive local treatment Indication to neoadjuvant or adjuvant chemotherapy
Dedifferentiated type [290, 305, 319]	More aggressive local treatment Indication to neoadjuvant or adjuvant chemotherapy Use of nivolumab or other immune checkpoint inhibitors
Clear cell subtype [310]	More aggressive local treatment
SOX4 overexpression, miR-30a and miR-335 downregulation [312, 314]	
Deregulation of FES, FER, SRC family kinases, PKC, ROCK, JNK, ERK1, p38 [315]	
Age >35 [306] or >40 [307] years old[c] (2 different cutoffs have been proposed)	
Encasement (contact for <25% of the vessel wall) of >2 major vessel [306]	More aggressive local adjuvant treatment

[a] Indication to adjuvant radiotherapy in grade II chondrosarcoma is debated [293]
[b] Preclinical evidence on grade III chondrosarcoma [304]
[c] Formally, this is not a tumor characteristic, rather a patient-related factor

a multidisciplinary setting was independently associated with decreased survival [326].

Overall, adjuvant radiotherapy is considered an essential part of treatment. Bai et al. demonstrated that postoperative radiotherapy should be delivered as adjuvant treatment following macroscopically complete surgery rather than salvage therapy after evidence of recurrence [327]. Radiation dose up to 78 Gy in 39 fractions proved beneficial over lower doses (<74 Gy) in terms of local control [328]. Both proton beam and carbon ion radiotherapy have shown remarkable efficacy in the postoperative treatment of chordomas, with 5-year local control exceeding 70% in most series [303, 329–332].

Chemotherapy has a limited role in the treatment of chordoma [333]. Interestingly, a computational drug repositioning study on FDA-approved agents identified cytarabine and tretinoin as drugs with a potential effect on chordoma, thus suggesting that their efficacy should be investigated in a preclinical setting to evaluate their potential clinical utility [334].

Akinduro et al. systematically reviewed the targeted therapy agents employed in clinical trials for chordoma [335]. They reported that 9-nitro-camptothecin, sunitinib, imatinib (with/without cyclophosphamide, with/without everolimus), lapatinib, sorafenib, dasatinib, nilotinib, and apatinib have been tested in a clinical setting,

with objective response rates ranging between 0 and 25%. Meng et al. suggested that targeted therapy for chordoma should be driven by gene mutation screening or immunohistochemistry by assessing the PDGFR, EGFR, VEGFR, and mTOR pathways [336].

The dedifferentiated, poorly differentiated (i.e., SMARCB1-deficient), and sarcomatoid subtypes have been associated with poorer disease-specific survival, whereas the chondroid variant had more favorable progression-free survival [321, 323, 327, 337–339].

If one considers the group of rare malignancies of the skull base from a molecular and genetic perspective, chordoma is among the most extensively explored lesions [314]. Zuccato et al. presented a comprehensive, methylomic-driven analysis of 68 chordomas and identified 2 clusters of hypomethylated tumors [340]. The first was associated with deregulation of immune- and transcription/translation-related pathways (referred to as "immune-infiltrated"), and the second with cell-to-cell interaction, extracellular matrix, angiogenesis, and metabolic pathways (referred to as "cellular"). Interestingly, the first cluster had significantly worse prognosis and cluster classification could be reliably performed non-invasively based on plasma cell-free DNA. The load of chromosomal deletions of 1p36 and 9p21 in the cellular population of chordoma has been found to predict progression-free survival [341]. This biomarker was found also to deeply impact the response to adjuvant radiotherapy in an analysis of 152 clival chordomas [342]. In macroscopically resected chordomas with low-to-intermediate chromosomal deletion burden, adjuvant radiotherapy did not increase progression-free survival, whereas it did in less than totally resected tumors with intermediate burden and in those with high burden irrespective of the degree of resection. Moreover, deregulation of ERK and HPGD expression was found to increase resistance to radiotherapy [343]. When considering molecular prognostic biomarkers, upregulation of asparagine synthetase, overexpression of c-Cbl, Cbl-b, PDGFR-β, TGF-α/β, VEGFR1/2, survivin, and ERK, and underexpression of SMARCB1, HPGD, and PTEN were associated with poor prognosis [339, 343–351]. On the other hand, duplication and overexpression of Brachyury (also called T gene) has an ill-defined prognostic role, but is thought to be involved early in chordoma development and was implicated in familial chordomas [314, 352, 353]. The prognostic effect of miRNA deregulation in chordoma is not fully elucidated and mostly relies on series including both cranial and spinal tumors [354].

Of note, as demonstrated by the Beijing Neurosurgical Institute's experience, several patient-related peripheral blood indexes have been associated with prognosis of chordoma patients. For instance, systemic immune-inflammation index, which summarizes the count of neutrophils, platelets, and lymphocytes, prognostic nutritional index, which includes serum albumin and lymphocytes count, and fibrinogen-albumin score have been associated with survival outcomes [355, 356]. Similarly, platelet and red cell distribution width was found to correlate with overall survival [357, 358].

The interaction between chordoma and the immune system is still far from being fully elucidated, but some inherent data have supported the design of immunotherapy clinical trials [359]. PD-L1 expression in non-tumor cells and high density of

macrophages, regulatory T cells, and "exhausted" tumor-infiltrating lymphocytes were associated with more rapid progression of the disease and worse prognosis [349, 360, 361]. Currently, a number of clinical trials are assessing the potential benefit of ICI and Brachyury vaccine in patients affected by chordoma [362]. Of note, a phase I trial on MVA-BN-brachyury-TRICOM vaccine showed some form of benefit in 5/10 patients affected by chordoma, with stable disease and partial response in 4 and 1 cases, respectively [363]. On the other hand, a phase II, double blind, placebo-controlled trial showed that yeast-Brachyury vaccine does not provide additional effect in patients with unresectable chordomas treated with standard-of-care radiotherapy [364]. Though evidence is limited to the preclinical setting, B7-H3 has been identified as a potential target for CAR-T cell therapy (Table 16.8) [365].

Table 16.8 Potential treatment-driving tumor characteristics (TDTC) of ventral skull base chordoma

TDTC		Putative treatment customization
Dedifferentiated, poorly differentiated SMARCB1-deficient, or sarcomatoid subtype [321, 327, 337, 338]		More aggressive local treatment
"Immune-infiltrated" chordoma according to Zuccato et al. [340]		
Upregulation of asparagine synthetase, overexpression of c-Cbl, Cbl-b, PDGFR-β, TGF-α/β, VEGFR1/2, survivin, ERK or underexpression of SMARCB1, HPGD, PTEN [339, 343–351]		
High systemic immune-inflammation index, low prognostic nutritional index, high fibrinogen-albumin score, high platelet/red cells distribution width[b] [355–358]		
PD-L1 overexpression in non-tumoral cells, high density of macrophages, regulatory T cells, and "exhausted" tumor-infiltrating lymphocytes [349, 360, 361]		
Percentage of 1p36 deletion and homozygous 9p21 deletion in chordoma cells [341, 342]	1p36 deletion >15% and homozygous 9p21 ≥25%	More aggressive local treatment (i.e., gross total resection should be pursued as it has an independent effect on survival, indication to adjuvant radiotherapy)
	1p36 deletion >15% or homozygous 9p21 ≥25%	Indication to adjuvant radiotherapy if less-than-gross total resection has been achieved
	1p36 deletion ≤15% and homozygous 9p21 <25%	Avoidance of adjuvant radiotherapy in patient who received gross total resection[a]
Deregulation of PDGFR, EGFR, VEGFR, and mTOR pathways [335, 336]		Use of respective targeted therapies

[a] No data are available on patients of this group who received less-than-total resection
[b] Formally, this is not a tumor characteristic, rather a patient-related factor

Conclusions

Malignant neoplasms that arise from or encroach on the ventral cranial base comprise a large group of different entities, each one with different behavior. The understanding of these cancers is rapidly evolving and is providing a wealth of information with potential therapeutic implications. The large majority of TDTC that were discussed in this chapter are based on theoretical grounds, and evidence of their value in the clinical setting is still lacking. However, several promising paths of treatment customization, which need to be appropriately validated, can be foreseen. Based on the complexity of the pathologies that we have reviewed in this chapter, the logical conclusion is that both research and clinical management should be performed at institutions with dedicated, multidisciplinary facilities.

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Chapter 17
Modern Day Reconstruction of the Facial Bones

David McGoldrick, Prav Praveen, and Sat Parmar

Introduction

Head and neck reconstruction has undergone significant evolution over the last 20–30 years. This period has seen the development and rapid evolution of digital systems to aid in surgical planning and treatment. Three-dimensional (3D) planning, also referred to as virtual surgical planning (VSP) or computer aided design/computer aided manufacture (CAD/CAM), has evolved to allow surgeons to digitally plan resection and reconstruction to a high degree of precision. It then allows for the manufacture of adjuncts to surgery such as cutting guides and patient specific plates, which aim to reduce operative time and increase accuracy of reconstruction. Recent years have seen the exponential growth in the use of these systems with improvements in intra-operative time, ease of use and cost [1, 2].

Reconstruction of the facial skeleton is a complex task with a number of aims that the surgeon must consider. The primary aims are to restore form and function. The surgeon must attempt to reconstruct the complex geometry of the face so as to maintain the continuity of the jaws and also replicate the curvature of the arches where possible. The amount of osseous and soft tissue required and the potential for dental rehabilitation must be considered carefully. Any reconstruction must also attempt to restore the complex functional requirements of this region, namely speech, swallow and mastication. Other considerations include the final aesthetic result, the potential impact on quality of life for the patient and the ability of the reconstruction to withstand radiotherapy, if this is likely to be required.

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Essential Steps

There are a number of essential steps required to achieve success in 3D planning. Firstly, accurate digital design is needed. A recent computed tomography (CT) scan in DICOM format with thin slices should be obtained. If planning will be used for both the ablative and reconstructive components, then a scan of the proposed donor bone is also necessary. Often the imaging will need to be 'cleaned-up' to remove artifacts. In the oral cavity, dental restorations may create significant scatter artifact and software systems may be needed to remove this.

Accurate planning is the next step to undertake. This should be multi-disciplinary in nature with the surgeon, technician and possibly restorative dentist involved. In modern practice most propriety companies will undertake these sessions virtually. If design and manufacture is undertaken 'in-house' then these sessions may be more frequent and also allow for rapid feedback and modification if required. Planning at this stage will consider the resection and surgical margins to be obtained as well as any involved or adjacent structures. Other issues that may need to be considered include how best to register cutting guides and the position of screws with regard to potential implant rehabilitation (Fig. 17.1).

Fig. 17.1 3D planning scan demonstrating a proposed resection

Indications

When 3D planning was initially developed, its use was often limited to complex reconstruction cases. As recent years have seen a reduction in costs and manufacturing time, some units would now consider 3D planning for the majority, if not all, of their cases. The process remains most beneficial in complex defects of the maxilla and mandible, such as those requiring large amount of soft and hard tissue resection and/or multiple osteotomies of the donor flap. The process is also very beneficial when dental implant rehabilitation is planned. Short span mandibular and low-level maxillary defects may not require 3D planning but may still benefit from the improved accuracy possible in both resection and reconstruction, as discussed below.

Resection

The primary aim of ablative head and neck surgery is to remove any lesion with adequate histological margins. In oncological practice this will generally be a 1 cm surgical margin. Where possible, aesthetic units and vital structures should be preserved although this should not take precedence over oncological clearance. In the mandible, if resection margins are close to the condyle, a decision must be made on whether to preserve this segment or to incorporate it into the resection. 3D planning allows for the creation of specific cutting guides to accurately achieve the planned resection intraoperatively. The guides and planned resection margins may also allow for the potential of interval tumour growth between the planning stage and the actual time of surgical resection.

After the lesion is exposed intra-operatively, the custom guides are seated on the mandible or maxilla. The guides should be constructed so as to seat passively and directly adjacent to the bone and may have special locators to assist in this (Fig. 17.2). The guides may then also be secured with mono-cortical screws. The bony resection is then undertaken without concern for stabilising the bony remnants. If planned, the guides may also contain guide holes to be drilled to allow later screw placement for securing the reconstruction plates.

Mandibular Reconstruction

Composite free flap reconstruction is now the gold standard in mandibular reconstruction. When planning the reconstruction, the height and vertical position of the donor bone is a key consideration. A lower position along the mandibular border maximises form while a higher position near the alveolus will allow for easier implant placement and dental rehabilitation. Once the bony position is confirmed, a 3D model of the

Fig. 17.2 3D planned cutting guides

planned reconstruction can be printed and a plate contoured to this. The plate length is determined so as to ensure there are three screws on either side of the resection.

Intra-operatively, the pre-bent plate is seated onto the native mandible following resection using temporary localisation guides soldered to the plate. This ensures an accurate fit prior to placement on the donor bone. If the cutting guides did not contain guide holes, then the plate can be used to pre-drill screws holes at this time. The plate is then removed and the localisation guides removed. The plate is secured onto the donor bone with mono-cortical screws so as to avoid damage to the vascular pedicle on the deep aspect of the bone. Usually, two screws are placed per bony segment. The plate and flap reconstruction is then inset into the defect and secured using the pre-drilled holes and bi-cortical screws (Figs. 17.3 and 17.4).

There are a number of bony flaps that may be used for mandibular reconstruction. A number of considerations such as the quantity and quality of hard and soft tissue required, if dental implant placement is planned and the pedicle length required will help guide selection. The fibula free flap remains the work-horse flap of mandibular reconstruction for a number of reasons. Its lower limb location makes it easily accessible and permits two team operating thus shortening operative time. It is straightforward to raise, provides a significant length of bone and results in low levels of donor site morbidity. Importantly, it also allows for 3D planning and the construction of specific cutting guides (Fig. 17.5) [3]. Although the bone stock may be limited in vertical and cross-sectional dimensions, dental implant rehabilitation is usually possible. Other flaps that may be considered include the deep circumflex
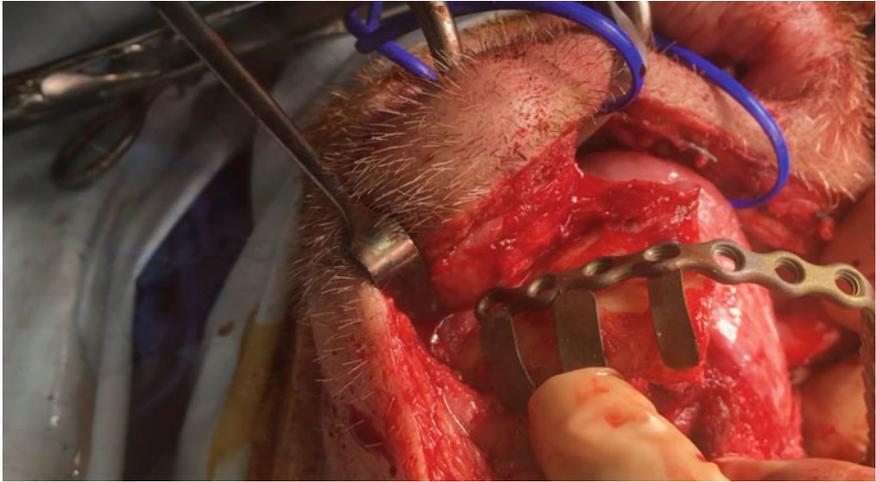
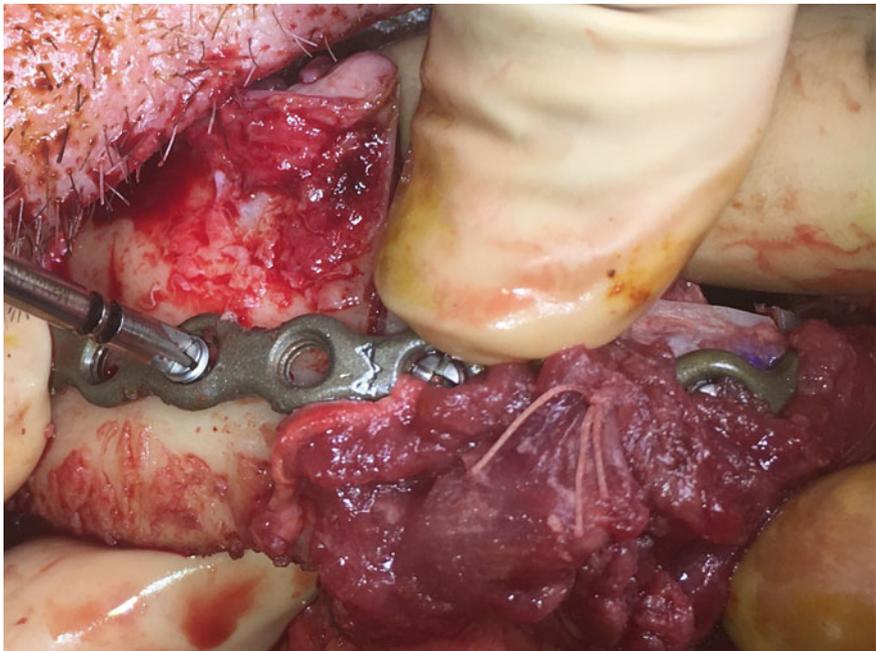Fig. 17.3 Intra-operative seating of pre-bent reconstruction plate onto the mandible
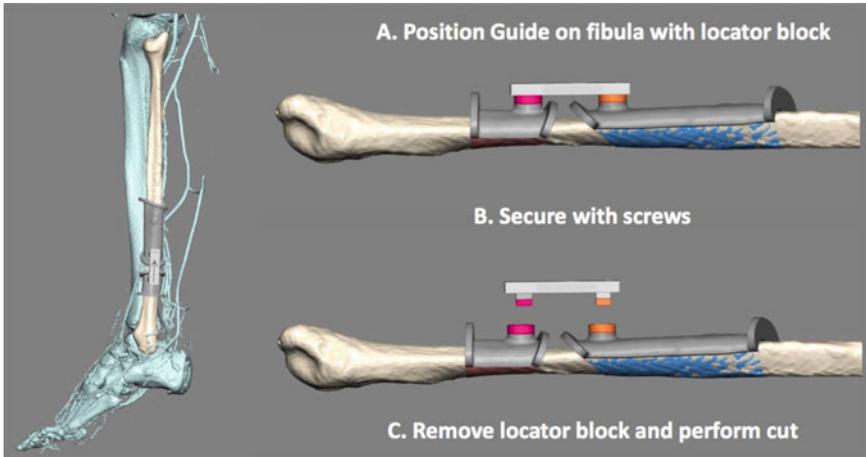

Fig. 17.4 Inset of flap using pre-bent plate

Fig. 17.5 3D planned fibula cutting guides

iliac artery (DCIA) flap, the composite scapula flap and the composite radial flap. All of these flaps have their own individual advantages and disadvantages with regard to mandibular reconstruction.

The DCIA flap provides good bone stock although the length of vascular pedicle is often a concern. A two-team approach is also permitted with this flap and although it may be raised as an osteo-cutaneous composite flap a myo-osseous flap utilising the internal oblique muscle is more commonly. Donor site morbidity is a concern with this flap and meticulous closure is required to minimise the risk of hernias. The composite scapula flap is often considered when large volumes of soft tissue is required in addition to bony reconstruction. The flap, based on the subscapular arterial system, may also be raised in a chimeric fashion to provide two or more reconstructive components. The composite radial free flap is less commonly used in mandibular reconstruction due to the limited amount of bone stock available and the potential for donor site morbidity.

Maxillary Reconstruction

Maxillary defects may create a number of issues that must be addressed when planning any reconstruction. There may be loss of vertical support with potential enophthalmos, orbital dystopia or reduced projection of the midface. Loss of horizontal support may also create issues with speech and swallow and may make the retention of prosthetic devices difficult. Maxillary defects are easily classified using the Brown classification system, which incorporates both vertical and horizontal components [4]. The vertical component is classified from class I to class V. Defects not creating an oro-antral defect and thus usually confined to the maxillary alveolus are referred

to as class I. Class II defects have a more vertical extent that result in an oro-antral defect but do not involve the orbit. If the defect extends to include the orbital contents, but with orbital retention, it is referred to as class III and if orbital exenteration or enucleation is required, as class IV. Class V and VI refer to orbitomaxillary and naso-maxillary defects respectively. This system can also be used to guide reconstruction as some options are more suitable than others depending on the defect.

In a similar fashion to the mandible, 3D planning will usually involve the printing of models that allow for the fabrication of cutting guides and plates that are pre-bent for the planned reconstruction. We have previously published our approach to reconstructing maxillary defects [5]. In low level, class I and some class II, defects we recommend soft tissue reconstruction and the use of prosthetic obturators. For larger or anterior class II defects a strut of bone is often required and the use of a fibula or scapula flap may be a good option in these cases. Class III defects pose particular challenges with the loss of alveolar bone, the orbital floor and cheek support. No single flap can solve all of these issues but the DCIA flap with a custom orbital reconstruction plate is perhaps the best option. The DCIA flap can be harvested with a shape and contour that readily reconstructs the lost buttresses of the maxilla. 3D planning allows for the construction of specialised cutting guides to simplify harvest. Inset can then be achieved using a pre-bent plate and customised orbital floor reconstruction plate (Figs. 17.6, 17.7 and 17.8).

Class V (orbitomaxillary) and VI (nasomaxillary) defects are less common and pose different challenges. In class IV cases, the primary aim is to create a base on

Fig. 17.6 3D planning for resection of maxillary lesion and position of cutting guide

Fig. 17.7 3D planned DCIA flap to reconstruct defect

Fig. 17.8 Pre-bent plate and customised orbital floor reconstruction plate

which to base a future prosthesis. There is no need to reconstruct the orbital floor however. Both the DCIA and scapula flap are good options to consider as they can provide good muscle bulk with which to fill the orbital defect. Reconstruction of class V defects requires only a small amount of bone and often the composite radial forearm free flap is a good option to utilise.

Limitations and Future Developments

While 3D planning clearly provides some advantages over conventional head and neck reconstructive surgery, there are some potential limitations and drawbacks. Firstly, there is a potential for surgeons to become reliant on these systems with a resultant loss in free hand skills. Although 3D planning aims to be highly accurate, there is always the potential for unexpected findings intra-operatively or problems with the hardware. In these scenarios the surgeon must be able to adapt and potentially revert to traditional techniques. Secondly, most current planning systems cannot account for soft tissue factors. In the past, cutting guides and hardware were bulkier and at times this posed problems in cases with a tight soft tissue envelope. Newer systems continue to adapt to this problem with lower profile guides and a move away from thicker metalwork. Finally, these systems can incur significant costs, both financially and in terms of time. Manufacturing costs may be significant although proponents of these systems point to the cost savings in reduced intra-operative time which some studies have reported [6, 7]. The use of 'in-house' planning and manufacture may also reduce costs significantly [8]. The time taken for planning and manufacture, and thus the potential for a delay in surgery, has been cited by some detractors of these systems in the past. As the technology has evolved, this concern has receded. Much if not all of the planning is now conducted virtually and manufacturing times have significantly improved. Indeed, recent studies have confirmed the oncological safety of this approach [9, 10].

The next decade is likely to see further refinements and progress in this field. Manufacturing time and costs are likely to reduce further as these systems gain widespread use. Although most current 3D planning focuses on bony reconstruction, future developments are likely to see a move towards soft tissue planning. The potential to plan soft tissue flaps based on the donor and recipient site vasculature would be a significant advantage and further evolution of this technique. Augmented reality is another rapidly emerging technology in surgery and may also play a role in head and neck reconstruction in future years [11, 12].

Conclusion

3D planning has evolved significantly in recent years and continues to do so. If a number of key principles are adhered to, it can allow for highly accurate resection

and reconstruction of the facial bones. As more centres use these systems, further advancements in manufacture times, cost and planning ability are likely.

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Chapter 18
Targeting Molecular Residual Disease Using Novel Technologies and Clinical Trials Design in Head and Neck Squamous Cell Cancer

Enrique Sanz-Garcia and Lillian L. Siu

Introduction

Despite therapeutic advances in locoregionally advanced head and neck squamous cell carcinoma (LA-HNSCC), there are patient subsets who remain at risk for disease relapse and death from their malignancies. For instance, the five-year overall survival (OS) of patients with stage III human papillomavirus (HPV)-positive oropharyngeal cancer, classified by the 8th edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) criteria, is about 55–60% [1]; whereas the three-year OS for HPV-negative oropharyngeal cancer is about 46% [2]. Patients with LA-HNSCC who develop clinical or radiological disease progression or relapse have limited curative options if salvage treatments are not possible. Although the immuno-oncology era has led to long-term survival in a small proportion of patients with recurrent or metastatic HNSCC [3], this only constitutes an incremental advancement in the field. In recent years, emerging technology has enabled the detection of microscopic quantities of nucleic acids (i.e. DNA, RNA), proteins, metabolites and other molecules secreted or shed by tumor cells into the blood stream. Such techniques, collectively referred to as "liquid biopsy", have garnered increasing attention due to their potential to detect the presence of cancer and associated molecular changes at a microscopic level [4].

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Liquid Biopsy and Molecular Residual Disease

There are multiple clinical applications of liquid biopsy to measure circulating tumor DNA (ctDNA) in body fluids, most frequently in plasma. These applications include the early detection of cancer before it becomes macroscopically visible; the assessment of molecular residual disease (MRD) after definitive treatment; the monitoring of response to treatment; and the evaluation of emerging resistance mechanisms. In all of these applications, the overarching hypothesis is that molecular detection of ctDNA precedes the clinical event (i.e. the development of cancer, clinical relapse, antitumor response, disease progression and resistance development, respectively). In this chapter, the focus will be on MRD, which describes the state post-definitive treatment such as surgery or (chemo)radiotherapy for LA-HNSCC in which conventional investigations such as physical examination and radiological imaging are unable to diagnose the presence of cancer, but residual disease is detectable by the presence of cancer-derived molecular biomarkers, using highly sensitive and specific assays [4].

Assays for MRD Detection

The detection of MRD can be performed using different types of ctDNA assays. Mutation-based assays rely on the detection of cell-free DNA (cfDNA) molecules that bear genomic alterations, suggesting that their source is likely from cancer cells rather than normal cells. Bespoke (i.e. personalized), mutation-based, tumor-informed assays rely on whole genome or exome sequencing of tumor tissue from which a limited number of somatic variants is selected based on proprietary algorithms to create patient-specific panels. These personalized panels are then used to track such variants in plasma samples of patients to assess quantitative changes in variant allele frequencies (VAF) or in number of mutant molecules per milliliter. There are also fixed gene panels that are not derived from next generation sequencing (NGS) of patients' tumor tissue, which propose to have faster turnaround time but harbor the risk of missing relevant aberrations which may thus lower their sensitivity and specificity [5]. In addition to mutation-based ctDNA assays, other cell-free DNA analyses may be utilized to monitor for MRD, such as viral sequences in the case of HPV-positive oropharyngeal cancer. Tumor-tissue modified viral DNA (TTMV) using a validated digital droplet PCR-based assay, can distinguish tumor-derived viral DNA from non-cancer associated sources of HPV DNA [6]. Methylated cfDNA analysis is another emerging method that does not rely on the detection of specific somatic mutations, but is based on the identification of unique DNA methylation patterns in different tumor types, which are also distinct from those found in normal tissues [7]. Table 18.1 summarizes the different assays that have evaluated MRD in HNSCC and their advantages and disadvantages.

Table 18.1 Circulating tumor DNA assays applied in the molecular residual disease setting in head and neck squamous cell cancer

Analysis	Assay	Pros	Cons	Evidence
HPV DNA	Quantitative PCR	Low cost and easy logistics High sensitivity in saliva	Low sensitivity in plasma Only for a specific serotype (HPV DNA 16)	Ahn et al. [8]
	Tumor tissue modified viral (TTMV) HPV DNA (digital PCR)	Tumor specific HPV-DNA High specificity High sensitivity Able to detect different strains	Not able to detect <1 copy per mL HPV DNA Only gives quantitative information about HPV DNA	Chera et al. [10], Berger et al. [11]
	HPV sequencing (HPV-seq)	Highly sensitive (limit of detection <1 copy) Provides qualitative information (discriminates serotype, maps location along the HPV genome and provides fragment length)	High cost Further validation in oropharyngeal cancer is needed	Leung et al. [14]
Mutation ctDNA	Bespoke informed ctDNA	Personalized assay for each patient Highly sensitivity and specificity Validated in other tumor types	Requires tumor availability for sequencing High cost Long turnaround time for the first sample	Flach et al. [21], Flach et al. [22]
	Tumor naïve fixed gene panels	No need for tumor sample (naïve) Short turnaround time	Less evidence currently available to confirm clinical utility No consistent data in follow up/surveillance	Burgener et al. [23], Chikuie et al. [24]
Methylated cfDNA	cfMeDIP-seq	Low amount of cfDNA (<10 ng) needed Do not rely on tumor availability Presence of mutations in tumor not needed	Not validated in large cohorts Complex bioinformatic process	Burgener et al. [23]

Evaluation of MRD in HNSCC

HPV DNA

The evaluation of MRD using HPV DNA in patients with HPV-positive oropharyngeal carcinoma has taken the lead in HNSCC, in the context of malignancies driven by this virus. Different assays have been tested to detect and track viral HPV DNA in plasma and saliva. Quantitative PCR (qPCR) for HPV serotype 16 was initially assessed in a retrospective cohort of 93 patients with locally advanced oropharyngeal carcinoma [8]. The presence of HPV DNA in saliva was predictive of recurrence and survival. However, HPV DNA detection in plasma during follow-up was not a predictive biomarker by itself. The combination of the presence of HPV DNA in both plasma and saliva was 90.7% specific and 69.5% sensitive in predicting recurrence within 3 years. The most contemporary validated assay for HPV DNA detection in HPV-positive HNSCC is TTMV. Sensitivity and specificity of this assay in plasma are 89% and 97% respectively; these values are significantly higher than previously reported qPCR assays [9]. This assay evaluates amplicons within the E6 and E7 genes for HPV strain 16 and E7 gene for strains 18, 31, and 33 using digital droplet PCR. Therefore, it is able to detect different viral genotypes. Moreover, it is considered to be a measurement of tumor specific HPV DNA as there is a high correlation between tumor and plasma HPV DNA as previously mentioned. This assay has prospectively been validated as a biomarker of MRD in a study comprising 115 patients with stage I-III p16-positive oropharyngeal carcinoma treated with definitive (chemo)radiotherapy [10]. Plasma samples were collected at different time points during follow-up starting at 6 months after the end of definitive therapy. The majority of patients (75%) did not demonstrate detectable ctDNA during follow-up and all these patients were free of recurrence after a median follow-up duration of 23 months (negative predictive value (NPV) = 100%). On the other hand, two consecutive positive detections of HPV DNA had a positive predictive value (PPV) of 94% for locoregional or distant recurrence. A transitory spike in HPV DNA was seen in some patients (less than 10% of patients) but those with spontaneous clearance in the next time point were also free of recurrence. Median lead time between HPV DNA detection and biopsy proven recurrence was 3.9 months (0.37–12.9). Therefore, it may be useful in the MRD setting to anticipate clinical progression. This assay has further been validated in a retrospective multicenter cohort of 1076 patients with non-metastatic HPV-driven oropharyngeal cancer treated with any definitive treatment [11]. HPV DNA was detected during surveillance in 80 patients (7.4%), 21 of them have active disease present concurrently at the sample collection time point. Among the remaining 59 patients, 55 patients (93%) were proven later to have recurrent disease. In contrast, only around 5% of patients with no detection of HPV DNA in the follow-up period showed recurrence any time later. Overall, in this cohort the PPV and NPV of a single TTMV test performed 3 months or later after the end of definitive treatment was 95% for both parameters, similar to the previous study. NRG-HN002 study, which evaluated a cisplatin-sparing approach in low risk p16-positive oropharyngeal cancer, has

reported recently correlative study results of its HPV DNA analysis. TTMV detection between 2 weeks to 1 month after definitive (chemo)radiation treatment showed a NPV of 95% for 2-year locoregional failure (LRF) and 93.3% for progression-free survival (PFS). When clearance (>95% reduction from baseline) was considered, NPV was 94.3 and 92.7% for 2-year LRF and PFS, respectively [12]. All these results support the potential value of HPV DNA in follow-up of MRD, opening the door to incorporate this biomarker into the surveillance strategy of HPV-positive oropharyngeal tumors.

Other assays to detect HPV DNA using an NGS approach are under evaluation in the MRD setting, one such assay involves viral genome hybrid-capture sequencing [13]. This is the case of HPV sequencing (HPV-seq) that can provide both quantitative and qualitative information regarding the sequenced cfDNA fragments. This assay has been validated in a study involving preclinical models and plasma samples from patients with cervical and oropharynx cancers driven by HPV [14]. The lower limit of detection (LLoD) has been established as less than 1 copy per milliliter enabling the detection of HPV DNA in low burden disease such as MRD. A very high correlation between HPV-seq and digital PCR (gold standard) was seen in patients with detectable HPV DNA. Moreover, some patients with undetectable HPV DNA using digital PCR were found to have detectable viral genome using HPV-seq, due to the lower LLoD of the latter assay. In this study, detection of HPV DNA using HPV-seq at the end of chemoradiation in patients with cervical cancer was associated with shorter PFS. This assay showed a sensitivity of 100% and a specificity of 67% for disease recurrence in cervical cancer. Further validation is being carried out during follow-up in patients with oropharyngeal carcinoma.

Mutation-Based ctDNA

Mutation-based ctDNA has been widely used in solid tumors from fixed gene panels to personalized (or bespoke) assays. In the MRD setting, bespoke mutation-based ctDNA has emerged as one of the most promising tools in different tumor types such as colorectal [15, 16], breast [17, 18], lung [19] and bladder cancers [20]. There are some recent encouraging findings suggesting that this approach may also be applicable in HNSCC. The LIONESS study evaluated MRD using a bespoke ctDNA assay in p16-negative HNSCC patients who received curative intent surgery [21]. Plasma samples were collected at different time points before and after surgery, adjuvant therapy (if applicable) and during follow-up. MRD in LIONESS was analyzed using the RaDar™ assay which uses multiplexed PCR and targeted NGS to track a median of 48 variants in plasma. These variants are identified in the tumor tissue by whole exome sequencing and are prioritized using an algorithm to build a patient-specific panel. Presence of one variant in plasma was considered as positive for ctDNA detection. Bespoke ctDNA was detected in all 17 patients at baseline. In post-surgery samples, ctDNA could be detected at levels as low as 0.0006% VAF. All patients

with clinical recurrence were positive for ctDNA during follow-up and before clinical progression with a lead time ranging from 108 to 253 days. An updated analysis with 46 patients presented recently confirmed the potential role of this assay for MRD. All 11 patients who recurred had ctDNA detected in plasma during follow-up [22]. However, there were 5 patients with detectable ctDNA and no recurrence up to the latest follow-up which reduces the specificity of the assay. Median lead time in this updated cohort between ctDNA detection and recurrence was 122 days (ranging from 1 to 260 days) with a median follow-up duration of 307 days. All the patients included in the LIONESS study were surgically treated patients. Therefore, the role of MRD detection in HNSCC patients treated with definitive (chemo)radiation is still unknown. PRE-MERIDIAN study (NCT04599309), conducted at the Princess Margaret Cancer Centre, will hopefully shed more light on the use of bespoke ctDNA and other assays in patients with high risk locally advanced HNSCC treated with this modality.

One of the limitations of bespoke ctDNA is tumor tissue availability to perform whole genome or exome sequencing. This could potentially limit the application of these assays in the MRD setting, especially in those patients without surgical specimen availability (i.e. those undergoing definitive radiation $\pm$ chemotherapy). While core tumor biopsies may be used, their tumor DNA quantities may limit success for genomic sequencing. One of the potential alternatives for mutation-based targeted ctDNA analysis is the Cancer Personalized Profiling by deep sequencing (CAPP-seq). This assay has been evaluated in 30 patients with LA-HNSCC who were treated with surgery [23]. It was performed using a panel designed to maximize the number of HNSCC-associated mutations, with ctDNA detected in 20 patients (66%) at baseline. However, CAPP-seq was not done in the follow-up samples so the impact of its detection during surveillance and its association with disease recurrence have not yet been studied. A recent study has evaluated a fixed 71-gene panel in 20 patients with LA-HNSCC. This study includes not only surgical patients but also patients who have been treated with definitive chemoradiation [24]. Clearance of ctDNA after treatment was observed in 10 patients, all of them were free of recurrence during follow-up. Similarly, detection of ctDNA post-definitive treatment was associated with shorter relapse free survival (RFS). Indeed, detection of ctDNA was observed in 5 of the 7 recurrent cases (71%). However, in only two of these patients, ctDNA preceded radiological progression thus limiting the application of results from this study in the MRD setting as most patients showed clinical progression at the time of ctDNA detection.

Methylated cfDNA

Methylated cfDNA analyzes epigenomic changes in cfDNA. Notably, it could potentially be applicable to more patients as it does not depend on HPV status, tissue availability or presence of mutations compared to the abovementioned strategies. However, methylated cfDNA has been challenging to be analyzed in plasma using

standard approaches. Cell-free methylated DNA immunoprecipitation and high-throughput sequencing (cfMeDIP-seq) is a bisulfite-free approach to track aberrant methylation in cfDNA and has been validated in different tumor types [7]. Methylated cfDNA analysis was performed in the same aforementioned cohort of patients with LA-HNSCC who were analyzed with CAPP-seq [23]. Methylated cfDNA was further refined by restriction of cfDNA by fragment size (100–150 bp), which is the usual size range of tumor derived cfDNA. A high correlation between both assays (CAPP-seq and cfMeDIP-seq) was observed in the baseline samples. Interestingly, follow-up samples in that study were also analyzed using cfMeDIP-seq. Patients without clearance of methylated cfDNA during radiation or post-treatment were more likely to show disease recurrence compared to those with a complete or partial (>90%) clearance. Indeed, all patients with increase in methylated cfDNA compared to baseline had disease recurrence or death at the time of the analysis. In contrast, 69% of those patients with no detection of methylated cfDNA by cfMeDIP-seq remained free of recurrence with a median follow-up of 44 months. However, among those with no detection of methylated cfDNA, there were 4 patients with persistent or recurrent disease. Further validation in larger cohorts and prospective studies (such as in the PRE-MERIDIAN study) are also ongoing with this assay.

MRD Clinical Trials Design

There are no prospective clinical trials focusing on cancer interception in the MRD setting of LA-HNSCC. As such, it seems reasonable to draw reference from reports in other malignancies whereby therapeutic intervention in MRD has led to improved clinical outcome. In the IMvigor010 phase III study (NCT02450331), 809 patients with high-risk, resectable, muscle invasive urothelial carcinoma were randomized to the anti-Programmed Death-Ligand 1 (anti-PD-L1) antibody atezolizumab versus observation. Based on the intention-to-treat analysis in unselected patients, the study did not meet its primary endpoint of improved disease-free survival (DFS) in the atezolizumab group compared to the observation group, nor in the secondary endpoint of OS [25]. However, in a follow-up report which focused on 581 patients from IMvigor010 who were ctDNA evaluable using the Signatera assay (a bespoke ctDNA assay), atezolizumab was found to improve DFS and OS compared to observation in those with detectable ctDNA post-surgery. No difference in these two clinical endpoints were observed in patients whose post-operative ctDNA levels were undetectable [26]. This biomarker-based evaluation suggests that ctDNA analysis is able to identify a molecularly high-risk group post-surgery who may benefit from additional therapeutic intervention to improve clinical outcome. The recently published DYNAMIC study in stage II colon cancer randomized patients in a 2 to 1 ratio to a prospective ctDNA-guided approach (Safe-Sequencing System tumor-informed ctDNA assays) versus treating physician decision based on standard clinico-pathological features to determine the administration of adjuvant chemotherapy [16]. The primary efficacy endpoint of RFS at 2 years using a ctDNA-guided strategy was

noninferior to standard management. This de-escalation approach proved to spare some patients from the toxicity of adjuvant chemotherapy without compromising RFS. Both IMvigor010 and DYNAMIC studies provide evidence that ctDNA results in the MRD setting are informative to guide treatment escalation in patients who are at high risk for clinical relapse, or treatment de-escalation in those who are at low risk for disease recurrence. Treatment escalation strategies may be considered in the MRD setting of LA-HNSCC post-curative therapy (e.g., surgery followed by post-operative [chemo]radiotherapy, or upfront definitive [chemo]radiotherapy), to compare additional investigational treatment versus standard observation in patients with detectable ctDNA. At the Princess Margaret Cancer Centre, such a study (MERIDIAN, NCT05414032) is about to be launched using the RaDaR™ assay to determine MRD in patients with high-risk HPV-positive and HPV-negative LA-HNSCC; patients who have MRD will be randomized to receive a novel immunotherapeutic agent versus observation. The primary endpoint of MERIDIAN is to assess the clearance of bespoke ctDNA at different time points (week 2 and week 10) after the end of MRD interception, which will be correlated with longer term clinical outcomes such as DFS and OS. Various ways to ascertain MRD status and follow-up of ctDNA kinetics will be applied using bespoke DNA, HPV DNA and methylated DNA assays.

Conclusions

Advances in ctDNA technology have led to the definition of MRD as a disease status not previously identifiable in solid tumors, since microscopic circulating quantities of nucleic acids such as DNA shed by tumor cells cannot be readily detected by conventional investigations such as radiological imaging. Various ctDNA assays currently exist in different stages of clinical development, such as TTMV to measure viral genomes in the case of HPV-positive malignancies, bespoke and other mutation-based assays to track variants in plasma, and methylated assays to evaluated differential methylated cfDNA patterns in HNSCC compared to normal states. These have been applied in retrospective and prospective studies and some assays have demonstrated clinical utility in predicting clinical outcome. Clinical trials in the MRD setting are beginning to accumulate evidence in multiple cancers. Such studies are being actively designed to investigate the impact of cancer interception of MRD in LA-HNSCC.

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Chapter 19
New Developments in Surgery for Malignant Salivary Gland Tumors

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Introduction

Salivary gland carcinomas (SGC), arising either from the major salivary glands (MaSGC) or the minor salivary glands (MiSGC), are rare entities comprising many different histologies with variable biological behavior. The surgical management of SGC is challenging, due to the often close proximity of the tumor to important anatomic structures such as the facial, lingual and hypoglossal nerves and adjoining vascular and musculoskeletal structures and due to the anatomic complexity of the regions involved, such as the nasopharynx and the skull base in case of MiSGMT's [1]. Given its rarity, its variety in histologic subtypes, its broad spectrum of involved anatomic locations and in addition, outcome studies which often have a retrospective design with a heterogeneous patient population, the optimal management of SGS has remained subject to controversy. However, there is agreement on following principles of treatment. First, primary surgery with achievement of clear surgical margins, followed by adjuvant radiotherapy as indicated based on the definitive pathological assessment of the surgical specimen, is commonly regarded as the primary treatment of choice for SGC [1–3]. Second, a rigorous pre-surgical work-up and appropriate planning of surgery and radiotherapy contribute to the success of treatment [1–3]. Third, the treatment needs to be tailored to the tumor and the patient, in order to minimize treatment-related morbidity and maximize postoperative function preservation,

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recovery and rehabilitation [1–3]. In recent years, interesting developments in ablative and reconstructive surgical procedures have emerged. They focus on reducing postoperative morbidity while maximizing function preservation, often via minimally invasive approaches (transoral laser or robotic surgery, transnasal endoscopic surgery etc.) on the one hand, and on optimization of the anatomic and functional reconstruction after tumor ablation on the other hand, leading to more rapid function rehabilitation and better esthetic outcomes.

Developments in Ablative Surgery

Transoral Surgery: TLM and TORS

Transoral endoscopic head and neck surgery, including both transoral laser microsurgery (TLM) and transoral robotic surgery (TORS), provides a means of accessing a range of anatomic sites in the upper aerodigestive tract that have traditionally been difficult to approach, such as the oropharynx, the supraglottic larynx and the hypopharynx. Additional advantages of TORS over TLM are enhanced visualization with 3-dimensional vision and tenfold magnification, elimination of physiological tremor leading to more surgical precision and restoration of proper hand–eye coordination. Furthermore, the use of multi-articulated instruments with 7 degrees of freedom improves dexterity and maneuverability, and as a result, overcomes the limits of the line-of-sight issue and typical tangential-only cutting plane as encountered in TLM. All this results in accessability in a selection of tumors, which are unapproachable by TLM [4, 5]. Whereas TLM and TORS have a proven track record in the primary surgical treatment of selected squamous cell carcinomas (SCC), with evidence being most abundant for treatment of laryngeal SCC by TLM and oropharyngeal SCC by TORS, only few reports have been published on transoral resection of MiSGC arising in the upper aerodigestive tract (oropharynx, larynx) [6–11]. The rationale for using transoral surgery is that it is a minimally invasive 'natural orifice' surgery that, compared to the classic transcervical and transmandibular approaches, dramatically reduces interference with healthy surrounding tissues thus resulting in less postoperative morbidity, less pain, faster recovery, shorter hospitalization and better functional outcomes. These advantages have been illustrated in comparative studies on open approaches versus TORS for oropharyngeal SCC (OPSCC), both in the primary and salvage settings [12, 13].

As a substantial part of the MiSGC arise in the oropharynx, with the base of tongue (BOT) being the most commonly affected subsite (78% of oropharyngeal MiSGC), classical TORS procedures such as radical tonsillectomy/lateral oropharyngectomy and BOT resection have been applied to MiSGC [14, 15]. Feasibility and safety of TORS for management of oropharyngeal MiSGCs was first illustrated by Villanueva et al. in 2013 in 10 patients with either T1 or T2 tumors. Free surgical margins were achieved in all cases, locoregional control after 2 years was 80% and functional

outcomes proved excellent with mean postoperative MD Anderson Dysphagia Index (MDADI) scores of 99/100. However, postoperative functionality was only measured in 6 patients at a random time point [16]. Schoppy and colleagues reported on 20 patients with MiSGCs of the oropharynx managed with endoscopic approaches, either TORS or TLM. Adenoid cystic carcinoma (AdCC) was the most common histology, accounting for 35% of cases and the BOT was the most commonly affected subsite (75%). Of the 20 patients included in the analysis, 10 underwent TORS followed by adjuvant radiation therapy. Postoperative complications were limited, with one patient (5%) returning to the operating theatre for control of post-operative oropharyngeal bleeding; no long-term tubefeeding or tracheotomy dependency were reported. On an average follow-up of 36 months, 90% of patients were alive with no evidence of recurrence [17].

In a recent retrospective analysis of the National Cancer Database (NCDB), perioperative outcomes and overall survival of patients with oropharyngeal MiSGC treated with TORS were compared to outcomes of patients treated by other approaches. In a total of 785 analyzed patients, no significant differences in positive margin rate, 30-day mortality or overall survival between groups were reported. Although the 30-day unplanned hospital readmission rate was higher in patients treated with TORS versus non-robotic resections (5.8 vs. 1.7%, p = 0.0004), when stratified by tumor subsite, there was a significant decrease in hospital length of stay in patients with BOT SGCs treated with TORS versus non-robotic resections (p = 0.029) [14]. Although current evidence is limited to retrospective studies reporting on outcomes of small patient populations with short follow-up, the abovementioned data suggest that transoral endoscopic head and neck surgery may be considered a valuable treatment modality in the multidisciplinary management of MiSGCs (Fig. 19.1) [18].

Additionally, TORS has recently been attempted for primary parapharyngeal space tumors, which often derive from the deep lobe of the parotid and present as a mass in the prestyloid compartment of the parapharyngeal space (PPS). Traditionally, these tumors are addressed by a transcervical, transparotid or transmandibular approach, as the classic, non-robot assisted transoral approach offers limited exposure to the PPS, with lack of control of the great vessels and cranial nerves and hence, possibility of neurovascular injury [19]. These limits can be overcome by TORS, offering better visualization and more precision compared to the conventional transoral approach. TORS candidates are patients with adequate exposure of the oropharynx and whose preoperative assessment reveals a well-circumscribed neoplasm with lateral displacement of the internal carotid artery and clear cleavage plane from the neurovascular bundle [19]. TORS may be used in both pre- and retrostyloid tumors, however, the far lateral and superior areas of the PPS are inaccessible by this technique and require transcervical assistance [20]. Although several case series and reviews confirmed safety and feasibility of TORS for selected PPS tumors, this needs to be interpreted with caution as only a small minority of PPS tumors treated with TORS were malignant [20–22]. Moreover, TORS for PPS lesions has some drawbacks such as the high rate of capsula rupture with resulting tumor fragmentation and spillage, lack of carotid artery protection and need for division of
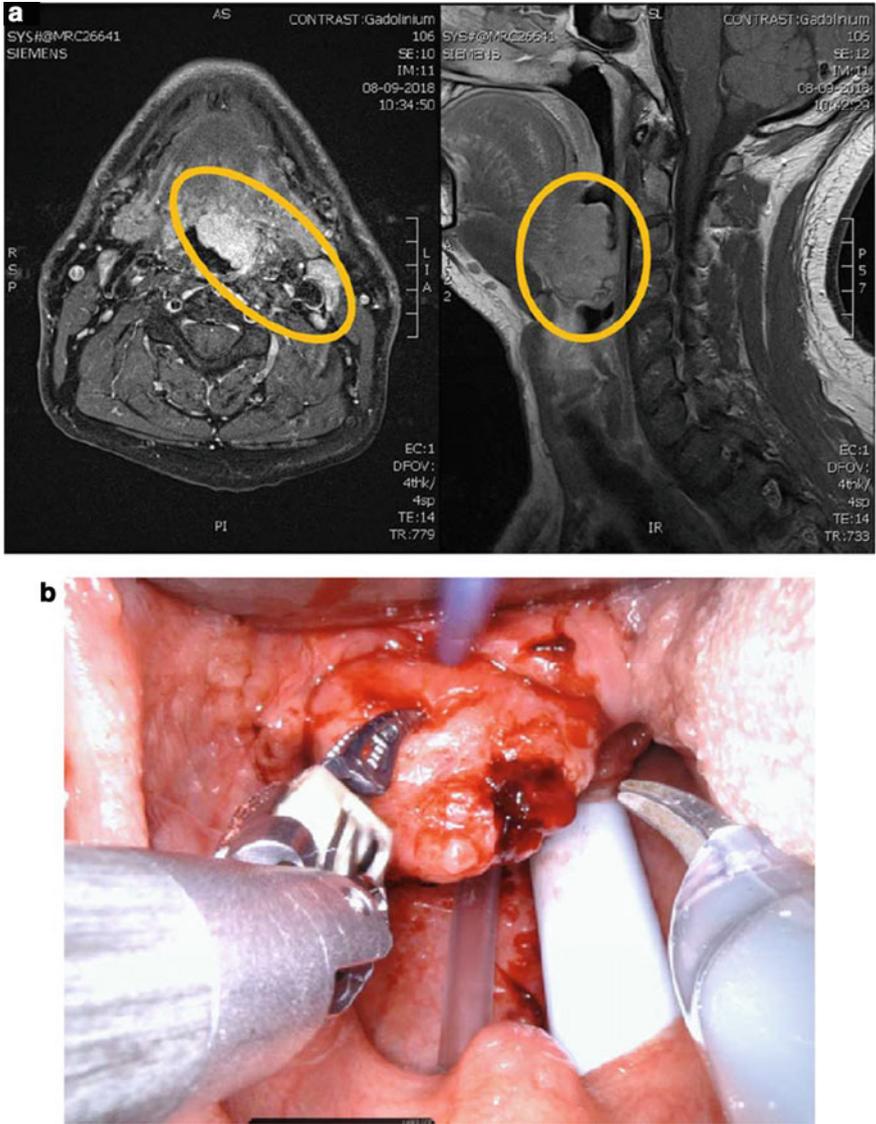
Fig. 19.1 Hyalinizing clear cell carcinoma (HCCC) of left base of the tongue. **a** Gadolinium-enhanced magnetic resonance imaging of clinical stage cT3N1 HCCC. The left panel shows the primary tumor and a level II lymphadenopathy on axial images. The right panel shows a sagittal T1-weighted image of the base of tongue tumor filling the vallecula and pushing the epiglottis down. Yellow circles show the tumor infiltration. **b** Transoral robotic resection and ipsilateral comprehensive neck dissection resulted in a pathologic stage pT3N2b HCCC. This figure was previously published elsewhere and approved for reproduction (18)

the parapharyngeal mucosa and superior constrictor muscle which is associated with considerable postoperative pain. Moreover, no comparative data of postoperative speech, swallowing and pain outcomes of TORS versus the transcervical approach exist. As such, there is currently no conclusive evidence that this approach is truly 'minimally invasive' [22]. Together with the lack of large case series and sound oncological outcome data, TORS for malignant PPS tumors should be considered only in very selected cases and should be performed by very experienced robotic surgeons, given the anatomic complexity of the PPS.

Finally, some technical developments related to the current robotic platforms, which have a suboptimal design for TORS, may optimize the capability of TORS for treating malignant salivary gland tumors arising in the upper aerodigestive tract. Monopolar electrocautery, the most common dissection and coagulation tool during TORS causes significant collateral tissue damage; the latter is far less common when using a CO_2 laser as a cutting device. As such, implementation of CO_2-laser technology during TORS could be of substantial benefit. In a recently published study, feasibility and safety of a newly developed steerable CO_2-laser fiber carrier compatible with the existing Endowrist® monopolar spatula of the Da Vinci Xi (Intuitive Inc, Sunnyvale, CA, USA) were illustrated in a preclinical setting, with the prototype successfully combining advantages of CO_2-laser with advantages of TORS [23].

Transnasal Endoscopic Surgery

For selected naso-ethmoidal MiSGMTs, especially AdCC of the ethmoid, small case series have supported the use of endoscopic transnasal surgery [1]. In a retrospective case series including 34 patients affected by sinonasal AdCC treated by an endoscopic endonasal approach, the authors report excellent oncological outcomes with 5-year disease-specific survival and recurrence-free survival rates of 86.5% and 71.8% respectively [24]. Similarly, it has been shown that MiSGCs localized in the nasopharynx without involvement of the internal carotid artery and minimal extension to the skull base can be effectively managed with transnasal endoscopic surgery [1].

For MiSGCs arising in the upper jaw, requiring maxillectomy, endoscopic approaches are also increasingly used in combination with and preceding standard external maxillectomy techniques. Before the en bloc resection, the retromaxillary and infratemporal tumoral extension is controlled endoscopically and the pterygo-maxillary junction is drilled to allow for a more precise and safer way to perform the posterior osteotomy. This combination of both open and endoscopic techniques optimizes the radicality of resection through better exposure of the medial and posterior extent of the lesion and by more precise delineation of the surgical margins, in particular the most difficult posterior margin (Fig. 19.2). This is illustrated by the high rates of clear margins posteriorly (96%) and the low incidence of local recurrence posteriorly (5.3%) as reported by Deganello et al. in a retrospective review of 79 patients who underwent endoscopic-assisted maxillectomy for nasoethmoidal,
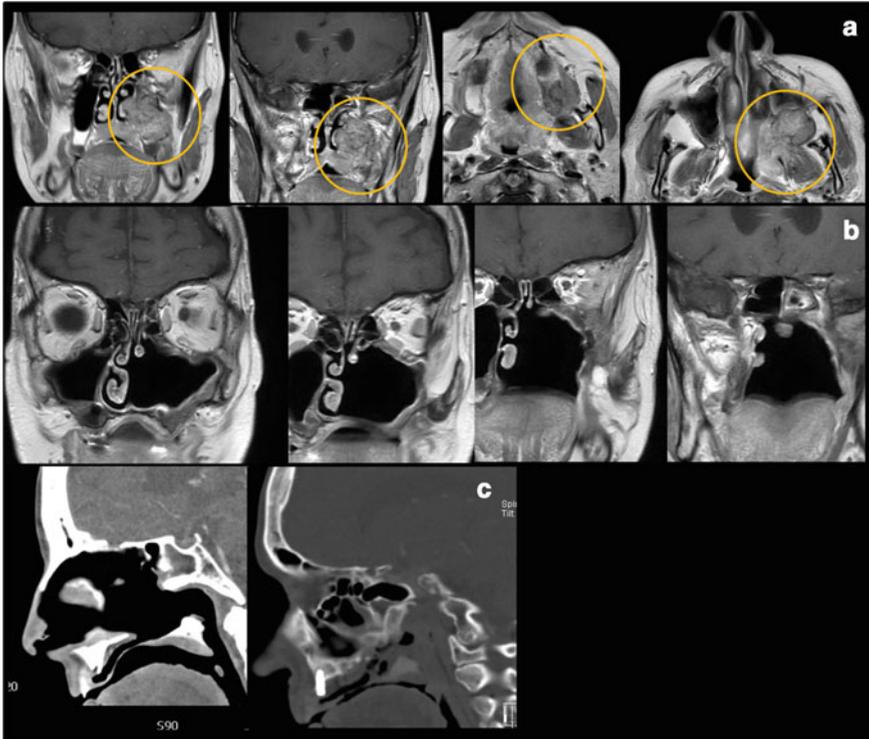
Fig. 19.2 **a** T1-weighted MRI of a T3N0 myoepithelial carcinoma ex pleomorphic adenoma of the left pterygopalatine fossa. **b** Postoperative MR showing the status after combined endoscopic—transoral resection. **c** CT scan showing implant retained obturator with good speech and swallowing function. Following postoperative image-guided radiotherapy (IMRT) to 66 Gy, the patient is now 3 years free of disease

maxillary, or hard palate cancer with a substantial portion of patients being affected by MiSGCs (17/79 or 21.5%) [25].

Developments in Reconstructive Surgery

Regarding recent evolutions in reconstructive surgery, mainly new developments in reconstruction following radical parotidectomy have emerged. The immediate reconstruction of the face in the setting of radical parotidectomy for malignancy represents a particular challenge because of the complexity of the defect, the frequent need for postoperative radiotherapy, the often advanced patient age, and possible limited life expectancy [1, 26].

Developments in Midface Reanimation

Common approaches to midface reanimation are the use of static slings, temporalis myoplasty and innervated free muscle transfers (most often the gracilis muscle). The eye is commonly protected through lid loading and lateral tarsorraphy or canthoplasty. Additionally, fasciocutaneous flaps (e.g., anterolateral thigh (ALT) flap) are routinely used for skin and soft tissue replacement, while reconstruction of the facial nerve is commonly performed with free nerve cable grafting. However, given the advanced age of most patients and the likelihood of postoperative radiotherapy, the recovery of spontaneous movement through free nerve grafting is slow, unpredictable and often suboptimal [27]. Moreover, development of troublesome synkinesis by misdirecting regenerating axons and simultaneous activation of multiple muscle groups frequently occurs [1].

When compared to free nerve grafts, the use of vascularized nerve grafts (VNGs), such as the radial forearm flap (RFF) with dorsal sensory branches of the radial nerve (DSBRN) and ALT with the lateral femoral cutaneous nerve (LFCN) or deep motor branch of the femoral nerve to vastus lateralis (DMBVL), are claimed to improve functional facial recovery outcomes, when compared to free nerve grafts [28]. In a retrospective review of 12 patients who underwent radical parotidectomy and immediate facial nerve reconstruction with VNGs, 8 patients (75%) regained at least resting symmetry [28]. The use of vascularized nerve grafts implies microvascular anastomosis and the donor nerve grafts are harvested together with adipofascial tissue to maintain nerve vascularity. As an additional advantage, the associated adipofascial component of these flaps (e.g., deepithelialized RFF) helps augment the soft tissue contour defect after tumor ablation. Hence, only 1 donor site is required to reconstruct both the contour and neuromuscular deficits. This contrasts with free nerve grafts, which are typically harvested from sites remote to the free flap.

Another option for reanimating the paralyzed face after radical parotidectomy, which recently became increasingly popular, is the use of the masseteric nerve, a motor branch of the mandibular nerve, for reinnervation of the midface and lip musculature (Fig. 19.3). Its position within the subzygomatic triangle and thus close proximity to the buccal branch of the facial nerve allows a tension-free coaptation without the need for cable grafting, which translates into a faster recovery of function; the regenerating axons have only a short distance to travel to reach the fascial muscles. Moreover, the masseteric nerve has a significantly higher axonal count as compared to the proximal stump of the facial nerve, which adds to the swift return of neural function which can be seen as early as 2 months postoperatively [29]. As a consequence of this high axonal density of the masseteric nerve, the masseter to buccal branch transfer (MBBT) produces strong oral commissure excursion with clenching, but lacks the spontaneity and resting tone achieved with interposition nerve grafting between the main trunk of the facial nerve and its distal branch(es). Given this consideration, several authors propagate a dual innervation approach in which a MBBT is combined with proximal facial nerve grafting to the remaining distal branches (which is only possible if the main trunk of the facial nerve could be
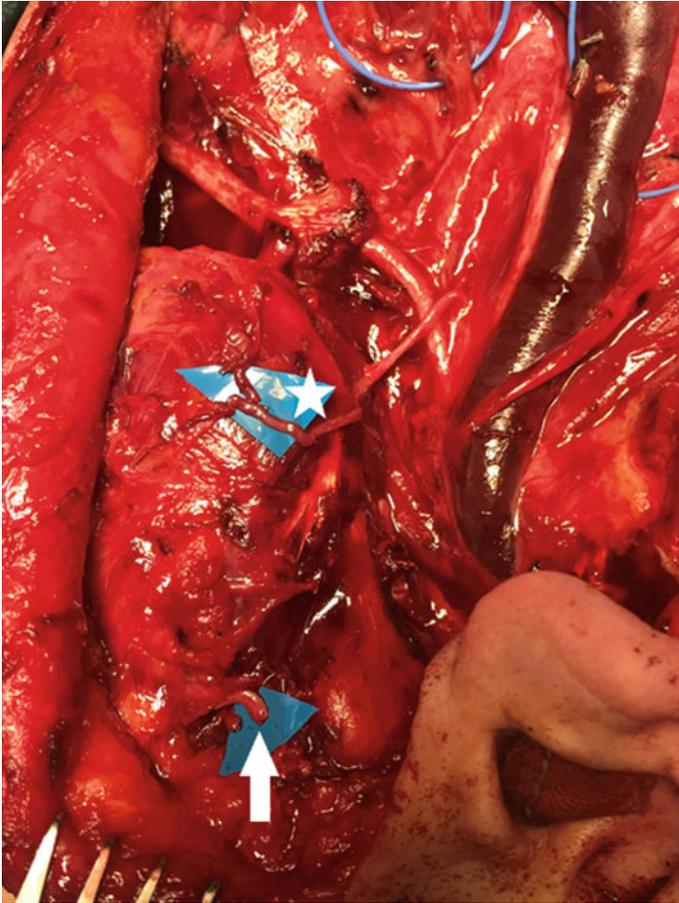
Fig. 19.3 Radical parotidectomy defect with sacrifice of the main trunk of the facial nerve. The masseteric nerve and the buccal branch of the facial nerve are identified and prepared for a masseter to buccal branch transfer (MBBT) (white arrow). The descending hypoglossal branch of the ansa cervicalis is reflected cranially and prepared for neural coaptation with the marginal branch of the facial nerve (white star)

spared during the ablative procedure), resulting in a more reliable but still voluntary smile [26, 29]. Moreover, this combined approach decreases the troublesome synkinesis by providing 2 separate nerve inputs to different facial muscle groups: the cable grafting restores tone to areas in the lower eyelid and midface whereas the MBBT is targeted to the lower facial muscle group, allowing for independent movement of the oral commissure [29]. It has to be noted that MBBT has minimal morbidity and that MBBT is also possible when the proximal stump of the facial nerve is unavailable due to the extent of the resection [26].

Developments in Single Stage Reconstruction of Complex Defects Using Free Flaps

Recently, new free flaps have been described which are suitable for single stage reconstruction of complex defects after radical parotidectomy. These include the ALT with dual chimeric innervated vastus lateralis free flap which is suitable for both cutaneous reconstruction and dynamic reanimation of the midface after radical parotidectomy with resection of the peripheral facial nerve branches. In this single stage reconstructive approach, 2 muscle units of the vastus lateralis muscle on separate nerves are harvested in combination with the ALT fasciocutaneous flap on a single vascular pedicle, creating a chimeric flap. The larger muscle unit is inserted directly to the oral commissure to suspend the midface and an end-to-end neural coaptation between the nerve to the vastus muscle and the masseteric nerve is performed. The smaller muscle unit is inserted into the upper eyelid to assist eye closure, followed by neural coaptation to the upper division of the facial nerve when available, or to the facial nerve stump. Due to the dense aponeurosis, the vastus lateralis muscle units provide a reliable static suspension until reinnervation kicks in. The ALT fasciocutaneous flap is used to restore the cutaneous defect or deepithelialized for contour restoration when no skin is required [27].

Another new flap described for single stage reconstruction of radical parotidectomy defects is the thoracodorsal artery perforator and nerve flap (TAPN) flap, which allows for skin or soft tissue reconstruction in combination with facial nerve reconstruction from the trunk of the facial nerve to 4–6 distal facial nerve branches [30, 31]. This flap can be designed according to the defect and the soft tissue required, including either an adipocutaneous paddle or only fat tissue if no skin resection was performed. Moreover, the main trunk of the thoracodorsal nerve is elevated together with the thoracodorsal artery and vein, in order to preserve the vascularization of the thoracodorsal nerve and its distal branches, which can be adapted to the facial nerve defect. As such, the thoracodorsal nerve and its branches are considered VNGs with inherent advantages compared to free nerve grafts (cfr supra) [30].

Conclusion

Although many promising developments in ablative and reconstructive surgical treatment of salivary gland malignant tumors have been reported, the current evidence supporting their added value remains limited. Reports are often small retrospective series that lack rigorous follow-up of both functional and oncological outcomes. As such, future comparative research is necessary in order to identify the most optimal ablative and reconstructive techniques in relation with specific indications, potentially allowing for future evidence-based patient-tailored approaches.

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Chapter 20
New Systemic Therapies in Salivary Gland Cancer

Ioannis A. Vathiotis, Jennifer M. Johnson, and Athanassios Argiris

Introduction

Salivary gland cancer (SGC) is a rare malignancy that accounts for less than 5% of all cases of head and neck cancer [1]. The annual age-standardized rate of SGC is 0.57 per 100,000 people worldwide, and is expected to increase by 50% by 2040 [2]. Although the causes remain largely unknown, several factors have been associated with the development of malignant salivary gland tumors, including radiation exposure, history of prior malignancy, viral infections (i.e., Epstein Barr virus [EBV], human immunodeficiency virus [HIV]), industrial chemicals (rubber manufacturing), and nickel compounds [3]. Malignant tumors arising from the major or minor salivary glands are also characterized by considerable diversity. The 5th edition of the World Health Organization (WHO) classification of head and neck tumors identifies 24 distinct histologic subtypes of SGC, with implications to disease biology and clinical features [4].

In general, malignant salivary gland tumors have prolonged clinical courses, characterized by slow growth, multiple local recurrences, and delayed development of

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distant metastases, most commonly to the lung, liver, and bone. Although histologic grading has been shown to possess some prognostic value, implementation of a universal grading scheme appears unable to explain inherent differences in tumor biology and thus, is not currently recommended [5]. Patients with recurrent or metastatic (R/M) disease have poor prognosis and effects of chemotherapy are moderate with a median overall survival (OS) of 15 months and five-year OS rates of about 15% [6]. Moreover, rarity as well as extensive heterogeneity of SGC have precluded the accumulation of prospective clinical trial data, and treatment decisions have been informed by non-randomized studies and/or retrospective series. According to the American Society of Clinical Oncologists (ASCO), systemic therapy should be reserved for patients with metastatic tumor deposits not amenable to palliative local therapy, threatened end-organ dysfunction, or lesions that have grown more than 20% within a period of six months [7].

Recent advances in molecular characterization of SGCs have uncovered subtype-specific genomic alterations with potential clinical significance (Table 20.1) [8]. These approaches have provided deeper understanding of the molecular pathogenesis and are presently included in the definition of several entities, aiding in proper diagnosis [4]. In addition, routine genomic profiling has enabled the development of novel, personalized therapeutic strategies, sometimes via direct extrapolation from progress made in more common tumor types.

Subtypes of Salivary Gland Cancers

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma (ACC) is the most common malignancy arising in the minor salivary glands and second most common overall [9–11]. Its clinical course is slow but relentless, marked by perineural invasion, with 40–50% of the patients experiencing disease recurrence after curative intent therapy [11–13]. Aside from site of origin and stage, the presence of "any solid" component and/or high-grade transformation/dedifferentiation on histology has been linked with prognosis, with high reproducibility, low interobserver variability, and high negative predictive value [14–17]. MYB overexpression as well as the presence of activating *NOTCH1* mutations have also been shown to confer a poor prognosis for patients with ACC [18–20].

ACCs typically harbor a low number of genomic alterations (GA; 1.6 GA/tumor) as well as low *TP53* GA frequency (4%) and tumor mutational burden (TMB; TMB >10 mut/Mb in 1% of the cases) [8]. *MYB-NFIB* fusions, generated by t(6;9)(q22-23;p23-24) translocations, represent the most common GA seen in patients with ACC, occurring in up to 80% of the cases [18, 19, 21]. The product of the *MYB* gene is a DNA-binding transcription factor that normally regulates stem and progenitor cells [22]. Persson et al. showed that such alterations disrupt repression of *MYB* resulting

Table 20.1 Frequent genomic alterations across different subtypes of salivary gland cancer

Subtype	Frequent genomic a alterations gene (%)	Over expression on IHC protein (%)
Adenoid cystic carcinoma	*MYB-NFIB/MYBLl-NFIB/5'-NFIB* (65–88), *NOTCH1* (11–29)	c-KIT (65–90), NICD (49–98), EGFR (24–85), VEGF (76)
Mucoepidermoid carcinoma	*CRTC1-MAML2/CTRC3-MAML2* (38–82), *PIK3CA* (20), *BRCA2* (17), *ERBB2* (13)	EG FR (46–100), HER2 (0–38)
Salivary duct carcinoma	*ERBB2* (32), *PIK3CA* (18–27), *HRAS* (16–23)	AR (78–98), HER2 (16–83), EGFR (53)
Mammary analogue secretory carcinoma	*EJV6-NTRK3* (95–98), *ETV6-nonNTRK3* (2–5)	NA
Acinic cell carcinoma	*HTN3-MSANTD3* (4–16)	NA
Polymorphous adenocarcinoma	*PRKD1/2/3* (50–80), *FGFR1* (20)	NA
Adenocarcinoma NOS	*PIK3CA* (20–24), *ERBB2* (17), *CDKN2A/B* (12–17), *HRAS* (14)	NA
Carcinoma ex pleomorphic adenoma	*FCFR1-PLAG* (9–86) *HMCA2* (29)	NA
Epithelial-myoepithelial carcinoma	*HRAS* (33), *KRAS* (18), MVS (18)	FGFR1 (86), c-KIT (69–83)
Myoepithelia carcinoma	*FWSR1* (39), *PIK3CA* (15)	NA
Intraductal carcinoma	*RET* (47)	NA
Poorly differentiated carcinoma	*PIK3CA* (20), *ERBB2* (15)	NA

in protein overexpression, with implications to apoptosis, cell cycle control, proliferation, cell adhesion, and angiogenesis [23]. Thus, *MYB* represents a *bona fide* human oncogene and true hallmark of ACC, irrespective of site of origin [24]. MYB overexpression has been the target of novel vaccination approaches, used synergistically with programmed cell death protein 1 (PD-1) inhibition in the ongoing MYPHISMO trial (NCT03287427) [25]. Additional preclinical efforts to reverse *MYB* overactivation in patients with ACC have targeted either the upstream IGF1R/AKT signaling axis or the downstream DNA-damage sensor kinase ATR with promising results [26, 27]. *MYBL1* rearrangements, including *MYBL1-NFIB* fusions that may in part be interchangeable with *MYB-NFIB*, as well as *5'-NFIB* fusions that do not involve either the *MYB* or *MYBL1* genes constitute less prevalent events in the genomic landscape of ACC [28].

Notch is a highly evolutionarily conserved pathway that acts as a stem cell fate determinant [29]. There are four NOTCH receptors (NOTCH1-4), and five

membrane-bound ligands, including delta-like (DLL1, -3, and -4), and Jagged (JAG1-2); upon interaction, two consecutive proteolytic cleavages of the receptor, the second mediated by the gamma-secretase complex, free the Notch intracellular domain (NICD) and allow it to enter the nucleus and form a transcriptional activation complex that controls the expression of target genes. Activating *NOTCH1* mutations have been shown to possess carcinogenic potential, driving 50% of T-cell acute lymphoblastic leukemias (T-ALLs) [30]. They have also been found in 11–29% of patients with ACC [21, 31, 32]. Such patients are more likely to have solid pattern on histology, advanced disease at diagnosis, non-lung metastases, including in liver, bone, or other atypical sites, and ultimately shorter relapse-free survival (RFS) and OS [20]. Several ways to target NOTCH1 have been utilized in patients with ACC. Brontictuzumab is a monoclonal antibody that binds to the negative regulatory region and inhibits NOTCH1; treatment with brontictuzumab led to an objective response rate (ORR) of 17% in the context of a phase I basket study that enrolled 12 patients with ACC, that either harbored a *NOTCH1* mutation or were NICD-high on immunohistochemistry (IHC; NCT01778439) [33]. In addition, the ACCURACY phase II clinical trial evaluated the nonspecific gamma secretase inhibitor AL101 in patients with R/M ACC and activating *NOTCH1-4* mutations. In cohort 1 (4 mg/week, n = 45), ORR was 15% and disease control rate (DCR) was 65% [34]. In cohort 2 (6 mg/week, n = 42), ORR was 9% and DCR was 70% [35]. The most common toxicity was diarrhea, which was mainly low-grade and tolerable. AL101 is currently being evaluated in the neoadjuvant setting in patients with NOTCH1-driven ACC (NCT04973683). Crenigacestat (LY3039478) represents another gamma secretase inhibitor that has demonstrated clinical activity in heavily pretreated patients with advanced or metastatic disease [36]. Regarding patients with ACC of the salivary gland, crenigacestat showed limited clinical activity with no confirmed responses [37]. Inhibition of cancer stemness kinases by amcasertib (BBI-503) has also demonstrated clinical safety with encouraging signs of antitumor activity, achieving sustained disease control, that warrant its further development in patients with ACC (NCT01781455) [38]. The transcriptional activation complex inhibitor CB-103 has been used to target the Notch pathway in patients with ACC as well (NCT03422679).

Protein arginine methyl-transferase 5 (PRMT5), a highly-conserved metabolic enzyme involved in multiple signal transduction pathways, is also being targeted in patients with ACC. Preliminary results of a phase I study (NCT02783300) demonstrated a partial response in 3 out of 14 patients with ACC that were treated with the PRMT5 inhibitor GSK3326595 [39]. Another PRMT5 inhibitor, PRT543, is currently in development for patients with ACC (NCT03886831).

Overexpression of KIT on IHC has been reported in 65–90% of patients with ACC; however, agents targeting c-KIT, including imatinib and dasatinib, have only achieved sporadic antitumor responses [1, 40]. IHC positivity for epidermal growth factor receptor (EGFR) has also been observed in the majority of ACC cases, but different EGFR-targeted therapies (cetuximab, gefitinib, lapatinib) have failed to produce any antitumor responses [1, 40]. In addition, approximately three out of four ACCs stain positive for vascular endothelial growth factor (VEGF) on IHC [40]. While older multitargeted receptor tyrosine kinase inhibitors (TKI; sunitinib, sorafenib,

pazopanib) have shown minor antitumor activity, newer agents have produced more promising results. Specifically, axitinib has been evaluated in the context of two phase II studies (NCT01558661 and NCT02859012) [41, 42]. In the largest study by Keam et al., 60 patients were randomized to axitinib or observation; administration of axitinib resulted in a ORR of 3%, DCR of 100% (versus 52% in the observation arm) and median progression-free survival (PFS) of 10.8 months (versus 2.8 months in the observation arm; hazard ratio [HR], 0.25; 95% confidence intervals [CI], 0.14–0.48; P < 0.0001) [42]. Matters of safety and efficacy for the multitargeted TKI lenvatinib have also been evaluated by two single-arm phase II studies in patients with ACC [43, 44]. The first study (NCT02780310) enrolled 33 patients with R/M ACC and met its primary endpoint of ORR with five partial responses (PR; 16%) [43]. Patients on lenvatinib had a median PFS of 17.5 months. Although there were no new safety signals, 18/32 patients discontinued lenvatinib due to toxicity. In the second study (NCT02860936), 28 patients received lenvatinib with an ORR of 12%, median PFS of 9.1 months and median OS of 27 months; quality of life (QOL) was found to deteriorate in some domains, including fatigue and dry mouth [44]. Recently, the VEGF receptor inhibitor apatinib demonstrated substantial antitumor activity in a single-arm phase II study that enrolled 68 patients with R/M ACC [45]. Patients on apatinib had an ORR and DCR of 46% and 99%, respectively; at a median follow-up of 25.8 months, the median PFS was not reached. As a result, in February 2021, apatinib gained Orphan Drug Designation by the FDA for patients with ACC.

Recently, relevant prostate-specific membrane antigen (PSMA)-ligand uptake has been detected in as high as 93% of ACCs, indicating that therapy with [177]Lutetium-PSMA may be beneficial in such patients [46].

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland tumor, accounting for approximately one third of all cases [47]. Most MECs are low-grade tumors and have a good prognosis; five-year cause-specific survival (CSS) rates are 98.8, 97.4, and 67.0% for low-, intermediate-, and high-grade tumors arising in the parotid gland, respectively, and 90.7% for those arising in the minor salivary glands [48, 49]. Age and stage (including nodal status, and the presence of intraparotid or distant metastasis) represent additional prognostic factors for patients with MEC [50, 51]. It should be noted that distant metastases are extremely rare in patients with low-grade MEC (0.2–0.3%) [48, 52].

High-grade tumors typically have more GAs than other-grade tumors (5 GA/tumor for high-grade versus 2.3 and 2.6 GA/tumor for low- and intermediate-grade tumors, respectively; P = 0.019) [53]. Moreover, GAs in *TP53*, *PIK3CA*, *BRCA1* and *BRCA2* are more frequent in high-grade tumors compared with low- or intermediate-grade. Other genomic events frequently reported in patients with MEC, though not grade-specific, include mutations in *CDKN2A*, *CDKN2B*, and *BAP1*. Translocations

between mastermind like gene 2 (*MAML2*) and CREB regulated transcription coactivators (*CTRC*) represent the most frequent GAs in patients with MEC [54]. *CRTC1-MAML2* fusions, most commonly as a result of a t(11;19)(q21;p13) translocation, are seen in the majority of cases, with a higher percentage documented in low- and intermediate-grade tumors; the *MAML2* gene occasionally fuses with *CRTC3* as well [55, 56]. The clinical significance of these fusions remains to be determined. *CRTC1-MAML2* fusion has been shown to upregulate the epidermal growth factor receptor (EGFR) ligand amphiregulin (AREG), causing autocrine EGFR signaling activation, and MEC cell growth and survival [57, 58]. Notably, *CRTC1-MAML2*-positive MEC tumors are sensitive to EGFR signaling inhibition both in vitro and in vivo in human xenograft models, making it a potential therapeutic target. Although EGFR overexpression on IHC may not be the case in all patients harboring the *CRTC1-MAML2* fusion, responses to EGFR inhibitors, such as erlotinib, gefitinib, or cetuximab, have been reported sporadically, making such approaches an attractive option for patients with MEC [40]. However, approaches that used different TKIs, including lapatinib, nintedanib or sorafenib, have failed to demonstrate consistent antitumor activity in patients with MEC [59].

Salivary Duct Carcinoma

Salivary duct carcinoma (SDC) represents 4–10% of all SGCs [40]. Histologically, it is similar to ductal carcinoma of the breast [60]. It typically presents as a rapidly growing mass within the parotid gland; 20–70% of the cases arise from a preexisting pleomorphic adenoma (carcinoma ex pleomorphic adenoma) [60]. SDC is an aggressive tumor with a tendency to metastasize in the lymph nodes (47–68% at presentation) [61, 62]. Notably, 54% of patients treated with curative intent develop locoregional recurrence and/or distant metastasis; brain metastases have been documented in 18% of patients with SDC. The presence of lymph node metastasis as well as the number of involved nodes are independent prognostic factors in patients with SDC [62, 63]. As highlighted by Nakaguro et al., the presence of prominent nuclear pleomorphism, ≥30 mitoses/10 HPF, vascular invasion, or ≥5 poorly differentiated clusters represent additional histologic features associated with poor prognosis [64].

SDCs harbor GAs in *ERBB2* in 32% of cases, whereas overexpression of human epidermal growth factor receptor 2 (HER2) by IHC and/or FISH has been recorded in 16–83% [65]. Trastuzumab coupled with docetaxel demonstrated an ORR of 70% in 57 patients with HER2-positive SDC and no prior exposure to HER2 targeted therapy; in this study, HER2 status was assessed with the combination of IHC and FISH and interpreted in accordance with the guidelines for HER2 assessment in breast cancer [66]. Median PFS and OS were 8.9 and 39.7 months, respectively. Addition of pertuzumab to amplify the anti-HER2 activity of first-line treatment regimens has also shown promising results [67]. In a retrospective case series, first line therapy with trastuzumab, pertuzumab and docetaxel achieved an ORRs of 58% (including one complete response [CR]) in patients with HER2-positive SDC (by IHC and/or

FISH); ado-trastuzumab emtansine (T-DM1) administered upon disease progression resulted in an ORR of 57% in the same patient population [68]. In addition, median OS reached 42.0 months. As of late, trastuzumab deruxtecan (T-DXd) has also shown promising antitumor activity. In a pooled analysis of two phase I studies (DS8201-A-J101, DS8201-A-A104), encompassing a total of 17 patients, ORR was 47% and DCR was 100%, with a median PFS of 14.1 months [69]. Notably, HER2 status was assessed by either IHC and/or ISH or next generation sequencing (NGS) and 14 patients had previously received treatment with HER2-targeted agents. No new safety signals were recorded—interstitial lung disease was documented in three cases (18%).

Androgen receptor (AR) is expressed in 78–96% of patients with SDC [40, 70]. The first report of androgen-deprivation therapy (ADT; with goserelin) prescribed for the treatment of SDC dates back to 1994 [71]. Combined androgen blockade with leuprorelin and bicalutamide as first-line therapy in patients with R/M SGC was evaluated in a single-arm, single-institution phase II study [72]. Out of 36 patients enrolled, 34 had SDC. AR status was assessed in accordance with the ASCO/College of American Pathologists (CAP) guidelines for the evaluation of breast cancer predictive factors, and tumors were considered positive if a minimum of 1% of tumor nuclei were immunoreactive for AR (AR $\geq$ 70% was seen in 83% of the cases) [73]. The ORR and DCR were 42% and 86%, respectively. Median PFS was 8.8 months and median OS was 30.5 months. Moreover, ADT with leuprorelin and bicalutamide demonstrated a favorable toxicity profile, with grade 3 or higher adverse events reported in two patients, leading to treatment discontinuation in one. Recently, Locati et al. reported on the efficacy of the combination of abiraterone with a luteinizing hormone-releasing hormone (LHRH) analogue in patients with castration-resistant, AR-expressing SGC (AR $\geq$ 70% by IHC) [74]. In this phase II study that enrolled 24 patients (19 with SDC), the ORR was 21% and DCR was 63%. In addition, the median PFS was 3.7 months and median OS was 22.5 months. Grade 3 toxicity was reported in 25% of the cases. Moreover, the European Organisation For Research And Treatment Of Cancer Head and Neck Cancer Group/United Kingdom Clinical Research Network (EORTC HNCG/UKCRN) 1206 phase II randomized study has completed accrual and compared ADT with bicalutamide and a gonadotropin-releasing hormone (GnRH) analogue to chemotherapy in previously untreated patients with AR-overexpressing tumors (cohort A; NCT01969578) [75]. Previously treated patients also received ADT as part of study cohort B. ADT has also exhibited clinical efficacy in the adjuvant setting; in a retrospective study, patients with completely resected, stage IVA, AR-positive SDC who received adjuvant ADT demonstrated significantly longer DFS (P = 0.02) and OS (P = 0.03) compared with those who did not [76].

Less frequent mutations seen in patients with SDC may also serve as potential targets for systemic therapy [70, 77]. Indeed, temsirolimus with bevacizumab have been utilized in *PIK3CA*-mutant tumors (18–27%) [78]. Also, *HRAS* mutations have been recorded in 16–23% of patients with SDC and treatment with tipifarnib, an inhibitor of farnesyltransferase that ultimately inactivates Ras by preventing it from binding to the membrane, has demonstrated modest antitumor activity with an ORR

of 8% and DCR of 62% in 13 previously treated patients with R/M SGC, including 4 with SDC [79]. Finally, the combination of dabrafenib and trametinib has shown clinical activity in a patient harboring *BRAF V600E* mutation (4–5%) [80].

Mammary Analogue Secretory Carcinoma

Characterized by histological and immunohistochemical resemblance to secretory carcinoma of the breast, mammary analogue secretory carcinoma (MASC) of the salivary gland was first described in 2010 [81]. It is a rare entity that most commonly involves the parotid gland [82]. Although it is marked by histologic diversity, 95–98% of the cases harbor a distinct, recurrent balanced chromosomal translocation t(12;15)(p13;q25), which leads to a fusion gene between the ETS Variant 6 (*ETV6*) gene on chromosome 12 and the neurotrophic receptor tyrosine kinase (*NTRK*)3 gene on chromosome 15 and is practically pathognomonic for MASC; the rest 2–5% of the cases harbor rearrangements involving *ETV6* and a non-*NTRK3* partner [83, 84]. *NTRK1-3* encode a family of tropomyosin receptor kinase proteins (TrkA, TrkB, and TrkC, respectively) implicated in the normal development of the nervous system [85]. Fusion of the intact tyrosine kinase domain of NTRK1, NTRK2, or NTRK3 with a variety of partners results in dysregulated activation of several biochemical signaling pathways that promote oncogenesis, including MAPK, PI3K and PKC, in a multitude of solid tumors [86, 87].

The first attempt to target NTRK was made by Drilon et al., who assessed matters of safety and efficacy of the TKR inhibitor larotrectinib, in the context of a phase I-II study in both adults and children with *TRK*-fusion positive tumors by IHC or FISH, encompassing a total of 17 unique cancer diagnoses [88]. Out of 55 patients enrolled, only one had CNS metastases at baseline. It should be noted that most patients had previously received at least one line of systemic therapy. In 12 patients with MASC, ORR was 83% and median duration of response (DOR) was not reached. In addition, larotrectinib was well tolerated, suggesting that long-term administration would be feasible for patients with *TRK*-fusion positive disease. Entrectinib is another potent pan-TRK inhibitor with increased antitumor activity in the CNS [89]. In a pooled analysis of three phase I-II studies, entrectinib demonstrated an ORR of 83% in patients with MASC of the salivary gland; out of 24 patients enrolled, 20 responded to entrectinib. Interestingly, ORR was highest among patients with MASC of the salivary gland compared with other tumor types. Again, this study enrolled mostly previously treated patients (63%). Patients with CNS metastases represented 21% of the study cohort; intracranial ORR with entrectinib was 53% in patients with measurable disease in the CNS and none of the patients without CNS metastases had confirmed progression in the CNS at data cutoff, achieving a 12-month event-free rate of 100%. PBI-200, a next-generation TRK kinase inhibitor that demonstrates clinical activity against relevant resistance mutations after treatment with a first-generation agent as well as enhanced brain penetration is currently being evaluated

in the PBI-200-101 phase I/II trial in patients with NTRK-fusion-positive advanced solid tumors (NCT04901806).

Acinic Cell Carcinoma

Acinic cell carcinoma is responsible for approximately 10% of all SGCs [47, 90]. This subtype most commonly arises in the major salivary glands [91]. It is a low-grade tumor, that is predominantly composed of acinic serous cells with zymogen-secreting granules and has a relatively slow growth pattern [92, 93]. Male sex, age >45 years, and tumor size >3 cm represent factors independently associated with prognosis [94]. The presence of aberrations in *MSANTD3* gene, most commonly *HTN3-MSANTD3* fusions, is highly specific for acinic cell carcinoma, characterizing 4–16% of the cases [95, 96]. However, their oncogenic potential as well as therapeutic relevance remain questionable. Nevertheless, *NTRK* gene fusion analysis is advised for all patients diagnosed with acinic cell carcinoma as MASC was formerly classified with the latter and has only been described as a separate entity since 2010.

Polymorphous Adenocarcinoma

Consisting of tumors previously classified as polymorphous low-grade adenocarcinoma (PLGA) or cribriform adenocarcinoma of the minor salivary gland (CAMSG), polymorphous adenocarcinoma (PAC) is characterized by cytologic uniformity but architectural diversity [97, 98]. PAC is the second most common malignancy of the minor salivary glands [98]. Overall, it is an indolent disease that rarely presents with distant metastases (4.3%) and has a good prognosis with 10-year DSS rates of 94% [99, 100]. PACs typically harbor GAs that affect the *PKRD* genes; the *PRKD1 E710D* hotspot mutation is present in >70% of PLGA cases, whereas 80% of CAMSGs display rearrangements involving *PRKD1*, *PRKD2*, or *PRKD3* (*PRKD1/2/3*); although these GAs appear mutually exclusive, PACs have marked genetic overlap, essentially representing a spectrum of lesions driven by GAs in the *PKRD* genes [101, 102]. Fusion-positive tumors are usually spotted at the base of the tongue, show papillary architecture, and have an increased risk of nodal metastasis [103]. Non-targetable GAs affecting the *FGFR1* gene have also been documented in 20% of all PLGA tumors [8].

Adenocarcinoma not Otherwise Specified (NOS)

By definition, adenocarcinoma NOS represents a residual group of salivary gland malignancies that cannot be classified into one of the other subtypes. The reported

rates of adenocarcinoma NOS range between 1.8 and 12.2% [47, 90]. However, these rates may overestimate its actual prevalence due to misclassification, and advances in molecular characterization of SGC are expected to curtail this remaining group. Similar to SDC, these tumors have a relatively increased load of GAs (4.1 GA/tumor), with GAs in *TP53* observed in 55% of the cases [8]. Although the rates of either HER2 or AR positivity are lower compared with SDC, it is reasonable to test all adenocarcinomas NOS for both targets and treat accordingly. Indeed, T-DM1 has demonstrated enhanced antitumor efficacy in *HER2*-amplified adenocarcinoma NOS [104, 105]. In addition, patients with adenocarcinoma NOS have been included in trials evaluating ADT in SGC, however results of this subgroup were not reported separately [72, 74]. Additional GAs that have been described in adenocarcinoma NOS involve the PI3K-pathway, cyclin dependent kinases, and RAS family of proteins, as mentioned above [106].

Carcinoma ex Pleomorphic Adenoma

Carcinoma ex pleomorphic adenoma accounts for 8–12% of all SGCs [47, 90, 107]. It arises within a preexisting polymorphous adenoma (PA) and primarily affects the major salivary glands, most commonly the parotid [107]. The extent of tumor invasion through the PA capsule into the surrounding tissue has been found to correlate with disease outcome [108, 109]. Gene fusions involving *PLAG1* and less frequently *HMGA2* have been documented in up to 86% of the cases [110]. Although of diagnostic importance, the clinical utility of these rearrangements has not been clearly delineated. As far as systemic therapy is concerned, adequate description of the subtype of the carcinoma component is crucial for optimizing therapeutic strategy; SDC represents the most common histologic subtype, followed by myoepithelial carcinoma [110].

Other Subtypes

Other subtypes of SGC are very rare, not characterized by targetable GAs or seldom require systemic therapy due to low rates of recurrence and/or metastasis. These are listed in Table 20.1.

Immunotherapy

Inhibition of the PD-1/ligand (PD-L1) immune checkpoint has achieved clinical responses in 15–20% of patients with R/M squamous cell carcinoma of the head and neck (SCCHN) [111–113]. However, data regarding the efficacy of immune

checkpoint blockade in patients with SGC are scarce. Overall, SGCs have low PD-L1 expression. The KEYNOTE-028 phase Ib basket trial enrolled a cohort of 26 patients with PD-L1-positive (PD-L1 combined positive score [CPS] ≥1) R/M SGC [114]. Pembrolizumab showed modest clinical activity with an ORR of 12% and DCR of 58%. The median PFS and OS reached 3.8 and 13.0 months, respectively. Antitumor efficacy was only slightly better with the addition of vorinostat [115]. In addition, single-agent PD-1 blockade with nivolumab achieved an ORR of 9% in unselected patients with ACC versus 4% in those with non-ACC histology, with a median PFS of 4.9 and 1.8 months, respectively [116].

The TMB is significantly lower in SGCs compared with tumor types where immunotherapy is currently approved (i.e., non-small cell lung cancer [NSCLC], melanoma). Specifically, less than 5% of clinically indolent tumors, such as ACC, acinic cell carcinoma, and MASC, harbor >10 mut/Mb, whereas for more aggressive subtypes, such as MEC, SDC, and adenocarcinoma NOS, the relative frequency does not exceed 15% [8]. Moreover, SDCs appear immune infiltrated and express immune checkpoints in abundancy in contrast to ACCs that have an immune-depleted tumor microenvironment, characterized by the presence of M2-polarized macrophages and myeloid-derived suppressor cells [117]. In line with the above, combination immunotherapy with nivolumab plus ipilimumab achieved an ORR of 6% (2/32) in patients with R/M ACC, compared with 16% (5/32) in those with non-ACC, in the context of a phase II trial (NCT0317624); importantly, responses were deep, durable and more common in patients with SDC [118, 119]. Similar results were reported in the SWOG S1609—DART trial, where patients with ACC histology had an ORR of 4% and patients with non-ACC histology had an ORR of 9% [120]. Ongoing clinical trials evaluating immune checkpoint inhibitors in patients with SGC are presented in Table 20.2.

Table 20.2 Ongoing immunotherapy clinical trials in salivary gland cancer. PD-1, programmed cell death protein-1; AR, androgen receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; VEGFR, vascular endothelial growth factor receptor; PD-L1, programmed death-ligand 1

Study identifier	Study type	Histology	Target	Agent	Primary endpoint	Status
NCT03942653	Phase II	All	PD-1, AR	Pembrollzumab, goserelin	ORR	Recruiting
NCT03146650	Phase II	All	PD-1, CTLA-4	Nivolumab, ipilimumab	PFS	Active, not recruiting
NCT04209660	Phase II	All	PD-1, VEGFR	Pembrollzumab, lenvatlnlb	ORR	Recruiting
NCT03990571	Phase II	ACC	PD-L1, VEGFR	Avelumab, lenvatinib	ORR	Recruiting

Conclusions

Both rarity and diversity of SGC pose challenges in conducting prospective clinical trials. As a result, we are lacking phase III data and quality of evidence that supports current clinical practice guidelines is low [7]. Recent advances in the molecular characterization of these tumors have unveiled multiple, oftentimes targetable, subtype-specific alterations. Thus, adequate pathological diagnosis by an expert salivary gland pathologist to determine the exact subtype of SGC is key in choosing the right systemic therapy. Participation in a clinical trial should be encouraged in all patients with SGC. For patients with ACC, a multitargeted tyrosine kinase inhibitor (i.e., lenvatinib, apatinib) may be offered. For those with non-ACC histology, therapy should be tailored to tumor molecular alterations (i.e., AR, HER2, NTRK). Next generation sequencing has to be considered for patients with SGC as it may offer the potential for targeted therapies. As far as immunotherapy is concerned, it should not be offered routinely, but may have a role in select patients, either in the context of a clinical trial or under local regulatory approval. The study of novel agents in prospective multicenter clinical trials, preferably in biomarker-selected populations, will be pivotal for the development of evidence-based approaches in SGC.

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Chapter 21
Exercise and Nutrition Interventions in Head and Neck Cancer

Paolo Bossi and Erika Stucchi

Introduction

Clinical outcome of patients with head and neck squamous cell carcinoma (HNSCC) is linked to patient, disease, and treatment characteristics, such as performance status, comorbidities, tumor stage, HPV status, and the availability and feasibility of adequate therapeutic approaches. However, the prediction of patient's prognosis is challenging and at the same time it is a critical point to offer the patient an adequate counseling and treatment planning.

There are also other patient-specific variables and host factors related to the immune, inflammation, and nutritional status that influence the survival of HNSCC patients. In the complex interaction between the host and the tumour, these factors play an important even if sometimes underestimated role.

In a recent work, Yu et al. [1] evaluated almost 600 primary HNSCC patients treated with definitive or post-operative RT. The authors showed through a machine-learning model that the main predictors of patients' overall survival were performance status, body-mass index (BMI) and the host factors reflecting the patients' nutrition and inflammation status.

Malnutrition and Nutritional Interventions

Weight loss, BMI, loss of muscle mass and biochemical examinations indicative of nutritional status are important predictive factors to consider in HNSCC patients. These patients are among the most vulnerable ones in terms of cancer-related malnutrition (defined as an unwanted weight loss of >5% (or >10%) in three (or six) months,

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or a reduction of BMI to less than 21 kg/m^2 [2]). Malnutrition in patients with HNSCC is a concern, as it is associated with increased treatment toxicity, number of admissions, health care costs, morbidity, and mortality [3]. In fact, the relative risk of dying of a severely malnourished patient is 1.8 times higher than for patients without malnutrition [4]. Furthermore, malnutrition in patients with HNSCC is associated with depression, [3] lower physical functioning and immune status, and also with impaired quality of life (QoL) [5].

According to these observations, it seems clear that a correct nutritional intervention is critical to improve the nutritional intake, general status, symptoms, and also the QoL of these patients [6].

In this regard, an individualized dietary counselling was found to be effective in maintaining weight and/or nutritional status when compared to standard nutritional advice, as demonstrated by the study of van den Berg et al. [7]. The individual approach of an expert allows to choose an appropriate diet that respects the needs of the individual subject also recommending, if necessary, additional nutritional supplements and/or enteral feeding [5].

In 2011, Jager-Wittenaar et al. reported that patients with head and neck cancer undergoing treatment with an intake of $\geq$ 35 kcal/kg/day and $\geq$1.5 g protein/kg/day lost significantly less body weight and lean mass than patient with a lower intake [8]. To achieve this caloric intake, patients with a BMI <20 kg/m^2 or who are malnourished at baseline might find benefit in using dietary supplements [9].

Furthermore, international guidelines suggest that in the presence of head and neck cancer that interferes with swallowing, enteral nutrition (EN) should be recommended. Prophylactic tube feeding is also recommended if severe local mucositis is expected, which could interfere with swallowing, particularly when the irradiation of large fields of oral pharyngeal mucosa is foreseen [10]. There are still many debates about the preference to be given to nasogastric tubes or percutaneous endoscopic gastrostomies (PEG). Each strategy has advantages and disadvantages and should be chosen according to clinical factors and patient's preference. Nasogastric tubes have a shorter duration, but a higher risk of tube dislodgement and may also cause more social discomfort to the patients. On the other hand, PEGs have other complications, including local wound infection, tube occlusion, tube leakage, cellulitis, eczema, or hypergranular tissue [11, 12].

Personalization of nutritional interventions should consider the baseline nutritional status, the biochemical inflammatory indexes, the planned radiation dose on oral/oropharyngeal mucosa and/or pharyngeal constrictor muscles and also the results of multiparameter risk scores (Fig. 21.1).

Immunonutrition

Beyond their purely nutritious function, some nutrients have been associated with pharmacologic-like effects. These "immuno-nutrients" comprise a wide range of molecules including fats (ex. n-3 fatty acids), amino acids (ex. arginine, glutamine),
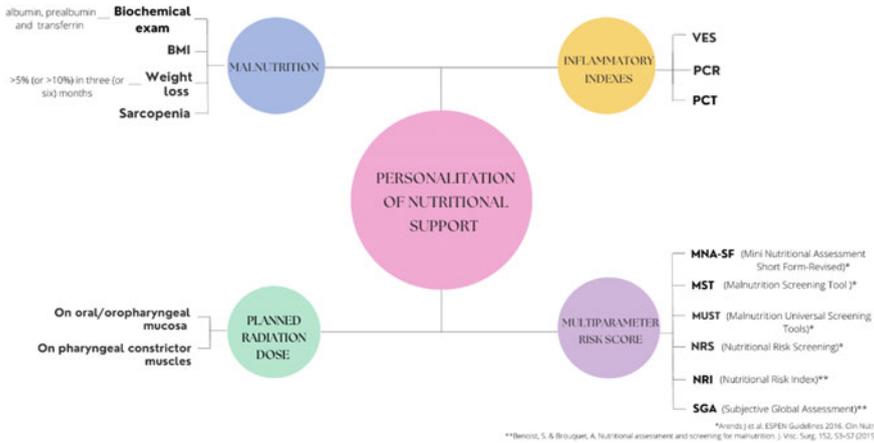
Fig. 21.1 Factors to be considered to tailor nutritional support in Head and Neck cancer patients

vitamins (ex. vitamin E), and other substances (ex. nucleotides, antioxidants), which can be administered either by the enteral or parenteral route [13]. In particular, these agents could modulate the non-infectious pro-inflammatory state associated with oxidative stress that characterizes patients with head and neck cancer. In fact, immuno-nutrition can enhance immune cell responses through the modulation of their phenotypes and functions [14]. Several studies have reported an increase of T lymphocytes and their subsets, respectively, in head and neck cancer patients after the nutritional support enhanced with arginine, ω-3 fatty acids, and ribonucleic acids [15, 16].

In patients with systemic inflammatory response syndrome and multiple organ failure, serum *C-reactive Protein* (CRP) levels were lower in patients receiving immuno-nutritional support than in those taking standard nutrition [17]. It has been hypothesized that because of this immunomodulating effect, immuno-nutrition can also lead to better local control, greater treatment efficiency, and, when used in a pre- or perioperative context, may decrease the length of hospital's stay and postoperative infectious complications [18]. However, we still need further evidence coming from well conducted clinical trials with large and homogeneous patient population before this strategy can be fully implemented in clinical practice.

Physical Activity and Quality of Life

Another aspect that needs to be considered in malnourished cancer patients is weight loss accompanied by muscle wasting. Muscle wasting may influence muscle function and leads to loss of strength, increased fatigue and decreased QoL [19]. In HNSCC patients, exercise has been shown to be feasible, safe and to have an impact on body

Fig. 21.2 Benefits of physical exercises in HNSCC survivors

composition, physical function, QoL, and fatigue management, during and after treatment [20]. Additionally, there is a growing body of evidence suggesting that regular physical activity leads to a reduction in the risk of cancer-specific mortality and all-cause mortality, compared with physically inactive patients. Exercise, including aerobic and active resistance exercises, can therefore be incorporated as a routine part of the HNSCC patient's care [21]. In addition to the positive effects on physical function, aerobic capacity, lean body mass, and muscle strength, it has been shown that physical activity can improve QoL, sleep, depressive symptoms, pain, and emotional and cognitive functioning [22]. Physical activity interventions improve also domains that historically plague HNSCC patients such as reductions in cigarette cravings [23], improved abstinence rates with alcohol and illicit drugs [12]; improved QoL in physical, emotional, and social domains [24]; and improved symptoms of depression and anxiety [25]. These beneficial effects have been found with both traditional and alternative physical activities such as yoga and Tai Chi, which have demonstrated improvements in heart rate variability, vascular endurance index, QoL, and immune function in patients with HNSCC [22] (Fig. 21.2).

Prophylactic Measures

To improve the QoL of patients with head and neck cancer, in addition to the management of malnutrition and loss of muscle strength, the negative consequences of treatment in terms of late adverse effects must be considered. Of these, dysphagia is the

most impactful. The sequelae of dysphagia include avoidance of eating or drinking, poor dietary intake, risk of ab ingestis pneumonia, reduced psychosocial functioning, and poor social engagement. Long-term swallowing function is strongly related to the ability to swallow before treatment in HNSCC patients. For this reason, it has been proposed to use a prophylactic approach to swallowing management, with "prehabilitation" programs. Prehabilitation aims to minimize the effect of dysphagia through the maintenance of muscle mass, strength, range of motion, coordination, and function. Prophylactic swallowing protocols have been found to improve functional swallowing outcomes, including the ability to manage a wider range of food and drinks; maintain muscle mass; improve mouth opening; improve taste, smell, and salivary function; and reduce the need for tube feeding [3]. The exercises performed are mainly aimed at training the mandible muscles, the tongue's and neck's mobility, and all movements necessary for swallowing. Some authors recommend starting with exercises that address anticipated function loss at a regimen of ten repetitions, three times a day, and supplementing these exercises with increased physical activity as well as consultation on nutrition and mental health [26] (Fig. 21.3).

It is therefore clear that to provide the greatest benefit to head and neck cancer patients, it is necessary to use a multimodal interdisciplinary rehabilitation approach, which combines nutritional and psychological support with physical exercise [27].

Fig. 21.3 The concept of pre-habilitation before the start of oncological treatments

Conclusion and Recommendations

It is important to increase the education and knowledge of physicians about the positive effects given by correct nutritional interventions and by physical exercise. The strategies to proactively engage physicians taking care of HNSCC patients in nutritional screening activities and in suggesting physical exercises to the patients may be different: integrating these educational aspects in their curriculum, putting these topics in each national guideline for cancer diagnosis and treatment, and creating checklists for assessment of nutritional status at baseline and periodically. Moreover, the possibility to support nutritional multidisciplinary working groups, the involvement of patients' association and the integration of nutritional and exercises issues into clinical trials may offer other possibility to increase the relevance of these topics in the routine care.

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Chapter 22
Digital Technologies in Supportive Head and Neck Cancer Care: A Promise?

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Introduction

Head and neck cancer (HNC) and its treatment can have a major impact on the physical, psychological and social aspects of health-related quality of life [1–6]. The overall aim of supportive cancer care is to reduce symptoms and improve health related quality of life in people living with and beyond cancer. In many countries, government policy statements and national guidelines reflect broad scientific and societal support for an integrated approach to supportive care, including rehabilitation, psychosocial care and lifestyle interventions [7–10]. Currently, much effort is undertaken to use the concept of value based health care to optimize care, which can be translated into three components: tailoring of care to the needs of the individual patient (patient-centered care), offering effective care (quality care) and offering cost-effective care (affordable care) [11, 12]. Self-management can be an effective part of value based health care and includes, among others, navigating across the cancer care trajectory, managing biopsychosocial sequelae of cancer and its treatment, applying healthy lifestyle behavior to reduce cancer recurrence and late effect risks and adjusting to the end of life phase in case of incurable cancer [13, 14]. Patient reported outcome measures are increasingly used to monitor symptoms and health

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related quality of life over time and to identify those patients who might benefit from supportive care [15, 16]. However, the diversity and unconnectedness of available supportive care options remain a problem and many patients have unmet needs [17]. Digital technologies may facilitate accessible and sustainable supportive care in cancer. This paper describes the use of digital technologies in supportive care, which is of relevance considering the ongoing shortage in healthcare services and the increasing incidence and survival rates in head and neck cancer.

Supportive Care and Self-management

The Multinational Association of Supportive Care in Cancer (MASCC) defined supportive care as the prevention and management of the adverse effects of cancer and its treatment. It involves the provision of services to meet physical, psychosocial, informational, practical, spiritual and lifestyle needs from diagnosis and treatment to long-term survivorship or end-of-life care [18–22]. Supportive care needs are diverse and vary between people and also over time. It is estimated that over 60% of HNC patients have unmet supportive care needs [23–26]. To improve the benefits of supportive care, people with cancer are expected to adopt an active role in managing their own care. Self-management is defined as "the individual's ability to manage the symptoms, treatment, physical and psychosocial consequences and lifestyle changes inherent in living with a chronic condition" [27] and "those tasks that individuals undertake to deal with the medical, role and emotional management of their health condition(s)" [28]. Self-management support is a dynamic, interactive and daily process, to help patients to engage in three self-management tasks—medical management, role management and emotional management—and six self-management skills—problem solving, decision making, resource utilization, the formation of a patient-provider partnership, action planning and self-tailoring [29]. In cancer, self-management interventions such as psycho-educational interventions, exercise programs and healthy lifestyle courses, aim to achieve optimal health and well-being, while living with and beyond cancer [30]. Benefits of self-management include reduction of symptoms, improvement of health related quality of life and its potential to be cost-effective [31, 32]. To better integrate self-management as part of high quality supportive cancer care, the Global Partners on Self-Management in Cancer described six priority areas for action [32] (Table 22.1). It is clear that a lot of work has yet to be done to achieve the goals of these actions. A promising development that may facilitate supportive care including self-management in cancer is the use of digital technologies.

Table 22.1 Six priority areas for action to better integrate self-management as part of high quality supportive cancer care, as described by the Global Partners on Self-Management in Cancer [32]

Action 1: Prepare patients and survivors for active involvement in care
Action 2: Shift the care culture to support patients as partners in co-creating health and embed self-management support in everyday health-care provider practices and in care pathways
Action 3: Prepare the workforce in the knowledge and skills necessary to enable patients in effective self-management and reach consensus on core curricula
Action 4: Establish and reach consensus on a patient-reported outcome system for measuring the effects of self-management support and performance accountability
Action 5: Advance the evidence and stimulate research on self-management and self-management support in cancer populations
Action 6: Expand reach and access to self-management support programs across care sectors and tailored to diversity of need and stimulation of research to advance knowledge

Digital Supportive Care

Digital technologies are part of our daily life and the use of these technologies in eHealth to ease the living with and beyond head and neck cancer is growing. eHealth is defined as "an emerging field in the intersection of medical informatics, public health and business, referring to health services and information delivered or enhanced through the internet and related technologies" [33, 34]. Behavioral intervention technologies can be used in supportive cancer care to support behavior change related to physical, psychological and social problems. Examples are websites, mobile apps and wearable devices to help users address or change behaviors, cognitions and emotional states. Interventions use varying formats, including text, audio, video or games. Some behavioral intervention technologies are designed to be used by users themselves (fully automated behavioral intervention technologies). Others are intended to be used as a component of care that is delivered by a health care provider (adjunctive behavioral intervention technologies) or as a key aspect of care with support from a health care provider (guided behavioral intervention technologies) [35].

Evidence about clinical and cost-effectiveness of digital technologies in supportive cancer care is growing but still limited and implementation remains a challenge [36]. To enhance adoption of digital care in clinical practice, it is essential to integrate research methods during both the development and evaluation of eHealth applications. By using participatory design methods in the development of digital care applications, the effectiveness and usefulness of these applications can be optimized. Participatory design is a method that actively involves users and other stakeholders in the design process of technological solutions, to make sure that the application fits the users' needs [37–39]. Participatory design generally consists of several iterative phases: (1) needs assessment or contextual inquiry: the identification of end users needs through active participation of users, (2) idea generation or value specification: generating ideas following the identification of needs, gaining insight

into the perceived benefits and barriers of the application and define requirements, resulting in prototypes that address the end users' needs, (3) testing and retesting, the design phase: testing the prototypes in pilot studies and further developing them before implementation, (4) operationalization: the phase in which the application is introduced into practice and (5) evaluation: assessment of effectiveness and contribution to the quality of care after implementation. The RE-AIM framework is often used to research the reach, effectiveness, adoption, implementation and maintenance of (digital) care options [40].

As an example of developing, researching and implementing digital supportive care, we describe our approach on an application called "Oncokompas".

The Case of Oncokompas

Oncokompas is a fully automated self-management application to monitor physical, psychological, social and spiritual domains of health related quality of life and lifestyle, to provide personalized information on health related quality of life and lifestyle, and to support people with and beyond cancer in finding optimal supportive care, adjusted to their personal well-being and preferences. A description of Oncokompas is provided in text Box 1.

Description of Oncokompas

The web-based self-management application 'Oncokompas' was developed with the aim to support people living with and beyond cancer in self-management by monitoring health-related quality of life (HRQOL), cancer-generic and tumor-specific symptoms and life-style, providing feedback and information on their personal scores, as well as a personalized overview of supportive care options.

Oncokompas consists of three components: Measure, Learn and Act. Based on patient-reported outcome measures (PROMs) (Measure), users get tailored information on multiple HRQOL, symptoms and lifestyle topics (Learn), and a personalized overview of supportive care options (Act).

Users log in at the Oncokompas website, and first complete a short questionnaire on e.g. marital status, treatment type and time since treatment (before, during or after treatment), to determine which topics are relevant. An overview with the relevant topics is provided from which users can choose which they want to complete. There are over 100 topics in Oncokompas.

In the component 'Measure', users complete PROMs for each of the selected topics. Oncokompas is a dynamic system, i.e. based on users' answers, follow-up questions or more in-depth questions are presented when necessary. Data from the Measure component is processed in real-time.

In the Learn component, users obtain an overview of their PROM scores. Feedback is provided by means of a 3-color system: green (no elevated well-being risks), orange (elevated well-being risks), and red (seriously elevated well-being risks). Users receive personalized information based on their PROM scores and background information on the topic, when they click on the topic. In case of (seriously) elevated well-being risks (orange or red scores), also tips and self-care advice is given, to support them in improving symptom burden themselves.

In the Act component, users obtain a personalized overview of supportive care options, tailored to their wellbeing risk and preferences. If the user has an orange score, self-help or low-intensive interventions are suggested, while contact with a medical specialist or their general practitioner or more intensive interventions are advised if the user has a red score.

Users can access Oncokompas at any time, from any place and Oncokompas can be used multiple times. When users login again, they can see the overview of PROMS scores of their previous visit and read the corresponding information in the components Learn and Act again, or they can complete Oncokompas once again and start with the component Measure again. When used repeatedly, users can see an overview of their scores over time. Repeated use is encouraged by sending reminders by e-mail every two months.

Oncokompas was developed using a stepwise, iterative and participatory design approach. People living with and beyond cancer, care providers and health care assurance companies were involved and several studies were conducted to optimally fit Oncokompas to patients' and care providers' preferences. The development consisted of five steps:

(1) Selection of relevant topics,
(2) Selection of validated questionnaires (Patient Reported Outcome Measures; PROMs),
(3) Composing of algorithms connecting PROM scores with well-being profiles and advices,
(4) Writing texts for well-being profiles and advices,
(5) Composing of strategies for self-help or seeking supportive care.

Steps 1 till 5 were carried out by the research group together with a team of experts including health care providers (medical specialist, nurse specialist and paramedics) and people living with and beyond cancer (representatives of patients associations and patients/survivors from participating medical centers). The PROMs were selected based on the COSMIN criteria (Consensus-based Standards for the selection of health Measurement Instruments) (www.cosmin.nl). A literature search was carried out to identify PROMs as candidates for Oncokompas according to the COSMIN checklist. Meetings were organized in which the expert teams were consulted regarding the results of the literature search and COSMIN checklist. In case a PROM did not

fulfil the necessary criteria, the expert team consented on selecting another PROM. Algorithms were developed that link the results of the PROMs of a user to personalized feedback on the symptoms (information and psychoeducation) and to advices on self-management and professional care. A national database with supportive care options was built in Oncokompas to allow personalized access to supportive care including self-help. In Oncokompas, users receive tailored information on their physical, psychological and social functioning, spiritual issues and lifestyle. Users with minor problems are informed on self-help interventions and on professional care in case of major problems (a stepped care approach). Based on the positive results of needs assessments among cancer patients and care professionals [41, 42], a plan of requirements for Oncokompas was formulated and clarified to the designer and programmers, who used their expertise to translate this plan into a prototype of Oncokompas. Usability tests identified some weaknesses in the user interface that resulted in adjustments, e.g. clearer user instructions. Studies among survivors of head and neck cancer and breast cancer showed that Oncokompas was feasible with an adoption grade of 64% and 75% respectively and a mean satisfaction score of 7.3 and 7.6 on a scale of 10 [43, 44].

From 2017 until 2021, three randomized controlled trials (RCTs) were conducted to investigate the reach, efficacy and cost-utility of Oncokompas among cancer survivors, among incurably ill patients and among their partners [45–51]. Main reasons for not reaching or using Oncokompas were no access to the internet, no symptom burden, no supportive care needs or lack of time [49–51]. Users selected many cancer-generic and tumor-specific topics to address, indicating added value of the wide range of available topics [50]. Oncokompas did not improve the amount of knowledge, skills and confidence for self-management. Among cancer survivors, the application improved health related quality of life and tumor-specific symptom burden [48] and was not more expensive than usual survivorship care [49]. Among incurably ill cancer patients and their partners, no significant effect of Oncokompas was found based on the RCTs (partly carried out during the COVID-10 pandemic) [51]; further publications are planned.

In 2015, we conducted a pilot study on the adoption and implementation of Oncokompas (at that time only available for cancer survivors) via care providers [52]. The study was carried out among 65 hospitals throughout the Netherlands. Health care providers filled out a questionnaire on the implementation of Oncokompas in their organization, consisting of study specific items and items based on the Measurement Instrument for Determinants of Innovations (MIDI) [53]. The MIDI comprises 29 determinants in 4 domains that predict the use of innovations: the innovation itself (Oncokompas), the user (healthcare professional), the organization (hospital) and socio-political context. In total, 20/65 eligible hospitals agreed to implement Oncokompas (adoption rate 31%). In these 20 adopting hospitals, the majority of the responding health care providers (44/61) indicated their patients were offered access to Oncokompas (implementation rate 72%). Comparing those health care providers who did and did not implement Oncokompas, the groups differed significantly on innovation-related (procedural clarity, complexity) and user-related determinants (importance of outcome expectations, professional obligation, social support

and self-efficacy). After this study, we observed that maintenance was a problem and many hospitals stopped offering Oncokompas.

To better understand adoption and implementation, we investigated drivers of resistance among oncology nurses towards online self-management tools in cancer care [54]. Drawing from earlier research, combining clinical and marketing perspectives, the Resistance to Innovation model (RTI-model) was developed. The RTI-model distinguishes between passive and active resistance, which can be enhanced or reduced by functional drivers (incompatibility, complexity, lack of value, risk) and psychological drivers (role ambiguity, social pressure from the institute, peers and patients). Both types of drivers can be moderated by staff-, organization-, patient- and environment-related factors. In total, 2500 nurses were approached of which 285 responded (11%). In line with the RTI-model we found that passive and active resistance among oncology nurses towards (online) self-management tools were driven by both functional and psychological drivers. Passive resistance towards online self-management tools was enhanced by complexity, lack of value and role ambiguity and reduced by institutional social pressure. Active resistance was enhanced by complexity, lack of value and social pressure from peers and reduced by social pressure from the institute and patients. In contrast to what we expected, incompatibility with current routines was not a significant driver of either passive or active resistance. This study further showed that these drivers of resistance were moderated by expertise, managerial support and influence from external stakeholders (government). The conclusion was that passive and active resistance in oncology nurses towards online self-management tools for cancer patients are driven by functional and psychological drivers, which may depend on expertise, managerial support and governmental influence.

Several meetings were organized during the project with representatives of patient societies, health care professionals, researchers, health care assurance companies and the technology transfer office in Amsterdam to develop a dissemination and valorization plan assuring sustainability of Oncokompas, also beyond the timeframe of the project. This resulted in the current (2022) collaboration with Sananet, an eHealth provider which has taken Oncokompas in their portfolio. Dissemination of Oncokompas is, among others, promoted by contacts in hospitals, announcements of results and products in digital newsletters and through social media. However, the digital care market is difficult and further implementation and upscaling efforts need to be continued.

Conclusion

Digital technologies in supportive head and neck cancer care are not a promise but a fact. Research and development following a participatory design approach and the RE-AIM framework helps to deliver patient-centered, effective and efficient applications ready to be used either as adjunctive, guided or fully automated

technology. Implementation and upscaling of evidence-based digital technologies in routine cancer care remains a challenge.

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