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Neoadjuvant immunotherapy for head and neck squamous cell carcinoma

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SUMMARY

The neoadjuvant immunotherapy approach marks a significant shift in the treatment paradigm of potentially curable HNSCC. Here, current therapies, despite being highly individualized and advanced, often fall short in achieving satisfactory long-term survival rates and are frequently associated with substantial morbidity.

The primary advantage of this approach lies in its potential to intensify and enhance treatment regimens, offering a distinct modality that complements the existing triad of surgery, radiotherapy, and chemotherapy. Checkpoint inhibitors have been at the forefront of this evolution. Demonstrating moderate yet significant survival benefits in the recurrent-metastatic setting with a relatively better safety profile compared to conventional treatments, these agents hold promise when considered for earlier stages of HNSCC.

On the other hand, a significant potential benefit of introducing immunotherapy in the neoadjuvant phase is the possibility of treatment de-escalation. By reducing the tumor burden before surgery, this strategy could lead to less invasive surgical interventions. The prospect of organ-sparing protocols becomes a realistic and highly valued goal in this context. Further, the early application of immunotherapy might catalyze a more effective and durable immune response. The induction of an immune memory may potentially lead to a more effective surveillance of residual disease, decreasing the rates of local, regional, and distant recurrences, thereby enhancing overall and recurrence-free survival.

However, neoadjuvant immunotherapy is not without its challenges. One of the primary concerns is the safety and adverse events profile. While data suggest that adverse events are relatively rare and manageable, the long-term safety profile in the neoadjuvant setting, especially in the context of curative intent, remains a subject for ongoing research. Another unsolved issue lies in the accurate assessment of treatment response. The discrepancy between radiographic assessment using RECIST criteria and histological findings has been noted, indicating a gap in current imaging techniques' ability to accurately reflect the true efficacy of immunotherapy. This gap underscores the necessity for improved imaging methodologies and the development of new radiologic and pathologic criteria tailored to evaluate the response to immunotherapy accurately.

Treatment combinations and timing represent another layer of complexity. There is a vast array of possibilities in combining immunotherapy agents with conventional chemotherapy, targeted therapy, radiation, and other experimental treatments. Determining the optimal treatment regimen for individual patients becomes an intricate task, especially when comparing small, single-arm, non-randomized trials with varying regimens and outcome measures.

Moreover, one needs to consider the importance of pre- and intraoperative decision-making in the context of neoadjuvant immunotherapy. As experience with this treatment paradigm grows, there is potential for more tailored surgical approaches based on the patient's remaining disease post-neoadjuvant

treatment. This consideration is particularly relevant in extensive surgeries, where organ-sparing protocols could be evaluated.
In practical terms, the multi-modal nature of this treatment strategy introduces complexities, especially outside clinical trial settings. Patients face challenges in navigating the treatment landscape, which involves coordination across multiple medical disciplines, highlighting the necessity for streamlined care pathways at specialized centers to facilitate effective treatment management if the neoadjuvant approach is introduced to the real-world.
These potential harms and open questions underscore the critical need for meticulously designed clinical trials and correlational studies to ensure patient safety and efficacy. Only these can ensure that this new treatment approach is introduced in a safe way and fulfils the promise it theoretically holds.

Content

ABBREVIATIONS

APM	antigen processing machinery
DC	dendritic cell
dMMR	mismatch repair deficiency
IMRT	intensity-modulated radiotherapy
irPRC	immune-related pathologic response criteria
HNSCC	head and neck squamous cell carcinoma
MPR	major pathological response
MSI-H	microsatellite instability-high
NPR	no pathological response
NSCLC	non-small cell lung cancer
pPR	pathologic partial response
PROM	patient-reported outcomes
RECIST	response evaluation criteria in solid tumors
RVT	residual viable tumor
SBRT	stereotactic body radiation therapy
scRNAseq	single-cell RNA sequencing
TANS	tumor-associated neutrophils
TIL	tumor infiltrating lymphocytes
TORS	transoral robotic surgery
Treg	regulatory T cell

1. Background

1.1 The unmet need for improved outcomes in HNSCC

In the primary setting, current treatment options for patients who have had a head and neck squamous cell carcinoma (HNSCC) – though highly differentiated and individualized in each specific case – remain limited to three main modalities: surgery or radiotherapy with or without chemotherapy. The treatment decision is guided by tumor stage and resectability, local surgical expertise, performance status, and patient preference. A high amount of treatment variability and individualization is introduced by tailoring the extent of surgery, the need for local reconstruction, and the dosage and scope of radiation. As the third treatment modality, classical cytoreductive chemotherapy is added in primary radiotherapy and adjuvant radiotherapy in the high-risk setting [1, 2]. The last decades have seen improvements and innovations across the treatment spectrum: transoral robotic surgery (TORS) can minimize surgical morbidity [3], intensity-modulated radiotherapy (IMRT) reduces off-target radiation of healthy tissue [4], and innovative monoclonal antibodies such as cetuximab may be used as an alternative in Cisplatin-ineligible cases [5]. However, the overall improvement in patient outcomes has been moderate and gradual [6] and was probably driven mostly by an increase in HPV-associated oropharyngeal cancers [7].

Of course, for patients and their families, the oncologic outcome measured as recurrence-free and overall survival times continues to be the most compelling concern. Here, the determining prognostic factor is still the tumor stage and location at diagnosis. Certainly, with the emergence of HPV-associated HNSCC as a separate biological and clinical entity, there is a subgroup of patients with good overall outcomes. However, this is driven by the underlying disease pathophysiology and as yet unimproved treatment algorithms [7]. For non-HPV-associated cancers, except for glottic and oral cavity disease which present early and in resectable stages, the survival outcomes have been unsatisfactory, especially given the highly invasive and sometimes extensive surgical procedures and intensive high-dose chemoradiotherapy regimen patients must undergo to achieve said outcomes. Indeed, a detailed analysis of SEER data comparing the changes in relative survival across four decades (1976–2015) shows a marked increase in survival only for oropharyngeal and oral tongue tumors, while the 40-year survival changed little for other subsites such as larynx, hypopharynx, nasopharynx, and non-tongue oral cavity when adjusting for other factors in multivariate analysis [8]. The study also outlines the unsatisfactory 5-year relative survival for the most recent 2006–2015 cohort of 38.4 % for non-tongue oral cavity, 31.2 % for hypopharyngeal, and 35.8 for laryngeal cancer in regional disease metastasized to the local lymph node basin. This is especially relevant as a large percentage of patients present in said locally advanced disease stage [9].

In those patients who do achieve long-term survival, it is associated with significant morbidity and reduced quality of life. Depending on the location of the primary tumors, one or more of the basic physiological and daily social functions such as breathing, speaking, swallowing, tasting, and olfaction might be disturbed [10]. The extent of functional deficit varies depending on the extent of the primary disease and the chosen treatment modality but is nonetheless comparable across therapies and patients. Voice and speech problems have been noted in two-thirds of HNSCC patients — even 10 years following primary radiotherapy [11]. Dysphagia and reduced oral feeding have been the most consistent concerns, with a high focus on reducing this morbidity through intensive speech therapy and rehabilitation [12]. Xerostomia and loss of taste have been mostly associated with primary radiotherapy and remain long-term issues with limited treatment options [13]. Other treatment sequelae that should be addressed by a multidisciplinary approach during head and neck cancer survivorship care are fatigue, sexual dysfunction, chronic pain, caries and dental issues, lymphedema, and cervical dystonia [14].

Surgery, whether in the primary or salvage setting, is associated with procedures that may result in long-term body image issues [15]. This is especially true for amputating surgeries such as laryngectomies, exenteratio orbitae, or ablatio nasi, as well as the need for pedicled or free flap tissue transfer. All this accumulates, creating a long-term reduction in quality of life in HNSCC survivors [16–18]. Alarmingly, reports show a two times higher risk to die from suicide in this population compared to other cancer types [19].

Taken together, this shows that even though considerable strides have been made to improve both mortality and morbidity in HNSCC patients, the current state-of-the-art therapy options do not provide satisfactory outcomes in terms of both long-term survival and treatment sequelae and, therefore, the resulting quality of life.

1.2 The introduction of immunotherapy as a fourth pillar of HNSCC treatment

In the last decade, the introduction of treatment options that harness the power of the immune system to detect and eliminate cancer cells has offered hopes for improved outcomes to both patients and providers [20]. For decades, there has been circumstantial clinical evidence about the role of the immune system in the tumor-host interaction. Examples include the spontaneous regression of cancers, at some point coinciding with febrile infections [21]; the recurrence of metastasis in transplanted organs, such as cases of melanoma metastases transferred from donor to kidney transplant patients [22]; and the increased incidence of cancers in individuals with genetic or acquired immunosuppression [23, 24]. In addition to these clinical observations, there were basic science findings that aligned with a hypothesized protective effect of the immune system against cancer. These include but are not limited to, a correlation between tumor infiltrating lymphocytes (TIL) and prognosis [25]. The idea of a protective effect of the immune system was underscored by more mechanical studies in immunosuppressed mouse models that resulted in the immunosurveillance hypothesis [26], which is discussed in more detail below. Following a long era of skepticism, these findings were at last translated into the clinic. First, cytokines that broadly stimulate the immune system, such as IL-2 for the treatment of metastatic renal cell carcinoma and melanoma [27] or alpha Interferon in hairy-cell leukemia [28], were FDA-approved. Other early innovative and pioneering interventions were the ex-vivo expansion and reinfusion of TIL [29]. Further studies into the role of T cells and their interaction with cancer cells (outlined in more detail below), especially the importance of checkpoint receptors in said interaction, paved the way for the current era of checkpoint inhibitors, monoclonal antibodies that block the inhibition of the antitumor immune response by cancer cells. Here the approval of ipilimumab, an anti-CTLA-4 antibody, for metastatic melanoma in 2011 marked the starting point for an explosion of indications [30]. In HNSCC, the CheckMate-141 trial demonstrated improved overall survival of nivolumab, an anti-PD1 antibody, compared to investigator's choice, in patients with recurrent or metastatic disease that is refractory to platinum-based treatments, leading it to be the current standard of care in this setting [31]. Further, the KEYNOTE-048 trial showed an overall survival benefit for pembrolizumab, an anti-PDL1 antibody, compared to the EXTREME (chemotherapy plus cetuximab) regimen in the first-line treatment of recurrent-metastatic setting [32]. It is now approved as monotherapy for patients expressing PD-L1 > 1 % and in combination with chemotherapy in expressing PD-L1 negative tumors. Further innovations in this field, which are beyond the scope of this review, are discussed in detail elsewhere [33]. Suffice it to say, the improvements in outcome, i. e., better survival and lower morbidity, that can be observed in the recurrent-metastatic setting underscore the viability of this treatment modality and inspire incorporation in earlier, curative therapy stages, such as adjuvant or neo-adjuvant settings.

1.3 Principles of immune-oncology and basics of current HNSCC immunotherapy

1.3.1 Immune elimination, equilibrium, and escape

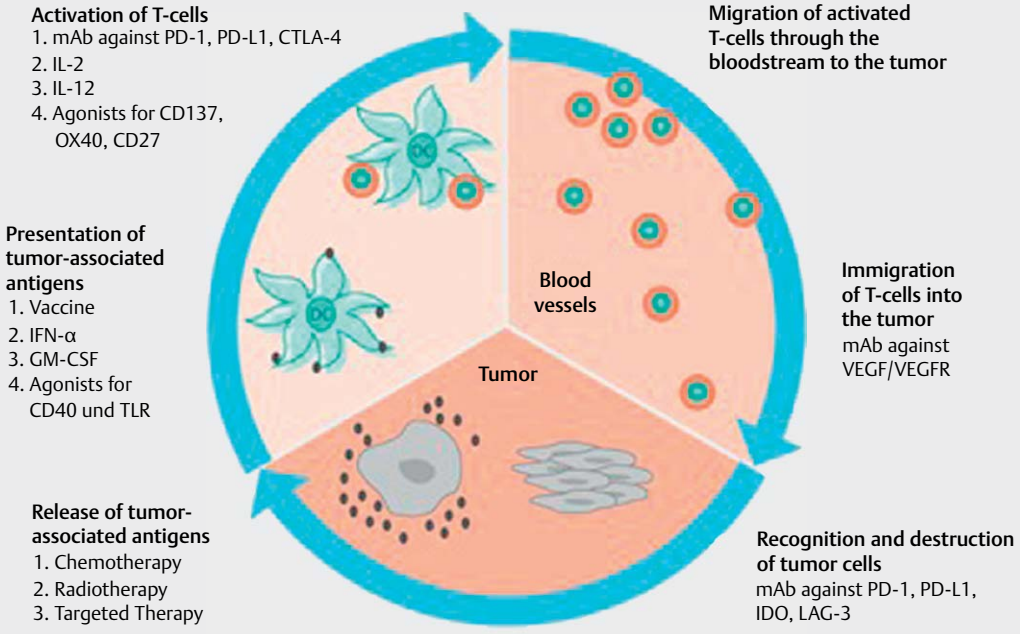
The basic function of the adaptive immune response is the development of a specific reaction against a structure, the antigen, identified as foreign by the immune system. This response differs according to cell type and context and can consist of the production of specific antibodies by B cells or the cytokine-driven organization of the immune response (CD4 + helper cells). Direct killing of structures recognized as foreign is the task of cytotoxic T cells [34]. The antigens can be detected in different contexts, namely infected somatic cells in acute or chronic infection, autologous tissue in autoimmunity, a donor organ in a rejection reaction, or mutated cells in anti-tumor immunity.

The idea that the immune system can recognize and eliminate cancer cells – termed the cancer immunosurveillance hypothesis – was proposed in the early 1960s, yet due to a lack of scientific evidence and insufficient experimental models, it was not pursued further [26]. As the research community focused on the cancer cell and its genetic perturbations, such as oncogenes and tumor-suppressor genes, there was a long period without progress in immuno-oncology [35]. Interest in the tumor-host interaction reemerged in the 2000s, building on new and encouraging evidence from the previous decade [36]. Here, the conceptual framework of immunosurveillance was expanded into the 3-Es of the immunoediting model, recognizing that the interaction between cancer and host did not stop with the destruction of clinically inapparent cancer lesions, the immunosurveillance or "elimination" state, but its "immunogenic phenotype is continuously shaped by the immunological forces in its environment" [36]. If the tumor is not eliminated, it enters an "equilibrium" state in which it is not clinically apparent, but cannot be cleared by the immune system. Following this, a third "escape" phase emerges in which the tumor outgrows the stalemate with the immune system and becomes clinically apparent.

1.3.2 T cell antigen recognition and activation

Due to the potential power of activated T cells, the initiation of cytotoxic effector function is highly regulated and involves multiple steps or signals [37, 38]. For an immune response to arise from recognition of a tumor antigen expressed on a cancer cell, the same antigen must be shown to the T cell a second time by an antigen-presenting cell, the first signal, along with co-stimulatory or co-inhibitory receptor, the second signal, as well as modulatory cytokine secretion, the third signal. If this process occurs for the first time in the context of an acute immune response, such as an infection or vaccination, a naive T cell, i. e., one that has never been in contact with its antigen before, develops into an effector T cell, which then divides and exerts its cytotoxic function. In parallel, memory T cells develop to rapidly provide an immune response in the event of a future reappearance of the antigen in the organism [40]. Depending on the location of these cells, they are subdivided into central memory T cells in lymphoid organs or effector memory cells in the tissue [41].

The cytotoxic effector T cell and its activation are at the center of the so-called tumor-immunity cycle (▶ **Fig. 1**) [39] in which tumor-favoring and tumor-preventing influences of the immune system can occur at each step: release of the tumor antigen, presentation of the tumor antigen, activation of the T cell, invasion of the tumor by the T cell, T cell-mediated recognition of the tumor, and killing of tumor cells. The cycle serves as a good model for under-



▶ **Fig. 1** Therapies that could influence the cancer immunity cycle. Source: Dietz A, Stöhr M, Zebralla V et al. Immunonkologie bei Kopf-Hals-Tumoren. Laryngo-Rhino-Otologie 2021; 100(04): 303–321. doi:10.1055/a-1337–0882

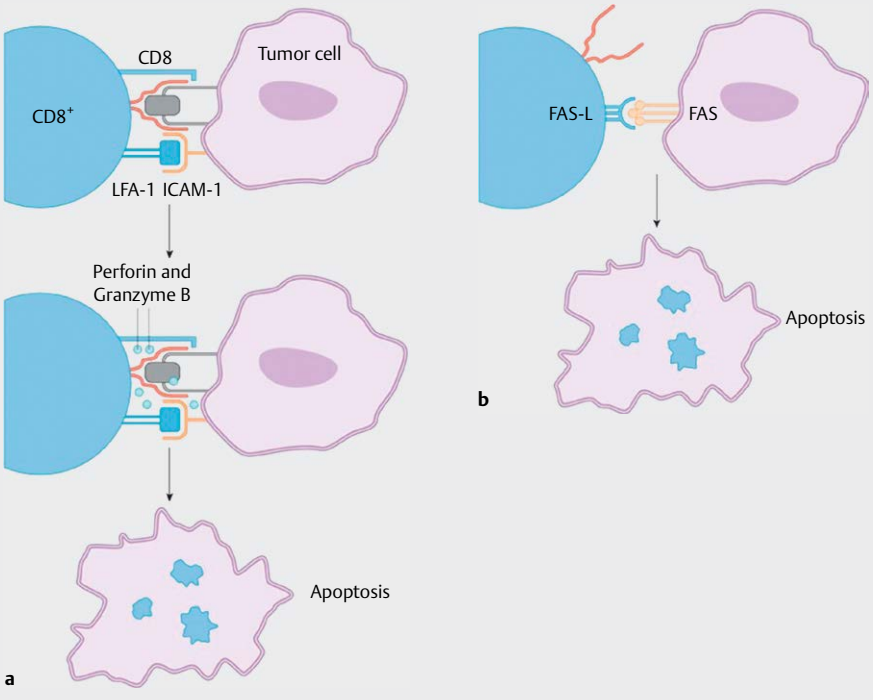
standing the main components and interactions, as well as potential therapeutic interventions. Analogous to the interaction between the host and infections, the tumor-immunity cycle centers on the recognition of antigens by the adaptive immune system. Immunogenic cell death releases antigens into the extracellular milieu. Dendritic cells (DCs) then capture these antigens and migrate to the tumor-draining lymph nodes, where they are presented to naive T cells via MHC molecules. The engagement of T cell receptors by MHC-antigen complexes in the presence of co-stimulatory molecules like CD28 – the immune synapse – leads to T-cell activation. The T cells then circle back to the tumor where they recognize their respective tumor antigen and initiate cancer cell killing via their cytotoxic properties. The cytotoxic effector function here consists of the release of perforin and granzyme B granules, activation of the Fas receptor by the Fas ligand, and activation of other immune cells by proinflammatory cytokines, such as interferon-gamma and TNF-alpha [40]. Perforin forms pores in the target cell membrane, allowing granzymes to enter and activate caspase-dependent apoptosis within the cancer cell, while Fas triggers extrinsic apoptotic pathways (▶ **Fig. 2**). The death of the cancer cell with the release of antigens can then lead to a feedback loop of anti-tumor response.

1.3.3 Cancer immune evasion in HNSCC

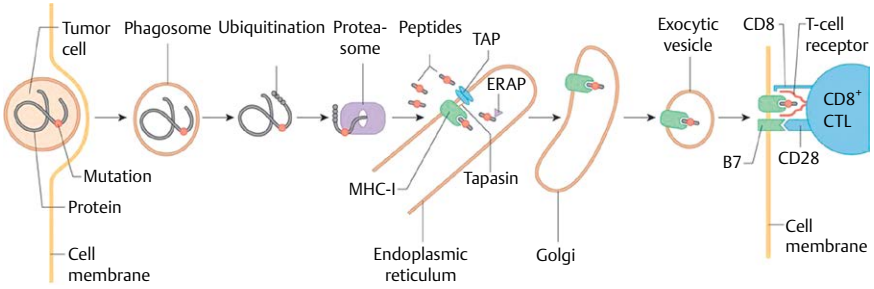
HNSCC evades immune recognition and destruction in a variety of ways that are linked to the principles discussed above. Components of the antigen processing machinery (APM) are under-expressed or mutated, leading to reduced tumor antigen presentation and less T cell recognition [43–46], although not to such an extent as to lead to activation of NK cells than eliminate cells that do not express HLA. Indeed, a deficient APM has been linked to worse outcomes in HNSCC [47]. (▶ **Fig. 3**)

In some cases, low MHC expression can be upregulated by an interferon-γ (IFN- γ) response. When IFN-γ connects with its receptor, it triggers the phosphorylation of Janus kinase 1/2 (JAK1/2) and signal transducer and activator of transcription 1 (STAT1), setting off the JAK/STAT signaling pathway. STAT1 functions as a transcription factor, boosting the production of interferon regulatory factor 1 (IRF1) and p48. This in turn leads to increased expression of MHC I. However, interferon-γ signaling can be reduced in HNSCC [48], leading to impaired antigen presentation and T-cell function [49].

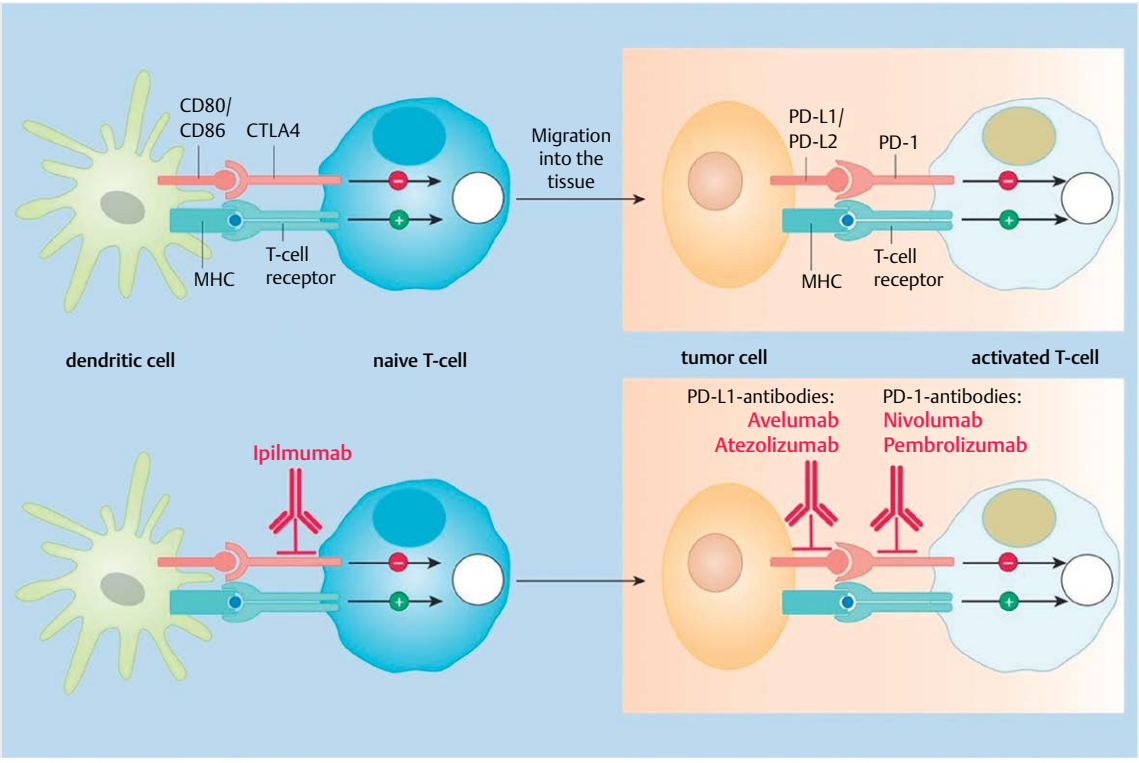
One clinically important way HNSCC can evade immune surveillance and destruction is the expression of immune checkpoints. In a physiological setting, these serve the role of limiting the overreaching immune destruction of healthy tissue in the setting of acute infections as well as preventing autoimmunity. Cancers can co-opt this mechanism by expressing inhibitory receptors such as PD1, CTLA-4, LAG-3, TIGIT, or Tim-3. Due to its clinical application, PD1 has been at the forefront of research interest. In the interaction between programmed death-1 (PD-1) receptor and its ligands, programmed death-ligand 1 (PD-L1), and programmed death-ligand 2 (PD-L2) [50], which are overexpressed on the surface of tumor cells as well as antigen-presenting cells, the PD-1 receptor transduces an inhibitory signal that attenuates T-cell activation and effector functions, essentially dampening the immune response (▶ **Fig. 4**). On the level of an individual T effector cell, an increasing dysfunctional (exhaustion) state of the T cell is induced, depend-



▶ **Fig. 2** Effector functions of CD8+ cytotoxic T cells. Source: Wagener C, Müller O, Hrsg. Molekulare Onkologie. 4., aktualisierte und erweiterte Auflage. Stuttgart: Thieme; 2022. doi:10.1055/b000000085



▶ **Fig. 3** P2C way of cross presentation. Source: Wagener C, Müller O, Hrsg. Molekulare Onkologie. 4., aktualisierte und erweiterte Auflage. Thieme; 2022. doi:10.1055/b000000085



▶ **Fig. 4** Principle of action of the immune checkpoint blockade. Source: Blum H, Müller-Wieland D, Hrsg. Klinische Pathophysiologie. 11., unveränderte Auflage. Stuttgart: Thieme; 2020. doi:10.1055/b000000121

ing on the strength, i. e., high antigen load, as well as on the duration of the stimulation [51, 52]. Basic features of depleted T cells include a decrease in effector functions (cytotoxicity, cytokine secretion), decreased proliferation, an altered metabolic cell program, epigenetic reprogramming, and increased expression of inhibitory checkpoint receptors [53].

A breakthrough that ultimately enabled the emergence of checkpoint inhibitors as a new class of drugs for the treatment of cancer was the recognition that T effector cells in chronic viral infections can be rejuvenated or revived by blocking checkpoint receptors [52, 53]. It is important to acknowledge the heterogeneity within dysfunctional T cells, as there is a hierarchical, graded development of T-cell exhaustion: CD127 + KLRG1- effector T cells develop into at least two subpopulations, PD1midT-bethigh Tex as well as PD1highEOMEShigh Tex, only the former of which can be revived by blockade of the PD1/PDL1 axis [56, 57]. Similarly, cells co-expressing different checkpoint receptors can be re-activated by a combination of checkpoint inhibitors [58]. In HNSCC, it has been shown that the extent of PD1 expression is a critical aspect with high frequencies of PD1high patients were associated with more dysfunctional T cells and worse outcomes [59].

1.3.4 The HNSCC tumor microenvironment

Separate from the immune synapse between the T cell and cancer cell, the surrounding tumor microenvironment has a powerful influence on the potential for immune elimination or evasion. Several immunosuppressive cell types have been described in HNSCC.

The role of regulatory T cells (Tregs) within the tumor microenvironment of head and neck squamous cell carcinoma (HNSCC) has been characterized, although their definitive prognostic or therapeutic significance remains unestablished [60]. Tregs misapply their physiological function – regulating T-cell hyperactivity and preventing autoimmunity – to foster an immunosuppressive milieu conducive to tumor growth [61]. Specifically, Tregs inhibit antitumor immunity by targeting cytotoxic T cells. Their suppressive mechanisms encompass the maintenance of high-affinity IL-2 receptor alpha chain expression, thereby mitigating IL-2-induced activation in effector cells; expression of immune checkpoint molecules like CTLA-4, which interact with co-stimulatory molecules CD80/CD86, thereby inhibiting T-cell activation; and secretion of immunosuppressive cytokines such as IL-10 and TGF-beta [62]. Emerging evidence from colon cancer research suggests that the conventional classification of CD4 + FoxP3 + Tregs is overly simplistic [63, 64]. A more nuanced stratification based on CD45RA and FoxP3 expression may be necessary, delineating naive (CD45RA- and FoxP3low, nTreg), non-suppressive (CD45RA + and FoxP3low, nsT-reg), and effector (CD45RA + and FoxP3high) Treg subtypes. Naive Tregs are recruited to the tumor site, where they subsequently transition into suppressive effector cells upon antigenic exposure. Specialized suppressive Treg subsets, characterized by CD39 + and Tim3 + expression, have been identified in HNSCC and demonstrated heightened immunosuppressive potential [65, 66].

Another cellular subset implicated in intratumor resistance to immunotherapy and pro-tumor immunity are neutrophil granulocytes. These cells occupy a dichotomous role, demonstrated to possess both tumor-promoting and tumor-inhibiting functions [67]. Research in recent years has illuminated the significant influence of tumor-associated neutrophils (TANs) on tumor angiogenesis and growth, largely mediated by the secretion of specific cytokines and growth factors [68]. Additionally, TANs facilitate metastatic spread and attenuate anti-tumor immune responses by creating a pre-metastatic niche [69]. Neutrophil plasticity is profoundly shaped by the tumor microenvironment, exemplified by neutrophil polarization modulated by factors such as type I interferons, TGF-beta, and G-CSF. Pro-tumoral neutrophils, notably those emerging in the absence of type I interferons – as seen in IFN knockout murine models – promote angiogenesis and tumor growth via the upregulation of proangiogenic molecules like VEGF and MMP9. Further, these neutrophils also exhibit extended longevity and increased chemokine secretion relative to their anti-tumoral counterparts [69–73]. In HNSCC, in vivo imaging models have shown a decreased contact between neutrophils and T cells in interferon receptor deficient (Ifnar1$^{-/-}$) mice, leading to dampened T-cell proliferation and activation [74]. The ratio of pro-tumor to anti-tumor neutrophils can fluctuate in line with tumor progression, consequently altering their cumulative impact on tumor dynamics [75]. In this context, recent studies have revealed that in HNSCC, antigen-loaded TANs migrate to lymph nodes, where they modulate T-cell dependent anti-tumor immune responses in a stage-dependent manner. In early phases, prior to lymphatic metastasis (cN0), neutrophils acquire an antigen-presenting phenotype (HLA-DR + CD80 + CD86 + ICAM1 + PD-L1-) and activate T-cells. At later cancer stages, lymph node metastases (cN +) produce GM-CSF, inducing the generation of PD-L1 + immunosuppressive neutrophils via STAT3 pathway activation, subsequently leading to the suppression of T-cell responses and further tumor progression [76].

Recently, single-cell RNA sequencing (scRNAseq) analyses of the head and neck tumor environment have helped to resolve the heterogeneity of the head and neck TME and identify prognostic cell types of interest. scRNAseq is a basic science and translational research technique that has evolved from a highly specialized niche method to a mainstream application [77]. Facilitated by various technical developments, there has been an explosion of experimental platforms and an associated popularity of the method in recent years [78]. In simple terms, single-cell RNA sequencing allows the representation of the entire transcriptome of a sample while maintaining single-cell resolution. A bioinformatic map of individual cells as well as their mRNA content is thus generated. Depending on the method, millions of cells with, on average, thousands of genes can be read out in this way. In HNSCC, scRNAseq has helped to delineate critical differences between the immune makeup of HPV-associated and non-HPV-associated HNSCC, showing that helper CD4 + T cells and B cells are divergent between these two etiologies [79]. In addition, CD4 + T follicular helper cell gene expression signature is associated with longer progression-free survival in HNSCC patients. Further, germinal center tumor-infiltrating B cells and tertiary lymphoid structures show the favorable outcome associated with HNSCC [80]. Further, one can use scRNAseq HNSCC to explore the role of non-immune cells and their interaction with the immune system, showing the cellular heterogeneity among cancer cells, pericytes, fibroblasts, and endothelial cells [81]. When analyzing the spectrum of intratumor T cells analogous to studies in other entities – malignant melanoma, colorectal carcinoma, hepatocellular carcinoma as well as non-small cell lung carcinoma – it becomes clear that the exhaustion state of T cells is a continuum.

2. Immunotherapy in the neoadjuvant setting

2.1 Potential risks and benefits of moving immunotherapy to the neoadjuvant setting

Before discussing the rationale for new adjuvant immunotherapy in detail, it is important to keep front and center in one's mind the important differences between checkpoint inhibition and classical to cytoreductive chemotherapies. While immunotherapy aims to enhance the body's own anti-tumor response, classical chemotherapy agents that are used in HNSCC interfere with the ability of rapidly dividing cells to replicate. Specifically, the most widely used agent, cisplatin, functions predominantly through the formation of intrastrand and interstrand DNA adducts [82]. This induces conformational changes, triggering a cascade of cellular responses, including impaired DNA repair mechanisms, cell cycle arrest, and apoptosis. In the primary or induction setting, they are therefore applied primarily to alleviate symptoms of large clinical disease and to debulk tumors before the start of primary radiotherapy. In the context of combination therapy, cisplatin serves as an effective radiosensitizer enhancing the cytotoxic effects of ionizing radiation on cancer cells. These DNA adducts formed by cisplatin act synergistically with radiation-induced breaks, complicating their repair and consequently promoting apoptosis.

Given the basic science and clinical background discussed so far, there are several reasons that support the use of immunotherapy, especially checkpoint inhibition, in the neoadjuvant, pre-surgical setting in HNSCC.

First and foremost, given the poor outcomes of current therapies, there is a clear unmet medical need to intensify treatment regimens. Yet, given the morbidity and deteriorating impact on the quality of life of patients undergoing current treatment, it is apparent that current modalities will be unable to achieve this. Here, adding a fourth modality with a distinct mechanism of action and adverse event spectrum to the primary setting seems common sense.

This is especially true given the known clinical efficacy and relatively good safety profile of checkpoint inhibition. Further, extensive clinical experience has been gathered in the last decade regarding monitoring and treating adverse events of checkpoint inhibitors in the palliative setting across a wide variety of primary cancers. As will be discussed in more detail below, immunotherapy has shown a moderate but clinically significant survival advantage in the recurrent-metastatic setting while maintaining a superior safety profile.

In fact, there is reason to believe that response rates might be higher in the presurgical compared to the adjuvant or recurrent-metastatic setting due to a variety of patient and tumor-related factors. First, in untreated cases, there is more tumor tissue and therefore increased opportunity for immune interaction with the cancer cells. This might be true for the absolute quantity of tumor antigens as well as the variety and quality of antigens since they have not been selected by the evolutionary pressures of long-term disease and multiple prior therapies. Therefore, there might be a stochastically higher likelihood of effective antitumor response at an initial stage. The same holds true in principle for the tumor-draining lymph nodes, which in HNSCC are usually removed during surgery or heavily radiated as part of primary radiotherapy. In the untreated setting, priming of T cells by dendritic cells can occur here unimpeded. On the patient side, there is a lower probability early in a treatment course of treatment-related impairment of the immune response. The indiscriminate cytotoxic effect of chemotherapy in the primary or adjuvant setting can particularly dampen the patient's ability to generate an effective anti-tumor response. Similarly, extensive surgery with the need for cumbersome recuperation or the strain of weeks-long radiotherapy might limit the immune system's capabilities.

Along the same lines, early stimulation of the immune system using checkpoint inhibition might not only lead to a deeper primary response but also a longer-lasting one, ideally even permanent remission. This can occur due to a more effective memory formation. An increase in the breadth and quantity of memory effector T cells that patrol the body after primary therapy might be better equipped to engage and eliminate microscopic residual disease locally or in distant micro-metastases. Clinically, this could lead to reduced local and regional, as well as distant recurrence and therefore improve overall and recurrence-free survival.

At the same time, new adjuvant immunotherapy might lead to a significant response of the tumor at presentation. This could – in theory and after rigorous evaluation in respective clinical trials that have identified reliable response markers – lead to a reduced need for extensive resection and reconstruction or improve the possibility of organ-sparing protocols. Similarly, size reductions in the primary tumor or local lymph node metastasis could lead to decreasing high-risk clinical features such as close-margin resections or extracapsular spread, which in turn could minimize the need for adjuvant therapies and their respective treatment sequelae. Thus, the addition of a fourth treatment modality could offer an opportunity for treatment de-escalation or at least a reduction in morbidity in select patient populations.

Last but not least, the period between panendoscopy and diagnosis and the surgical primary treatment offers an aptly termed window of opportunity. This can be used to study multiple basic science and translational research questions [83]. These include, but are not limited to, details of the tumor-host interaction, mechanisms of resistance, the effects of immunosuppressive cell populations as well as biomarkers for response or high-risk scenarios warranting adjuvant therapy. Ideally, this induces a positive feedback loop from innovations going from bench to bedside and back.

Given the possible advantages of early immunotherapy in the untreated setting, it is important to consider possible disadvantages so that these can be monitored or avoided. Given the theoretical increase in treatment response in the primary setting, there is a similar risk of more pronounced side effects in populations with a healthy immune system. This could mean a higher frequency as well as a more severe extent of adverse events, especially autoimmune diseases. In the neoadjuvant treatment context, it is important to consider that these patients are in a potentially curable disease stage and have to live with permanent side effects, especially autoimmune diseases such as diabetes, hypothyroidism, or hypophyseal dysfunction, for the rest of their lives. Also, Grade 3 or 4 adverse events might interfere with the patient's scheduled surgery date or allow the tumor to progress while the patient recuperates. Further, it is important to consider the possibility of a negative trial in which all patients do not benefit sufficiently from adding immunotherapy prior to primary treatment.

One final practical aspect, is that the multi-modal approach of integrating neoadjuvant immunotherapy in to the standard of care may present a unique set of challenges, particularly in its applicability outside the controlled environment of clinical trials. It requires a collaboration among various medical disciplines, including oncology, surgery, radiology, and pathology specialists. Coordinating care across these diverse fields can be complex, potentially leading to logistical and communication challenges. For patients, navigating this treatment landscape in the real-world setting can be daunting, especially when transitioning between different phases of therapy and dealing with multiple healthcare providers. The complexity of scheduling, understanding the different aspects of the treatment, and managing the side effects that may arise from such a comprehensive approach could pose significant challenges. These considerations highlight the importance of optimized care pathways and treating patients at specialized centers.

In conclusion, while the potential of neoadjuvant immunotherapy in the treatment of HNSCC is indeed promising, it is imperative to apply it in this early setting with a measured and cautious approach. To what extent it can be implemented depends critically on the design and execution of carefully crafted clinical trials and correlational studies. These must be structured to not only investigate the potential bene-

fits but also to thoroughly assess any associated long-term harm or rare adverse effects. The clinical trials should be tailored to capture a broad spectrum of patient responses, ensuring that the findings are representative and applicable to the diverse patient population affected by HNSCC. One example of such a clinical study is the PIONEER - Window of opportunity study of **p**reoperative **i**mmun**o**therapy with atezolizumab (Tecentriq) in local head and neck squamous cell carcinoma (NCT04939480) - trial currently recruiting in Essen.

2.2 Lessons learned from neoadjuvant chemotherapy in HNSCC

Neoadjuvant treatment strategies have been applied to HNSCC in the curative pre-surgical setting before the era of immunotherapy with a similar intention – hoping to improve unsatisfying patient outcomes. Here, high-level data from a randomized Phase-3 trial in resectable Stage III or IVA HNSCC randomized 256 patients to chemotherapy (Docetaxel, Cisplatin, Fluorouracil) plus surgery or surgery alone, with adjuvant radiotherapy in both groups [84]. Even though this study proved that there is good feasibility, with 91.6 % of patients undergoing surgery within four weeks of chemotherapy, and good clinical response, with an 80.6 % RECIST response rate and a 27.7 % pathological response rate, the study missed its primary endpoint, showing no benefit in overall or recurrence-free survival. Similarly, for chemotherapy before radiotherapy – termed "induction" in this context – the large, updated MACH-NC meta-analysis of 93 trials and 17,493 patients shows no benefit from this approach (hazard ratio 0.96, 95 % CI 0.90–1.02) [85].

Given the discrepancies between clinical response and its translation into a survival benefit for the patient, which ultimately guides treatment decisions, this provides a cautionary tale for current neoadjuvant trials.

2.3 Lessons learned from immunotherapy in the RM setting in HNSCC

Since the introduction of checkpoint inhibition to the recurrent/metastatic setting in the first line as well as in platinum-refractory patients, there has been ample opportunity to study treatment effects, safety profiles, and biomarkers of response.

The initial trials that led to the FDA approval of nivolumab and pembrolizumab have already shown a moderate but significant survival benefit of immunotherapy over conventional chemotherapy in this setting. Long-term follow-up of these cohorts as well as ongoing Phase IV studies highlighted that there is a small but persistent subgroup of patients who experience a durable response, even cure from their palliative disease. In a 2-year follow-up of the Checkmate-141 trial, overall survival was 16.9 % in the nivolumab versus 6.0 % in the investigator's choice group [86]. Further, long-term, 4-year follow-up data from the KEYNOTE-048 trial suggests a plateau in overall survival at around 20 % in the pembrolizumab alone group and the pembrolizumab-chemotherapy group (total cohort and CPS ≥ 1) [87]. Similar results can be inferred from a pooled analysis of the initial and the expansion cohort of the KEYNOTE-012 trial where 71 % of the patients responded to pembrolizumab, and this response lasted more than a year [88]. This suggests that even though a relatively small fraction of patients benefit, those who do have a durable response are likely due to memory formation of the adaptive immune system.

Importantly, the adverse event profile of checkpoint inhibitors proved to be favorable in the long term with 7.2 % percent of patients in the Check-Mate-141 trial experiencing serious adverse events compared to 15.3 % in the conventional therapy arm [86]. Along the same line, long-term data from KEYNOTE-048 underlines the relatively better safety profile of checkpoint inhibition: 17.0 % of patients receiving pembrolizumab monotherapy experiencing adverse events greater Grade 3 versus 71.7 % in the pembrolizumab-chemotherapy and 69.3 % cetuximab-chemotherapy group [87].

Especially relevant and interesting in this setting is the introduction of quality-of-life measures as exploratory endpoints. In the Checkmate-141 trial, patient-reported outcomes (PROM) were collected using three questionnaires [89]. In the EORTC Quality of Life Questionnaire-Core 30 (QLQ-C30), clinically meaningful deterioration was observed in 8/15 (53 %) domains in the chemotherapy group, while PROM stabilized in the nivolumab group. A similar effect was seen in the EORTC head and neck cancer-specific module (EORTC QLQ-H&N35). Further, the three-level European Quality of Life-5 Dimensions (EQ-5D), an overall health measure, was also able to show a benefit in the nivolumab group.

Trials in the current metastatic setting have been used to extract predictive biomarkers. Expression of PDL1 is an obvious but imperfect predictor of response to checkpoint inhibition targeting the PD1-PDL1 interaction [90]. Exploratory biomarker analysis of the Checkmate 141 trial suggested that a tumor PD-L1 expression ≥ 1 % might have a more pronounced effect [31]. In KEYNOTE-040, a randomized, open-label, Phase 3 trial of pembrolizumab versus investigator's choice in HNSCC that progressed under platinum-based therapy, there was a clear survival advantage depending on PDL1 staining. In the combined positive score (CPS) ≥ 1 versus < 1, the hazard ratio was 0.74 (95 % CI 0.58–0.93, p = 0.0049) versus 1.28 (95 % CI 0.80–2.07, p = 0.8476), respectively [91], with comparable results for a tumor proportion score (TPS) ≥ 50 % or < 50 %. Similarly, a subgroup analysis of the KEYNOTE-048 trial, using pembrolizumab in the first-line setting, showed an increased survival benefit with higher PD-L1 expression, measured as CPS [92].

2.4 Lessons learned from adjuvant immunotherapy in HNSCC

Seeing the fact that the low overall survival in HNSCC patients is primarily driven by local or regional recurrence rather than patients showing distant metastasis primarily or secondarily, there seemed to be sufficient rationale to introduce immunotherapy to the treatment regimen in the adjuvant setting following primary chemoradiation in the hopes of mitigating loco-regional recurrence.

The Javelin Head and Neck 100 trial was a randomized, double-blinded, placebo-controlled study designed to assess the superiority of the anti-PD-L1 antibody avelumab over placebo after primary chemoradiotherapy in 697 patients [93]. The trial was stopped at a preplanned interim analysis, showing that the primary objective, progression-free survival as determined through RECIST criteria, was not met.

In a similar setting and cohort, it was announced that KEYNOTE-412, a randomized, double-blind, Phase III trial testing pembrolizumab or placebo concurrently with primary chemoradiotherapy and as maintenance treatment, did not meet its primary endpoint of event-free survival, though published results are not yet

available. A third randomized Phase III trial, the IMvoke010, which tests atezolizumab versus placebo after primary chemoradiotherapy, is currently actively recruiting without any information on results.

These negative results came unexpectedly, as adjuvant immunotherapy has proven advantageous in other disease entities [94]. Translational studies have since offered a compelling explanation of the missing effect of adjuvant immunotherapy in HNSCC, while at the same time underlining its possible value in the neoadjuvant setting. After developing a murine neck dissection model, it was shown that the surgical removal of the tumor-draining lymph nodes inhibited the response to subsequent anti-PD1 or anti-CTLA4 therapy. Similarly, radiation to the local lymph node basin led to diminished anti-CTLA4 response [95]. Further, elective nodal irradiation, as it is performed as standard-of-care in the current primary and adjuvant protocols of HNSCC, was evaluated in another murine model. Here, reduced tumor control, systemic immunity, and T-cell-specific immune response were shown [96]. These findings are supported by ex-vivo analysis of systemic biomarkers of patients undergoing chemoradiotherapy that showed an increase in immunosuppressive cell types when comparing pre- to post-treatment samples [97]. Taken together, these studies can help to explain why therapies targeting the local tumor-draining lymph nodes might limit the host's anti-tumor immune response. Furthermore, this suggests treatment should be timed so that checkpoint inhibition is administered prior to surgical or radiotherapeutic lymph node ablation, as done in neoadjuvant treatment regimen.

2.5 Lessons learned from neoadjuvant immunotherapy in non-HNSCC cancers

Considering the need for improved outcomes in diseases outside the head and neck, it is unsurprising that immunotherapy has been incorporated in other disease entities in the neoadjuvant setting. Indeed, there have been remarkable clinical responses and patient outcomes even leading to FDA approval for some of these indications.

Given the pathological and clinical similarities, trials in non-small cell lung cancer (NSCLC) can provide an apt comparison and model for head and neck cancer trials. Indeed, the first clinical trial investigating the preoperative use of anti-PD1 therapy was conducted in NSCLC in which 21 patients received nivolumab prior to surgical tumor resection [98]. Even this early and small trial provided insights that foreshadow future investigations. Nearly half of the patients showed major pathologic response with >10 % tumor regression in their surgical specimens. Also, there was a marked difference between radiological response as measured by RECIST criteria and the histological findings in the tumor specimen, the former significantly underestimating the effect of the neoadjuvant treatment. Also, this trial showed the treatment to be safe, with no major adverse events or delays of surgery. Consequently, a large Phase III trial, Checkmate-816, randomized 385 patients with resectable NSCLC patients to chemotherapy with or without nivolumab for three months followed by surgery [99]. There was no disadvantage in terms of safety, additional toxicity, or delays in surgery in the treatment arm with added immunotherapy. Indeed, there was less need for extensive surgery in this group. In terms of outcomes, the event-free survival (EFS) was increased by approximately one year in the experimental group. Analogous to these findings, the percentage of patients showing complete pathological response increased from 2.2 % in the chemotherapy alone group to 24.0 % in patients receiving additional immunotherapy. This landmark study underlines the profound potential effect of edit immunotherapy in the pre-surgical setting.

Another disease entity in which neoadjuvant immunotherapy is now the standard of care is triple-negative breast cancer, where it was previously only neoadjuvant chemotherapy. Keynote 522, a Phase III trial that randomized 1174 patients to chemotherapy plus either pembrolizumab or placebo followed by surgery, was the first trial that ultimately led to FDA approval for a neoadjuvant checkpoint inhibition (DOI: 10.1056/NEJMoa1910549). It was able to show an increase in event-free survival from 76.8 % in the chemotherapy group to 84.5 % experimental group, as well as an increase in pathological complete response from 56 % to 63 %.

One further entity that can serve as an interesting case study for the power of identifying highly predictive biomarkers for response in neoadjuvant immunotherapy is colon cancer with mismatch repair deficiency (dMMR). dMMR constitutes an important molecular aberration in a small subset of colon cancers closely associated with the microsatellite instability-high (MSI-H) phenotype. This deficiency arises from the loss of function in key proteins involved in the mismatch repair system – namely MLH1, MSH2, MSH6, and PMS2 – resulting in a failure to correct base-pair mismatches during DNA replication. Consequently, dMMR leads to an accumulation of errors in microsatellite sequences, manifesting as MSI-H. This hypermutated state not only contributes to tumorigenesis but also leads tumors to be more immunogenic due to a higher tumor mutational burden and the presentation of novel neoantigens. Clinically, tumors exhibiting dMMR are generally associated with a better prognosis in early-stage cases but paradoxically may be less responsive to traditional chemotherapy agents like fluoropyrimidines, which form the backbone of colon cancer treatment. This chemoresistance necessitates alternative treatment paradigms for dMMR patients. In a small single-arm Phase 2 study, dostarlimab, an anti-PD-1 monoclonal antibody, was given for 6 months prior to planned chemoradiotherapy and surgery. However, 12/12 patients (100 %, 95 % CI, 74–100) showed no sign of residual tumor on endoscopic evaluation with biopsy, MRT, or PET-imaging [100], leading to none of the patients receiving subsequent treatment. This highlights the potential curative power of immunotherapy monotherapy in a highly targeted subgroup of patients.

2.6 Clinical results from neoadjuvant immunotherapy trials in HNSCC

Neoadjuvant immunotherapy trials that have published results are summarized in ▶ **Fig. 5** (▶ **Fig. 5**). One can appreciate the great diversity of treatment regimens, with variables including the treatment drug(s), dosing, number of cycles, immunotherapy combinations, duration of the neoadjuvant phase, adjuvant therapy, and even combination with pre-operative radiation or targeted therapy.

2.6.1 Immunotherapy-only trials

Several trials have investigated presurgical treatment with immunotherapy only. In the first published report, a multicenter Phase II trial administered a single dose of pembrolizumab in 36 patients with non-HPV-associated HNSCC in a 2–3-week window before sur-

Author, Year		Arm	N	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	pCR	MPR
Immunotherapy only																	
Uppaluri et al	2020	1	36	Pembro		Surgery										0%	-
Schoenfeld et al	2020	1	14	Nivo		Nivo	Surgery									0%	8%
		2	15	Nivo + Ipi		Nivo	Surgery									7%	20%
Vos et al	2021	1	6	Nivo		Nivo		Surgery								0%	17%
		2	26	Nivo + Ipi		Nivo		Surgery								4%	35%
Knochelmann et al	2021	1	12	Nivo		Nivo		Nivo	Re-Staging	Nivo	Surgery					0%	-
										Surgery							
Wise-Draper et al	2022	1	92	Pembro		Surgery										-	7%
Ferris et al	2021	1	52	Nivo		Nivo		Surgery								0%	6%
Ferrarotto et al	2020	1	14	Durva			Durva				Surgery					8%	8%
		2	14	Durva + Treme			Durva + Treme				Surgery					8%	8%
Hanna et al	2020	1	28	Nivo + Lirilumab		Surgery										0%	14%
Immunotherapy + other agents																	
Luginbuhl et al	2022	1	20	Nivo		Nivo		Surgery								5%	5%
		2	25	Nivo	Tadalafil daily	Nivo		Surgery								8%	12%
Oliva et al	2021	1	10	Nivo	Sitravatinib daily		Surgery									10%	30%
Ju et al	2023	1	20	Camre		Camre		Camre	Surgery							10%	40%
						Apatinib daily											
Redman et al	2022	1	14	bintrafusp alfa		bintrafusp alfa		Surgery								0%	0%
Zhang et al	2022	1	30	Camre + paclitaxel + cisplatin			Camre + paclitaxel + cisplatin			Camre + paclitaxel + cisplatin				Surgery		37%	74%
Huang et al	2023	1	20	Toripa + paclitaxel + cisplatin			Toripa + paclitaxel + cisplatin			Surgery						30%	60%
Huang et al	2022	1	23	Toripa + gemcitabine + cisplatin			Toripa + gemcitabine + cisplatin			Surgery						17%	44%
Wang et al	2023	1	22	Pembro + paclitaxel + cisplatin			Pembro + paclitaxel + cisplatin			Surgery						36%	55%

▶ **Fig. 5** Overview of published neoadjuvant immunotherapy trials in HNSCC. Pembro = Pembrolizumab, Nivo = Nivolumab, Ipi = Ipilimumab, Durva = Durvalumab, Treme = Tremelimumab, Camre = Camrelizumab, Toripa = Toripalimab, pCR = pathological complete response, MPR = major pathological response

gery, followed by risk-adapted adjuvant chemoradiotherapy. This protocol proved safe, as no Grade 3 or 4 adverse events or delays in surgery occurred. In terms of response, a pTR ≥ 50 % was achieved in 22 % of patients and 10–49 % in another 22 %. There was no pCR [101]. A single-center Phase II trial in 29 patients with oral cavity cancers randomized to either nivolumab at Week 1 and 3 or nivolumab plus ipilimumab at Week 1 and nivolumab at Week 3 followed by surgery within one Week. Here, 13 % of patients developed Grade 3 or 4 adverse events in the nivolumab arm and 33 % in the nivolumab plus ipilimumab arm. There, again, was no delay in surgery dates. A pTR 10–49 % was observed in 38 % of the patients receiving nivolumab and 40 % in the combination arm, while a pTR ≥ 50 % was seen in only 15 % of patients receiving nivolumab but 33 % in the nivolumab plus ipilimumab arm, including one patient with pCR (7 %) [102]. A similar protocol and patient cohort were studied in the IMCISION trial; in the safety run-in Phase Ib part of the trial, 6 patients were treated with nivolumab in Weeks 1 and 3, while 6 patients received nivolumab plus ipilimumab at Week 1 followed by nivolumab at Week 3. The trial was prolonged to a single-arm IIa extension cohort with 20 patients receiving the latter combination treatment. Safety evaluation showed 33 % of patients in the nivolumab group and 38 % of nivolumab plus ipilimumab patients having Grade 3 or 4 adverse events, with none resulting in the delay of surgery. Patients were classified into major pathological response (MPR), partial pathological response (PPR), or no pathological response (NPR) based on previously described criteria from melanoma studies [103]. In the nivolumab group, an MPR was observed in 17 % of patients, while in the combination arm, 35 % of patients had an MPR including 4 % with pCR [104]. A more individualized single-arm trial was conducted in 12 patients with oral cavity squamous cell carcinomas, where, after giving nivolumab three times biweekly, a clinical and radiographic re-evaluation determined whether a fourth dose was given [105]. There were no Grade 3 or 4 adverse events definitely or possibly related to neoadjuvant immunotherapy, and there was no delay in surgery. Response was measured by comparing the surgical specimen's maximum tumor diameter with the single greatest tumor dimension on pretreatment imaging, defining a partial response as a > 30 % reduction. Of the patients, 33 % showed stable disease, 33 % showed a partial pathologic response, 0 % a complete pathological response, and 33 % a disease progression. One more recent, larger, multicenter Phase II trial evaluated single-dose pembrolizumab one to three weeks prior to surgery in 96 patients. Partial pathological response, defined as tumor regression ≥ 20 % to < 90 % was achieved in 32 % of patients, while major pathological response, ≥ 90 % tumor regression, was seen in 7 % [106].

Two trials investigated the neoadjuvant immunotherapy paradigm predominantly in the context of oropharyngeal cancer. CheckMate 358, a multi-center multi-cohort trial contained neoadjuvant HNSCC cohorts of HPV-associated and non-HPV-associated cancers, recruiting 52 patients who received nivolumab in Week 1 and Week 2 followed by surgery in Week 4 [107]. Grade 3 or 4 adverse events were observed in 19.2 % of the HPV-associated and 11.5 % of the non-HPV-associated cancers, with no delays in surgery due to adverse events. Pathological response was judged by evaluating residual viable tumor (RVT) with pCP equaling 0 % RVT, major pathologic response (MPR) ≤ 10 % RVT, and pathologic partial response (pPR, > 10 %- 50 %RVT). Out of 34 evaluable patients, 7 % of HPV-associated HNSCC, achieved MPR and 18 % pPR, while non-HPV-associated patients achieved pPR in 6 % of cases. In a similar patient cohort, the CIAO trial randomized patients to two cycles of durvalumab versus durvalumab plus tremelimumab. Severe adverse events in Grades 3 or 4 were observed in 20 % of patients in the durvalumab group versus 7 % in the durvalumab plus tremelimumab group. Major pathologic response (MPR), defined as ≤ 10 % viable tumor, was achieved in 7 % of the primary tumor in both arms and 50 % of the lymph nodes in the durvalumab group versus 22 % in the combination group [108].

One trial that is particularly specialized in its treatment and study population was a Phase II trial of single-dose nivolumab and lirilumab (anti-KIR) in 28 patients with recurrent but surgically salvageable HNSCC [109]. There were no delays in surgery due to adverse events. For the patients in the study, ≤ 10 % viable tumor cells, defined as a major pathological response (MPR), were achieved by 14 % of patients, and pathologic partial response (pPR) (≤ 50 % tumor viability) by 29 % of the patients.

2.6.2 Immunotherapy in combination with other agents

There are multiple published studies that combine preoperative immunotherapy with other treatment modalities, including targeted therapy, chemotherapy, and radiotherapy.

In a two-arm, multi-institutional trial, nivolumab at Weeks 1 and 2 was combined with daily phosphodiesterase-5 inhibitor (tadalafil) followed by surgery at Week 4 in 45 patients. There were no Grade 3 or 4 adverse events and no delays in surgery. Patients with pathological tumor response ≥ 20 % were defined as responders, > 0 %- < 20 % as minimal responders, 0 % as non-responders, and 100 % as complete responders. Across both cohorts, 51 % had a response with an additional 7 % experiencing a complete response. There was no difference in terms of pathological response in patients receiving tadalafil [110].

In another combination trial, 10 patients with oral cavity carcinomas were treated with one dose of nivolumab at Week 2, combined with daily Sitravatinib, an oral receptor tyrosine kinase inhibitor, followed by surgery at Week 3 [111]. There was one Grade 3, but no Grade 4, treatment-related adverse event. There was one Grade 2 thrombocytopenia, which led to a two-week delay in surgery. Of the patients, 10 % achieved complete pathological tumor response (cPTR) with 0 % residual tumor cells, 20 % of patients major response (mPTR) with < 10 % residual viable tumor, and the other 70 % incomplete response.

A Phase I trial of 20 patients with oral squamous cell carcinoma combined camrelizumab, an anti-PD-1 monoclonal antibody, at Weeks 1, 2, and 4 with four weeks of oral apatinib, a tyrosine kinase inhibitor that inhibits vascular endothelial growth factor 2. There were no Grade 3 or 4 events in the preoperative phase, one surgery was postponed by one week due to elevation in cardiac troponin I, which recovered spontaneously. Residual viable tumor cell content was evaluated, with 40 % of patients showing major pathologic response (< 10 % residual viable tumor), including 10 % of complete pathological response. Notably, 95 % of patients had a tumor response of ≥ 50 % [112].

In a Phase I trial, 14 patients were treated with 1 or 2 doses of bintrafusp alfa, a bifunctional fusion protein composed of the TGF-β

receptor II linked to anti–PD-L1, followed by surgery. Of the patients, 7.1 % developed Grade 3 adverse events. There were no complete or major pathologic responses, and 36 % of patients showed a partial response (> 50 % regression) [113].

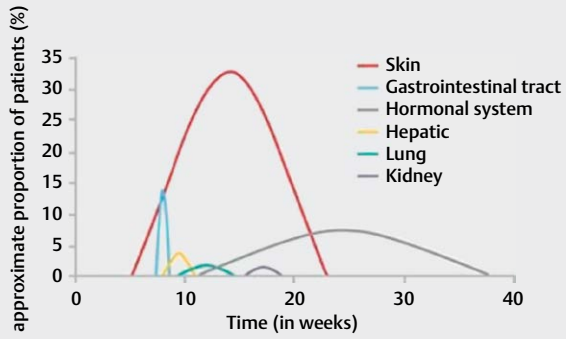
Several recent trials have explored the combined effect of chemotherapy and immunotherapy in the neoadjuvant setting. A single-center, single-arm Phase II trial evaluated the effect of paclitaxel or docetaxel plus cisplatin in combination with camrelizumab, an anti-PD1 monoclonal antibody, for three cycles. In terms of safety, 6.3 % of patients experienced Grade 3 adverse events, but there were no Grade 4 toxicities, delays of surgery, or trial discontinuation. Major pathologic response, ≤ 10 % residual viable tumor cells, was achieved in 74.1 %, including 37.0 % with a complete pathological response [114]. In a similar study of oral cavity squamous cell carcinoma, 20 patients received two cycles of paclitaxel, cisplatin, and toripalimab, an anti-PD1 monoclonal antibody. Of the patients, 15 % experienced Grade 3 or 4 adverse events, none of which led to treatment discontinuation or delay of surgery. Major pathologic response showing ≤ 10 % residual viable tumor cells was seen in 60 % of patients, with 30 % achieving complete pathological response [115]. In a further study, a single-arm Phase Ib trial, two cycles of gemcitabine and cisplatin with toripalimab were administered to a mixed cohort of 23 patients with HNSCC. Grade 3 adverse events occurred in 13.0 % of patients and Grade 4 in 8.7 %, with no treatment-related delays of surgery. Of the patients, 44.4 % achieved major pathological response, including 16.7 % who achieved complete pathological response [116]. Another single-arm, single-center study with a mixed cohort of HNSCC investigated 2–3 cycles of pembrolizumab with cisplatin and paclitaxel in 22 patients. There were Grade 3 toxicities in 9.2 % of patients, but no Grade 4 events and no treatment-related delays in surgery. Major pathological response was 54.5 %, including 36.4 % with pathological complete response [117].

One distinct single institution Phase Ib trial investigated the role of neoadjuvant nivolumab with added stereotactic body radiation therapy (SBRT) before surgery in 21 patients divided into four treatment groups differing by the amount of radiation, 40 Gy versus 24 Gy, HPV-status, and nivolumab treatment. There were no delays in surgery due to adverse neoadjuvant treatment effects. Across all cohorts, mPR was 86 %, including 67 % pathological complete response [118].

3. Open questions and challenges in neoadjuvant immunotherapy

3.1 Safety and adverse events profile

Considering the curative intent in non-metastatic HNSCC, the feasibility and safety of a neoadjuvant approach were the main concerns in Phase I and II studies published thus far. As has been reviewed extensively above, adverse events seem to be rare and manageable (▶ **Fig. 6**). A meta-analysis of 344 patients, not including the more recent trials of combined immunochemotherapy, calculated the rate of preoperative Grade 3 to 4 adverse events to be 8.4 % [119]. Importantly, across all the studies reviewed above there were only two delays in surgery reported, one for two weeks due to thrombocytopenia [111] and one for one week due to self-limiting troponin increase [112]. Thus, unless large Phase III trials report rare severe or long-term adverse events, checkpoint inhibition is to be considered safe in the neoadjuvant setting. Even though some studies have reported surgical complications, their impact following neoadjuvant (chemo)immunotherapy and how this impacts morbidity and quality of life has not been explored consistently and systematically.



▶ **Fig. 6** Occurrence of various toxicities depending on the duration of therapy with PD-1 and PD-L1 inhibitors (Daten aus [32]). Aus: https://cme.thieme.de/cme-webapp/#journals/0935–8943/a_1337_0882_toc/10.1055-a-1337–0882

3.2 Radiographic assessment of response

Similar to the observations made in lung cancer [98], there has been a marked discrepancy between imaging, measured using RECIST criteria, and the response seen in histology. Even in the first reported study, two of the three patients with progressive disease as measured by RECIST had a pathological tumor response of 10–49 % and > 50 %, respectively [101]. Analogous observations have been made in other studies. The test validity criteria for MRI in detecting major pathological response were evaluated in one study, showing a high specificity of 100 % but a low sensitivity of 29 % [104]. Conversely, in the same cohort, a metabolic tumor volume or total lesion glycolysis decrease pre- and post-immunotherapy was identified as a potential marker to identify response [120]. Taken together these studies suggest that though hybrid imaging might be able to identify responders, conventional imaging using MRT or CT is unable to differentiate stable or progressive disease from a successful anti-tumor immune response.

3.3 Pathologic assessment of response

Due to the limitations of radiographic imaging to determine a tumor response in the context of immunotherapy, pathological response criteria in the surgical specimen seemed to be the optimal candidate to assess efficacy. Leaning on experience from neoadjuvant chemotherapy from the pre-immunotherapy era, respective criteria have been developed and shown to be prognostic markers in several cancer types. Complete pathological response, with no viable tumor cells, as well as major pathological response, meaning < 10 % viable tumor cells, are the most consistently used and described methods in this context [121]. Even though these metrics have been applied to some extent in the neoadjuvant immunotherapy trials described above, there is great variability in how they are used. Some studies created customized criteria such as

pTR 10–49 % versus pTR > 50 % [101] or comparing the surgical specimen maximum tumor diameter with the single greatest tumor dimension on pretreatment imaging [105]. Other studies reported pCR and MPR but set individual cut-offs for partial pathological response such as 20 %- 90 % [106], ≤ 50 % tumor viability [109], or > 20 % [110]. It is important to consider that most of these criteria were developed based on response to chemotherapy and not immunotherapy. This has been highlighted in the context of NSCLC, where the pathologic features might be different in the neoadjuvant immunotherapy setting, leading to the development of separate immune-related pathologic response criteria (irPRC) [122]. However, these might not be applicable to non-NSCLC entities, as even within NSCLC the optimal cutoff of percent viable tumor differed between adenocarcinoma and squamous cell carcinoma [123]. This holds true even though a pan-tumor pathologic scoring system was developed that included HNSCC samples [124].

One further point of controversy is to what extent the tumor-draining lymph nodes should be incorporated into the pathological response metric, where a divergence between the primary and lymph node response has been described in several HNSCC studies [108, 115, 125]. This highlights the importance of developing HNSCC-specific pathologic response criteria and cut-offs, most likely using 10 % increments of residual viable tumor – possibly separately for the primary and tumor-draining lymph nodes, which can then facilitate the comparison across multiple studies. Alternatively, the pan-tumor scoring system needs to be further validated in HNSCC.

3.4 Pre- and intraoperative decision-making

One aspect that has thus far not been explored in detail is the determination of the extent of surgery. In this early phase of the neoadjuvant treatment paradigm in HNSCC, most studies reported to have operated in the pre-therapeutic tumor borders with some taking detailed care by tattooing tumor borders or using pre-immunotherapy imaging and photography as guidance [115]. With larger trials, more experience, and better data and presurgical markers of response, it might be possible to tailor the surgery more directly to the patient's remaining disease after neoadjuvant treatment. This is especially relevant in the setting of disfiguring surgeries such as laryngectomies, exenteratio orbitae, or ablatio nasi. Here, new organ-sparing protocols could be tested.

3.5 Treatment combination and timing

Bearing in mind the large possible number of combinations using immunotherapy agents (e. g., anti-PD1, anit-CTLA4, anti-LAG3, etc.), conventional chemotherapy, targeted therapy, radiation, and even more experimental treatment such as oncolytic viruses or therapeutic vaccination, finding the optimal treatment regimen for each individual patient remains the most challenging task. One must be careful when comparing small single-arm non-randomized trials with varying regimens and divergent outcome measures. In terms of response data, complete and major pathological responses have been the most widely and consistently reported measures. Given the data we have thus far, there seems to be an added benefit of adding targeted therapy or chemotherapy to checkpoint inhibition. Here, the reported complete response rates have increased from nivolumab (0 %) [101], nivolumab plus ipilimumab (7 %) [102], or 4 % [104] to 10 % in two reports combining immunotherapy with targeted therapy [111, 112]. This increased further in the setting of combination with chemotherapy to 37.0 % [114], 30 % [115], 16.7 % [116], and 36.4 % [117], an assessment which is supported by a recent metanalysis of ORR comparing neoadjuvant immunotherapy to immunochemotherapy [126].

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Lacas B, Carmel A, Landais C et al. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): An update on 107 randomized trials and 19,805 patients, on behalf of MACH-NC Group. Radiother Oncol 2021; 156: 281–293. DOI: 10.1016/j.radonc.2021.01.013

[2] Bernier J, Cooper JS, Pajak TF et al. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (#22931) and RTOG (# 9501). Head Neck 2005; 27: 843–850. DOI: 10.1002/hed.20279

[3] Hussain A, Kim EY, Khachemoune A. Systematic review of benefits and practical challenges for application of Mohs surgery for oral tumors [published online ahead of print, 2023 May 12]. Arch Dermatol Res 2023. DOI: 10.1007/s00403-023-02632-3. DOI: 10.1055/a-1647-8650

[4] Nutting CM, Morden JP, Harrington KJ et al. Parotid-sparing intensity modulated versus conventional radiotherapy in head and neck cancer (PARSPORT): a phase 3 multicentre randomised controlled trial. Lancet Oncol 2011; 12: 127–136. DOI: 10.1016/S1470-2045(10)70290-4

[5] Mehra R, Cohen RB, Burtness BA. The role of cetuximab for the treatment of squamous cell carcinoma of the head and neck. Clin Adv Hematol Oncol 2008; 6: 742–750

[6] Pulte D, Brenner H. Changes in survival in head and neck cancers in the late 20th and early 21st century: a period analysis. Oncologist. 2010; 15: 994–1001. DOI: 10.1634/theoncologist.2009-0289

[7] Chaturvedi AK, Engels EA, Pfeiffer RM et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011; 29: 4294–4301. DOI: 10.1200/JCO.2011.36.4596

[8] Kim YJ, Kim JH. Increasing incidence and improving survival of oral tongue squamous cell carcinoma. Sci Rep. 2020; 10: 7877. Published 2020. DOI: 10.1038/s41598-020-64748-0

[9] Huang SH, Xu W, Waldron J et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. J Clin Oncol 2015; 33: 836–845. DOI: 10.1200/JCO.2014.58.6412

[10] Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma [published correction appears in Nat Rev Dis Primers. 2023 Jan 19;9(1):4]. Nat Rev Dis Primers 2020; 6: 92. Published 2020 Nov 26. DOI: 10.1038/s41572-020-00224-3

[11] Kraaijenga SA, Oskam IM, van Son RJ et al. Assessment of voice, speech, and related quality of life in advanced head and neck cancer patients 10-years + after chemoradiotherapy. Oral Oncol 2016; 55: 24–30. DOI: 10.1016/j.oraloncology.2016.02.001

[12] Kuhn MA, Gillespie MB, Ishman SL et al. Expert Consensus Statement: Management of Dysphagia in Head and Neck Cancer Patients. Otolaryngol Head Neck Surg 2023; 168: 571–592. DOI: 10.1002/ohn.302

[13] Nathan CO, Asarkar AA, Entezami P et al. Current management of xerostomia in head and neck cancer patients. Am J Otolaryngol 2023; 44: 103867. DOI: 10.1016/j.amjoto.2023.103867

[14] Verdonck-de Leeuw I, Dawson C, Licitra L et al. European Head and Neck Society recommendations for head and neck cancer survivorship care. Oral Oncol 2022; 133: 106047. DOI: 10.1016/j.oraloncology.2022.106047

[15] Melissant HC, Jansen F, Eerenstein SE et al. Body image distress in head and neck cancer patients: what are we looking at? Support Care Cancer 2021; 29: 2161–2169. DOI: 10.1007/s00520-020-05725-1

[16] Rathod S, Livergant J, Klein J, Witterick I, Ringash J. A systematic review of quality of life in head and neck cancer treated with surgery with or without adjuvant treatment. Oral Oncol 2015; 51: 888–900. DOI: 10.1016/j.oraloncology.2015.07.002

[17] Hammerlid E, Silander E, Hornestam L, Sullivan M. Health-related quality of life three years after diagnosis of head and neck cancer-a longitudinal study. Head Neck 23: 113–125

[18] Liao LJ, Hsu WL, Lo WC et al. Health-related quality of life and utility in head and neck cancer survivors. BMC Cancer 19: 425 2019. DOI: 10.1186/s12885-019-5614-4

[19] Osazuwa-Peters N, Simpson MC, Zhao L et al. Suicide risk among cancer survivors: Head and neck versus other cancers. Cancer 2018; 124: 4072–4079. DOI: 10.1002/cncr.31675

[20] Esfahani K, Roudaia L, Buhlaiga N, Del Rincon SV, Papneja N, Miller WH Jr. A review of cancer immunotherapy: from the past, to the present, to the future. Curr Oncol 2020; 27: S87–S97. DOI: 10.3747/co.27.5223

[21] Kucerova P, Cervinkova M. Spontaneous regression of tumour and the role of microbial infection--possibilities for cancer treatment. Anticancer Drugs 2016; 27: 269–277. DOI: 10.1097/CAD.0000000000000337

[22] MacKie RM, Reid R, Junor B. Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. N Engl J Med 2003; 348: 567–568. DOI: 10.1056/NEJM200302063480620

[23] Simard EP, Pfeiffer RM, Engels EA. Cumulative incidence of cancer among individuals with acquired immunodeficiency syndrome in the United States. Cancer 2011; 117: 1089–1096. DOI: 10.1002/cncr.25547

[24] Haas OA. Primary Immunodeficiency and Cancer Predisposition Revisited: Embedding Two Closely Related Concepts Into an Integrative Conceptual Framework. Front Immunol 2019; 9: 3136. Published 2019 Feb 12. DOI: 10.3389/fimmu.2018.03136

[25] Uppaluri R, Dunn GP, Lewis JS Jr. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in head and neck cancers. Cancer Immun 2008; 8: 16. Published 2008 Dec 4

[26] Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 2002; 3: 991–998. DOI: 10.1038/ni1102-991

[27] Margolin KA. Interleukin-2 in the treatment of renal cancer. Semin Oncol 2000; 27: 194–203

[28] Ahmed S, Rai KR. Interferon in the treatment of hairy-cell leukemia. Best Pract Res Clin Haematol 2003; 16: 69–81. DOI: 10.1016/s1521-6926(02)00084-1

[29] Rosenberg SA, Yang JC, Sherry RM et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 2011; 17: 4550–4557. DOI: 10.1158/1078-0432.CCR-11-0116

[30] Morad G, Helmink BA, Sharma P, Wargo JA. Hallmarks of response, resistance, and toxicity to immune checkpoint blockade [published correction appears in Cell. 2022 Feb 3;185(3):576]. Cell 2021; 184: 5309–5337. DOI: 10.1016/j.cell.2021.09.020

[31] Diabetic Retinopathy Clinical Research NetworkWells JA, Glassman AR et al. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. N Engl J Med 2015; 372: 1193–1203. DOI: 10.1056/NEJMoa1602252

[32] Burtness B, Harrington KJ, Greil R et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study [published correction appears in Lancet. 2020 Jan 25;395(10220):272] [published correction appears in Lancet. 2020 Feb 22; 395 (10224): 564] [published correction appears in Lancet. 2021 Jun 12; 397 (10291): 2252]. Lancet 2019; 394: 1915–1928. DOI: 10.1016/S0140-6736(19)32591-7

[33] Ruffin AT, Li H, Vujanovic L, Zandberg DP, Ferris RL, Bruno TC. Improving head and neck cancer therapies by immunomodulation of the tumour microenvironment. Nat Rev Cancer 2023; 23: 173–188. DOI: 10.1038/s41568-022-00531-9

[34] Pennock ND, White JT, Cross EW, Cheney EE, Tamburini BA, Kedl RM. T cell responses: naive to memory and everything in between. Adv Physiol Educ 2013; 37: 273–283. DOI: 10.1152/advan.00066.2013

[35] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57–70. DOI: 10.1016/s0092-8674(00)81683-9

[36] Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 2004; 21: 137–148. DOI: 10.1016/j.immuni.2004.07.017

[37] Ferris RL. Immunology and Immunotherapy of Head and Neck Cancer. J Clin Oncol 2015; 33: 3293–3304. DOI: 10.1200/JCO.2015.61.1509

[38] Kürten CH et al. Stimulierende und inhibierende Signalwege der APZ- und T-Zell-Interaktion sowie Einfluss von TLR-Agonisten auf APZ. HNO 2020; 68, no. 12: 916–921. DOI: 10.1007/s00106-020-00960-8

[39] Chen SD, Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. Immunity, 2013, Vol.39 (1), p.1-10, Article 1. Doi: 10.1016/j.immuni.2013.07.012

[40] Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T-cell differentiation: human memory T-cell subsets. Eur J Immunol 2013; 43: 2797–2809. DOI: 10.1002/eji.201343751

[41] Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. Nat Rev Immunol 2014; 14: 24–35. DOI: 10.1038/nri3567

[42] Demers KR, Reuter MA, Betts MR. CD8(+) T-cell effector function and transcriptional regulation during HIV pathogenesis. Immunol Rev 2013; 254: 190–206. DOI: 10.1111/imr.12069

[43] Lawrence MS, Stojanov P, Polak P et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013; 499: 214–218. DOI: 10.1038/nature12213

[44] Ogino T, Shigyo H, Ishii H et al. HLA class I antigen down-regulation in primary laryngeal squamous cell carcinoma lesions as a poor prognostic marker. Cancer Res 2006; 66: 9281–9289. DOI: 10.1158/0008-5472.CAN-06-0488

[45] Ferris RL, Whiteside TL, Ferrone S. Immune escape associated with functional defects in antigen-processing machinery in head and neck cancer. Clin Cancer Res 2006; 12: 3890–3895. DOI: 10.1158/1078-0432.CCR-05-2750

[46] Ferris RL, Hunt JL, Ferrone S. Human leukocyte antigen (HLA) class I defects in head and neck cancer: molecular mechanisms and clinical significance. Immunol Res 2005; 33: 113–133. DOI: 10.1385/IR:33:2:113

[47] Concha-Benavente F, Srivastava R, Ferrone S, Ferris RL. Immunological and clinical significance of HLA class I antigen processing machinery component defects in malignant cells. Oral Oncol 2016; 58: 52–58. DOI: 10.1016/j.oraloncology.2016.05.008

[48] Gillison ML, Akagi K, Xiao W et al. Human papillomavirus and the landscape of secondary genetic alterations in oral cancers. Genome Res 2019; 29: 1–17. DOI: 10.1101/gr.241141.118

[49] Leibowitz MS, Andrade Filho PA, Ferrone S, Ferris RL. Deficiency of activated STAT1 in head and neck cancer cells mediates TAP1-dependent escape from cytotoxic T lymphocytes. Cancer Immunol Immunother 2011; 60: 525–535. DOI: 10.1007/s00262-010-0961-7

[50] Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 2008; 26: 677–704. DOI: 10.1146/annurev.immunol.26.021607.090331

[51] Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 2015; 15: 486–499. DOI: 10.1038/nri3862

[52] Parsa AT, Waldron JS, Panner A et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 2007; 13: 84–88. DOI: 10.1038/nm1517

[53] Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. Trends Immunol 2015; 36: 265–276. DOI: 10.1016/j.it.2015.02.008

[54] Barber DL, Wherry EJ, Masopust D et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006; 439: 682–687. DOI: 10.1038/nature04444

[55] Hirano F, Kaneko K, Tamura H et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res 2005; 65: 1089–1096

[56] Blackburn SD, Shin H, Freeman GJ, Wherry EJ. Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1 blockade. Proc Natl Acad Sci U S A 2008; 105: 15016–15021. DOI: 10.1073/pnas.0801497105

[57] Paley MA, Kroy DC, Odorizzi PM et al. Progenitor and terminal subsets of CD8 + T cells cooperate to contain chronic viral infection. Science 2012; 338: 1220–1225. DOI: 10.1126/science.1229620

[58] Blackburn SD, Shin H, Haining WN et al. Coregulation of CD8 + T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol 2009; 10: 29–37. DOI: 10.1038/ni.1679

[59] Kansy BA, Concha-Benavente F, Srivastava RM et al. PD-1 Status in CD8 + T Cells Associates with Survival and Anti-PD-1 Therapeutic Outcomes in Head and Neck Cancer. Cancer Res 2017; 77: 6353–6364. DOI: 10.1158/0008-5472.CAN-16-3167

[60] Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol 2017; 14: 717–734. DOI: 10.1038/nrclinonc.2017.101

[61] Wing JB, Tanaka A, Sakaguchi S. Human FOXP3 + Regulatory T Cell Heterogeneity and Function in Autoimmunity and Cancer. Immunity 2019; 50: 302–316. DOI: 10.1016/j.immuni.2019.01.020

[62] Tanaka A, Sakaguchi S. Regulatory T cells in cancer immunotherapy. Cell Res 2017; 27: 109–118. DOI: 10.1038/cr.2016.151

[63] Miyara M, Yoshioka Y, Kitoh A et al. Functional delineation and differentiation dynamics of human CD4 + T cells expressing the FoxP3 transcription factor. Immunity 2009; 30: 899–911. DOI: 10.1016/j.immuni.2009.03.019

[64] Saito T, Nishikawa H, Wada H et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. Nat Med 2016; 22: 679–684. DOI: 10.1038/nm.4086

[65] Liu S, Li S, Hai J et al. Targeting HER2 Aberrations in Non-Small Cell Lung Cancer with Osimertinib. Clin Cancer Res 2018; 24: 2594–2604. DOI: 10.1158/1078-0432.CCR-17-1350

[66] Schuler PJ, Schilling B, Harasymczuk M et al. Phenotypic and functional characteristics of CD4 + CD39 + FOXP3 + and CD4 + CD39 + FOXP3neg T-cell subsets in cancer patients. Eur J Immunol 2012; 42: 1876–1885. DOI: 10.1002/eji.201142347

[67] Granot Z, Jablonska J. Distinct Functions of Neutrophil in Cancer and Its Regulation. Mediators Inflamm 2015; 2015: 701067. DOI: 10.1155/2015/701067

[68] Jablonska J, Leschner S, Westphal K, Lienenklaus S, Weiss S. Neutrophils responsive to endogenous IFN-beta regulate tumor angiogenesis and growth in a mouse tumor model [published correction appears in J Clin Invest. 2010 Nov 1;120(11):4163]. J Clin Invest 2010; 120: 1151–1164. DOI: 10.1172/JCI37223

[69] Jablonska J, Lang S, Sionov RV, Granot Z. The regulation of pre-metastatic niche formation by neutrophils. Oncotarget 2017; 8: 112132–112144. Published 2017 Nov 30. DOI: 10.18632/oncotarget.22792

[70] Pylaeva E, Lang S, Jablonska J. The Essential Role of Type I Interferons in Differentiation and Activation of Tumor-Associated Neutrophils. Front Immunol 2016; 7: 629. Published 2016 Dec 21. DOI: 10.3389/fimmu.2016.00629

[71] Andzinski L, Kasnitz N, Stahnke S et al. Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. Int J Cancer 2016; 138: 1982–1993. DOI: 10.1002/ijc.29945

[72] Andzinski L, Wu CF, Lienenklaus S, Kröger A, Weiss S, Jablonska J. Delayed apoptosis of tumor associated neutrophils in the absence of endogenous IFN-β. Int J Cancer 2015; 136: 572–583. DOI: 10.1002/ijc.28957

[73] Jablonska J, Wu CF, Andzinski L, Leschner S, Weiss S. CXCR2-mediated tumor-associated neutrophil recruitment is regulated by IFN-β. Int J Cancer 2014; 134: 1346–1358. DOI: 10.1002/ijc.28551

[74] Hussain B, Xie Y, Jabeen U et al. Activation of STING Based on Its Structural Features. Front Immunol 2022; 13: 808607. Published 2022 Jul 19. DOI: 10.3389/fimmu.2022.878959

[75] Fridlender ZG, Albelda SM. Tumor-associated neutrophils: friend or foe? Carcinogenesis 2012; 33: 949–955. DOI: 10.1093/carcin/bgs123

[76] Pylaeva E, Korschunow G, Spyra I et al. During early stages of cancer, neutrophils initiate anti-tumor immune responses in tumor-draining lymph nodes. Cell Rep 2022; 40: 111171. DOI: 10.1016/j.celrep.2022.111171

[77] Papalexi E, Satija R. Single-cell RNA sequencing to explore immune cell heterogeneity. Nat Rev Immunol 2018; 18: 35–45. DOI: 10.1038/nri.2017.76

[78] Svensson V, Vento-Tormo R, Teichmann SA. Exponential scaling of single-cell RNA-seq in the past decade. Nat Protoc 2018; 13: 599–604. DOI: 10.1038/nprot.2017.149

[79] Cillo AR, Kürten CHL, Tabib T et al. Immune Landscape of Viral- and Carcinogen-Driven Head and Neck Cancer. Immunity 2020; 52: 183–199.e9. DOI: 10.1016/j.immuni.2019.11.014

[80] Ruffin AT, Cillo AR, Tabib T et al. B cell signatures and tertiary lymphoid structures contribute to outcome in head and neck squamous cell carcinoma. Nat Commun 2021; 12: 3349. Published 2021 Jun 7. DOI: 10.1038/s41467-021-23355-x

[81] Kürten CHL, Kulkarni A, Cillo AR et al. Investigating immune and non-immune cell interactions in head and neck tumors by single-cell RNA sequencing. Nat Commun 2021; 12: 7338. Published 2021 Dec 17. DOI: 10.1038/s41467-021-27619-4

[82] Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 2014; 740: 364–378. DOI: 10.1016/j.ejphar.2014.07.025

[83] Marron TU, Galsky MD, Taouli B et al. Neoadjuvant clinical trials provide a window of opportunity for cancer drug discovery [published correction appears in Nat Med. 2022 Aug;28(8):1723]. Nat Med 2022; 28: 626–629. DOI: 10.1038/s41591-022-01681-x

[84] Zhong LP, Zhang CP, Ren GX et al. Randomized phase III trial of induction chemotherapy with docetaxel, cisplatin, and fluorouracil followed by surgery versus up-front surgery in locally advanced resectable oral squamous cell carcinoma. J Clin Oncol 2013; 31: 744–751. DOI: 10.1200/JCO.2012.43.8820

[85] MACH-NC Collaborative GroupPignon JP, le Maître A, Maillard E, Bourhis J. Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. Radiother Oncol 2009; 92: 4–14. DOI: 10.1016/j.radonc.2009.04.014

[86] Ferris RL, Blumenschein G Jr, Fayette J et al. Nivolumab vs investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck: 2-year long-term survival update of CheckMate 141 with analyses by tumor PD-L1 expression. Oral Oncol 2018; 81: 45–51. DOI: 10.1016/j.oraloncology.2018.04.008

[87] Harrington KJ, Burtness B, Greil R et al. Pembrolizumab With or Without Chemotherapy in Recurrent or Metastatic Head and Neck Squamous Cell Carcinoma: Updated Results of the Phase III KEYNOTE-048 Study. J Clin Oncol 2023; 41: 790–802. DOI: 10.1200/JCO.21.02508

[88] Mehra R, Seiwert TY, Gupta S et al. Efficacy and safety of pembrolizumab in recurrent/metastatic head and neck squamous cell carcinoma: pooled analyses after long-term follow-up in KEYNOTE-012. Br J Cancer 2018; 119: 153–159. DOI: 10.1038/s41416-018-0131-9

[89] Harrington KJ, Ferris RL, Blumenschein G Jr et al. Nivolumab versus standard, single-agent therapy of investigator's choice in recurrent or metastatic squamous cell carcinoma of the head and neck (CheckMate 141): health-related quality-of-life results from a randomised, phase 3 trial. Lancet Oncol 2017; 18: 1104–1115. DOI: 10.1016/S1470-2045(17)30421-7

[90] Cohen R, Bennouna J, Meurisse A et al. RECIST and iRECIST criteria for the evaluation of nivolumab plus ipilimumab in patients with microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: the GERCOR NIPICOL phase II study. J Immunother Cancer 2020; 8: e001499. DOI: 10.1093/annonc/mdy507

[91] Cohen EEW, Soulières D, Le Tourneau C et al. Pembrolizumab versus methotrexate, docetaxel, or cetuximab for recurrent or metastatic head-and-neck squamous cell carcinoma (KEYNOTE-040): a randomised, open-label, phase 3 study [published correction appears in Lancet. 2019 Jan 12;393(10167):132]. Lancet 2019; 393: 156–167. DOI: 10.1016/S0140-6736(18)31999-8

[92] Burtness B, Rischin D, Greil R et al. Pembrolizumab Alone or With Chemotherapy for Recurrent/Metastatic Head and Neck Squamous Cell Carcinoma in KEYNOTE-048: Subgroup Analysis by Programmed Death Ligand-1 Combined Positive Score. J Clin Oncol 2022; 40: 2321–2332. DOI: 10.1200/JCO.21.02198

[93] Lee NY, Ferris RL, Psyrri A et al. Avelumab plus standard-of-care chemoradiotherapy versus chemoradiotherapy alone in patients with locally advanced squamous cell carcinoma of the head and neck: a randomised, double-blind, placebo-controlled, multicentre, phase 3 trial. Lancet Oncol 2021; 22: 450–462. DOI: 10.1016/S1470-2045(20)30737-3

[94] Antonia SJ, Villegas A, Daniel D et al. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. N Engl J Med 2017; 377: 1919–1929. DOI: 10.1056/NEJMoa1709937

[95] Saddawi-Konefka R, O'Farrell A, Faraji F et al. Lymphatic-preserving treatment sequencing with immune checkpoint inhibition unleashes cDC1-dependent antitumor immunity in HNSCC. Nat Commun 2022; 13: 4298. Published 2022 Jul 25. DOI: 10.1038/s41467-022-31941-w

[96] Darragh LB, Gadwa J, Pham TT et al. Elective nodal irradiation mitigates local and systemic immunity generated by combination radiation and immunotherapy in head and neck tumors. Nat Commun 2022; 13: 7015. Published 2022 Nov 16. DOI: 10.1038/s41467-022-34676-w

[97] Sridharan V, Margalit DN, Lynch SA et al. Definitive chemoradiation alters the immunologic landscape and immune checkpoints in head and neck cancer. Br J Cancer 2016; 115: 252–260. DOI: 10.1038/bjc.2016.166

[98] Forde PM, Chaft JE, Smith KN et al. Neoadjuvant PD-1 Blockade in Resectable Lung Cancer [published correction appears in N Engl J Med. 2018 Nov 29;379(22):2185]. N Engl J Med 2018; 378: 1976–1986. DOI: 10.1056/NEJMoa1716078

[99] Forde PM, Spicer J, Lu S et al. Neoadjuvant Nivolumab plus Chemotherapy in Resectable Lung Cancer. N Engl J Med 2022; 386: 1973–1985. DOI: 10.1056/NEJMoa2202170

[100] Cercek A, Lumish M, Sinopoli J et al. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. N Engl J Med 2022; 386: 2363–2376. DOI: 10.1056/NEJMoa2201445

[101] Uppaluri R, Campbell KM, Egloff AM et al. Neoadjuvant and Adjuvant Pembrolizumab in Resectable Locally Advanced, Human Papillomavirus-Unrelated Head and Neck Cancer: A Multicenter, Phase II Trial [published correction appears in Clin Cancer Res. 2021 Jan 1;27(1):357]. Clin Cancer Res 2020; 26: 5140–5152. DOI: 10.1158/1078-0432.CCR-20-1695

[102] Schoenfeld JD, Hanna GJ, Jo VY et al. Neoadjuvant Nivolumab or Nivolumab Plus Ipilimumab in Untreated Oral Cavity Squamous Cell Carcinoma: A Phase 2 Open-Label Randomized Clinical Trial. JAMA Oncol 2020; 6: 1563–1570. DOI: 10.1001/jamaoncol.2020.2955

[103] Tetzlaff MT, Messina JL, Stein JE et al. Pathological assessment of resection specimens after neoadjuvant therapy for metastatic melanoma. Ann Oncol 2018; 29: 1861–1868. DOI: 10.1093/annonc/mdy226

[104] Vos JL, Elbers JBW, Krijgsman O et al. Neoadjuvant immunotherapy with nivolumab and ipilimumab induces major pathological responses in patients with head and neck squamous cell carcinoma. Nat Commun 2021; 12: 7348. Published 2021 Dec 22. DOI: 10.1038/s41467-021-26472-9

[105] Knochelmann HM, Horton JD, Liu S et al. Neoadjuvant presurgical PD-1 inhibition in oral cavity squamous cell carcinoma. Cell Rep Med 2021; 2: 100426. Published 2021 Oct 19. DOI: 10.1016/j.xcrm.2021.100426

[106] Wise-Draper TM, Gulati S, Palackdharry S et al. Phase II Clinical Trial of Neoadjuvant and Adjuvant Pembrolizumab in Resectable Local-Regionally Advanced Head and Neck Squamous Cell Carcinoma. Clin Cancer Res 2022; 28: 1345–1352. DOI: 10.1158/1078-0432.CCR-21-3351

[107] Ferris RL, Spanos WC, Leidner R et al. Neoadjuvant nivolumab for patients with resectable HPV-positive and HPV-negative squamous cell carcinomas of the head and neck in the CheckMate 358 trial [published correction appears in J Immunother Cancer. 2021 Aug;9(8):]. J Immunother Cancer 2021; 9: e002568. DOI: 10.1136/jitc-2021-002568

[108] Ferrarotto R, Bell D, Rubin ML et al. Impact of Neoadjuvant Durvalumab with or without Tremelimumab on CD8 + Tumor Lymphocyte Density, Safety, and Efficacy in Patients with Oropharynx Cancer: CIAO Trial Results. Clin Cancer Res 2020; 26: 3211–3219. DOI: 10.1158/1078-0432.CCR-19-3977

[109] Hanna GJ, O'Neill A, Shin KY et al. Neoadjuvant and Adjuvant Nivolumab and Lirilumab in Patients with Recurrent, Resectable Squamous Cell Carcinoma of the Head and Neck. Clin Cancer Res 2022; 28: 468–478. DOI: 10.1158/1078-0432.CCR-21-2635

[110] Luginbuhl AJ, Johnson JM, Harshyne LA et al. Tadalafil Enhances Immune Signatures in Response to Neoadjuvant Nivolumab in Resectable Head and Neck Squamous Cell Carcinoma. Clin Cancer Res 2022; 28: 915–927. DOI: 10.1158/1078-0432.CCR-21-1816

[111] Oliva M, Chepeha D, Araujo DV et al. Antitumor immune effects of preoperative sitravatinib and nivolumab in oral cavity cancer: SNOW window-of-opportunity study. J Immunother Cancer 2021; 9: e003476. DOI: 10.1136/jitc-2021-003476

[112] Ju WT, Xia RH, Zhu DW et al. A pilot study of neoadjuvant combination of anti-PD-1 camrelizumab and VEGFR2 inhibitor apatinib for locally advanced resectable oral squamous cell carcinoma. Nat Commun 2022; 13: 5378. Published 2022 Sep 14. DOI: 10.1038/s41467-022-33080-8

[113] Redman JM, Friedman J, Robbins Y et al. Enhanced neoepitope-specific immunity following neoadjuvant PD-L1 and TGF-β blockade in HPV-unrelated head and neck cancer. J Clin Invest 2023; 133: e172059. Published 2023 Jun 1. DOI: 10.1172/JCI172059

[114] Zhang Z, Wu B, Peng G et al. Neoadjuvant Chemoimmunotherapy for the Treatment of Locally Advanced Head and Neck Squamous Cell Carcinoma: A Single-Arm Phase 2 Clinical Trial. Clin Cancer Res 2022; 28: 3268–3276. DOI: 10.1158/1078-0432.CCR-22-0666

[115] Huang Y, Sun J, Li J et al. Neoadjuvant immunochemotherapy for locally advanced resectable oral squamous cell carcinoma: a prospective single-arm trial (Illuminate Trial). Int J Surg 2023; 109: 2220–2227. Published 2023 Aug 1. DOI: 10.1097/JS9.0000000000000489

[116] Huang X, Liu Q, Zhong G et al. Neoadjuvant toripalimab combined with gemcitabine and cisplatin in resectable locally advanced head and neck squamous cell carcinoma (NeoTGP01): An open label, single-arm, phase Ib clinical trial. J Exp Clin Cancer Res 41: 300 2022. DOI: 10.1186/s13046-022-02510-2

[117] Wang AX, Ong XJ, D'Souza C, Neeson PJ, Zhu JJ. Combining chemotherapy with CAR-T cell therapy in treating solid tumors. Front Immunol 2023; 14: 1140541. Published 2023 Mar 6. DOI: 10.3389/fimmu.2023.1189752

[118] Leidner R, Crittenden M, Young K et al. Neoadjuvant immunoradiotherapy results in high rate of complete pathological response and clinical to pathological downstaging in locally advanced head and neck squamous cell carcinoma. J Immunother Cancer 2021; 9: e002485. DOI: 10.1136/jitc-2021-002485

[119] Masarwy R, Kampel L, Horowitz G, Gutfeld O, Muhanna N. Neoadjuvant PD-1/PD-L1 Inhibitors for Resectable Head and Neck Cancer: A Systematic Review and Meta-analysis. JAMA Otolaryngol Head Neck Surg 2021; 147: 871–878. DOI: 10.1001/jamaoto.2021.2191

[120] Vos JL, Zuur CL, Smit LA et al. [^{18}F]FDG-PET accurately identifies pathological response early upon neoadjuvant immune checkpoint blockade in head and neck squamous cell carcinoma. Eur J Nucl Med Mol Imaging 2022; 49: 2010–2022. DOI: 10.1007/s00259-021-05610-x

[121] Hellmann MD, Chaft JE, William WN Jr et al. Pathological response after neoadjuvant chemotherapy in resectable non-small-cell lung cancers: proposal for the use of major pathological response as a surrogate endpoint. Lancet Oncol 2014; 15: e42–e50. DOI: 10.1016/S1470-2045(13)70334-6

[122] Cottrell TR, Thompson ED, Forde PM et al. Pathologic features of response to neoadjuvant anti-PD-1 in resected non-small-cell lung carcinoma: a proposal for quantitative immune-related pathologic response criteria (irPRC). Ann Oncol 2018; 29: 1853–1860. DOI: 10.1093/annonc/mdy218

[123] Qu Y, Emoto K, Eguchi T et al. Pathologic Assessment After Neoadjuvant Chemotherapy for NSCLC: Importance and Implications of Distinguishing Adenocarcinoma From Squamous Cell Carcinoma. J Thorac Oncol 2019; 14: 482–493. DOI: 10.1016/j.jtho.2018.11.017

[124] Stein JE, Lipson EJ, Cottrell TR et al. Pan-Tumor Pathologic Scoring of Response to PD-(L)1 Blockade. Clin Cancer Res 2020; 26: 545–551. DOI: 10.1158/1078-0432.CCR-19-2379

[125] Merlino DJ, Johnson JM, Tuluc M et al. Discordant Responses Between Primary Head and Neck Tumors and Nodal Metastases Treated With Neoadjuvant Nivolumab: Correlation of Radiographic and Pathologic Treatment Effect. Front Oncol 2020; 10: 566315. Published 2020 Dec 2. DOI: 10.3389/fonc.2020.566315

[126] Chen S, Yang Y, Wang R, Fang J. Neoadjuvant PD-1/PD-L1 inhibitors combined with chemotherapy had a higher ORR than mono-immunotherapy in untreated HNSCC: Meta-analysis. Oral Oncol 2023; 145: 106479. DOI: 10.1016/j.oraloncology.2023.106479