Immunotherapy of Head and Neck Cancer: summary of a National Cancer Institute Head and Neck Cancer Steering Committee Clinical Trials Planning Meeting, November 9-10, 2014, Bethesda, Maryland

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A particular note is made of the enduring contributions of the late Holbrook Kohrt, MD, PhD, a visionary translational immunotherapist, who thoughtfully contributed to this CTPM from his extensive experience in the field.

Abstract

Recent advances now permit therapeutic targeting of components of the immune system that play a key role in the development, establishment, and progression of head and neck squamous cell carcinoma (HNSCC). These new immunotherapeutic targets and agents are being rapidly adopted by the oncologic community and hold considerable promise. The National Cancer Institute sponsored a Clinical Trials Planning Meeting (CTPM) to address how to further investigate the use of immunotherapy in HNSCC. The goals of the meeting were to consider phase II or III trial designs primarily in three different patient populations: previously -untreated, human papillomavirus (HPV)-initiated oropharyngeal cancers; previously-untreated, HPV-negative HNSCC; and recurrent/metastatic HNSCC. In addition, a separate committee was formed to develop integrative biomarkers for the clinical trials. The meeting started with an overview of key components of immune-infiltrating cells in the tumor microenvironment and immunological principles related to HNSCC including immunosurveillance and immune escape. Four clinical trial concepts were developed at the meeting integrating different immunotherapies with existing standards of care. These designs were presented for implementation by the head and neck committees of the NCI-funded National Clinical Trials Network. This paper summarizes the proceedings of this CTPM, the purpose of which was to facilitate the rigorous development and design of randomized phase II and III immunotherapeutic trials in HNSCC. While usually reviews are published immediately after the meeting was held, this report is unique since we now have tangible clinical trial designs that have been funded and put into practice and the studies are being activated to accrual.

Introduction

Cancer immunotherapy is based on the premise that tumors can be recognized as foreign rather than as self and can be effectively attacked by an activated immune system (1). A greater understanding of the dysregulation and evasion of the immune system in the development and evolution of head and neck squamous cell carcinoma (HNSCC) should lead to improved therapies and outcomes for patients. There has been a recent renaissance in the idea that nascent premalignant cells are destroyed by the immune system before tumor formation can occur, termed "immune surveillance." Derangements in the immune system or alterations in the transformed cells may allow immune escape which then enables the cancer to manifest. Tumors themselves produce cytokines such as transforming growth factor-beta (TGF-β), interleukin (IL)-6, and IL-10, which suppress cell-mediated antitumor immunity, while activation of immune-stimulatory signal transducers and activators of transcription-1 (STAT1) is suppressed (2, 3). Inflammatory transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and STAT3 are aberrantly activated in tumor cells and are intensively studied as possible targets for therapeutic intervention.

Tumor progression depends upon the acquisition of traits that allow cancer cells to evade immune surveillance and an effective immune response. HNSCC is an immunosuppressive disease, and patients have lower absolute lymphocyte counts than healthy subjects (4), impaired NK cell activity (5, 6), and poor antigen-presenting function (7, 8). Impairment of tumor infiltrating T lymphocytes has also been reported in HNSCC and other cancers (9, 10) with a strong impact on clinical outcome (11). In addition, suppressive regulatory T cells (T_{reg}) have been linked to HNSCC tumor progression. T_{reg} cells secrete suppressive cytokines such as TGF-β and IL-10, express CTLA-4, and correlate with tumor progression (12). Therefore,

immunomodulatory therapies that overcome immune suppressive signals in HNSCC patients have therapeutic promise. These include cancer vaccines using tumor peptide antigens, or viral, bacterial and DNA-based vectors - as well as tumor antigen (TA)-specific monoclonal antibodies (mAb). The recent clinical efficacy of FDA-approved mAb targeting immune checkpoint receptors, including anti-CTLA-4 and anti-PD-1, provide further potential for patient benefit as positive clinical data emerge.

Immune Escape and Immunosuppression in Head and Neck Cancer

The meeting (held November 9-10, 2014 in Bethesda at the NCI Clinical Center) began with a series of scientific overview presentations focused on the mechanisms of immune escape in HNSCC, as well as different targets, classes of agents, and information gained from immunotherapy in other diseases, such as melanoma, lung and renal cell carcinoma. The concept was established that in order to establish effective immunotherapies, understanding the different pathways of tumor immune evasion is necessary. The profound though apparently selective immunosuppression in HNSCC ranges from lymphopenia, to altered secretion of normal cytokines and inflammatory signaling pathways, to aberrant skewing of cellular immunity, abetted by suppressive populations such as CD4+ T_{reg}, macrophages, and myeloid derived suppressor cells (MDSC).

Alteration of Immunogenicity by HNSCC Cells

HNSCC cells reduce their inherent immunogenicity (**Table 1**) and they actively suppress the antitumor immune response (**Figure 1**). A key component for the immune system's recognition of different or altered cells is the human leucocyte antigen (HLA) complex, which

presents processed TA peptides to T lymphocytes (8). Tumor cells can reduce T cell-mediated recognition by altering HLA class I expression. Recently, mutations in specific HLA alleles, β-2 microglobulin (β-2m), and antigen processing machinery (APM) components have been observed in large-scale next generation HNSCC sequencing efforts, such as The Cancer Genome Atlas, or TCGA (13), paralleling lung cancer. Chromosomal (14) and regulatory expression defects (7) in the HLA/APM-encoding genes themselves can cause selective loss of HLA and APM component expression in a substantial fraction of HNSCC and are correlated with poor prognosis (15, 16). Cells with complete loss of HLA may evade immune response by T cell recognition but represent a strong trigger for NK cell activation, as the absence of HLA removes a key inhibitory signal for NK cells. HNSCC cells that express HLA I and TA can still evade T cell recognition through decreased expression or mutation of APM components, but still maintain moderate HLA I expression in order to avoid recognition by NK cells. Activation of the epidermal growth factor receptor (EGFR) by HNSCC is well understood to cause aberrant mitogenic signaling, however EGFR also mediates immunosuppressive effects such as downregulation of HLA, APM components and STAT1 activation, and upregulation of suppressive STAT3 signaling, cytokines and ligands on HNSCC cells.

Monoclonal Antibody-Based Immunotherapy of HNSCC

Today the most widely used form of cancer immunotherapy is mAb therapy (17), including TA-targeted mAbs, cytokine-targeted mAbs, tumor necrosis factor receptor (TNFR)-family costimulatory targeted mAbs and immune checkpoint-targeted mAbs. Currently available mAbs under investigation in HNSCC are listed in Table 2. The most extensively studied (and FDA approved for HNSCC) of these is cetuximab, a mouse–human chimeric IgG1 anti-epidermal growth factor receptor (EGFR) mAb (18). EGFR is an attractive target in HNSCC

because it is overexpressed in 80–90% of HNSCC and leads to tumor cell proliferation, invasion, angiogenesis, tumor survival, and consequently, poor prognosis (19).

Anti-EGFR mAb can mediate antigen-specific immune responses to targeted tumors through two major mechanisms, direct killing via lytic immune cell (NK cell or monocytes) and complement fixation, or opsonization of tumor for phagocytosis and subsequent antigen processing. The latter would induce TA-specific cytotoxic T lymphocytes (CTL) to recognize and lyse tumor cells. Specific T cell activation has been demonstrated in HNSCC patients treated with cetuximab (20, 21), alone or in combination with cisplatin chemotherapy. In addition to extensive clinical and correlative immune response data using cetuximab, MEHD7945A**,** an anti-HER3/EGFR human mAb targeting human epidermal growth factor receptor 3 (HER3) and EGFR is currently being tested in a phase I/II clinical trials for HNSCC (NCT01577173, NCT01911598). Enhancing the secondary immune response to TA-targeted mAb by combination with other immune-targeted therapies is a particularly appealing approach for HNSCC, given that cetuximab is a standard, FDA approved agent in locally advanced or recurrent/metastatic disease.

Immune Checkpoints and Co-Stimulatory Receptors in HNSCC

T cell activation occurs through a combination of T cell receptor engagement and co-stimulatory molecules. The duration and extent of immune responses, for example to infections, is regulated by “immune checkpoints” or inhibitory pathways that prevent excessive inflammatory responses as well as development of autoimmunity. Immune checkpoints have also been shown to play an important role in the tumor microenvironment (TME) and can be manipulated as a mechanism of tumor immune evasion (22). The immune checkpoint pathways

are mediated by ligand and receptor interactions. Examples include CTLA-4 and its ligands CD80 and CD86 and programmed death-1 (PD-1) and its ligands PD-L1 and PD-L2. Blocking anti-CTLA-4 mAb therapy results in rejection of syngeneic murine cancers (23). A mAb against CTLA-4, ipilumumab, was the first drug in this class to demonstrate clinical benefit and was approved by the FDA for patients with metastatic melanoma in 2011 (24). Tremelimumab is also available for CLTA-4 targeting. More recently, anti-PD-1 or PD-L1 Abs have demonstrated clinical efficacy, alone (25-27) or in combination with ipilumumab (28).

Tumor immune evasion can occur by high tumor expression of PD-L1 and/or tumor immune infiltration by PD-1+ T lymphocytes.(29) Preliminary analyses indicate that PD-L1 is expressed in 50-60% of HNSCC, and that tumor infiltration by PD-1+ T_{reg} cells may be more common for human papillomavirus (HPV)-positive than HPV-negative HNSCC (REF). Strome and colleagues reported membrane and/or intracytoplasmic PD-L1 expression in 66% (16 of 24) of HNSCC (REF). Importantly, these studies also demonstrated that expression of PD-L1 can be induced by IFN-γ, suggesting that the TME dictates tumor expression of PD-L1 and that measurement of PD-L1 at a single time point or location may not accurately reflect the natural history of its expression.(30) Badoual and colleagues reported tumor infiltration by PD-1+ CD8+ and PD-1+CD4+ lymphocytes was more common among HPV-positive than HPV-negative HNSCC. In 33 (55%) of 64 HNSCC, high levels of PD-L1 expression were observed, but there was no association between PD-L1 expression and tumor HPV status (31). A higher expression of immune-checkpoint receptors (CTLA-4 and PD-1) in intratumoral T_{reg} cells than on matched peripheral blood samples has been observed from patients with HNSCC (32). These data strongly support a role for PD-1 inhibition in the therapy of HNSCC. Seiwert and colleagues recently reported promising preliminary efficacy associated with the anti-PD-1 mAb

pembrolizumab in a large (>130 patient) phase Ib cohort with refractory, recurrent/metastatic HNSCC, as measured by response rate and overall survival (OS) (Seiwert, ASCO 2015). In a recently completed, randomized phase III trial of nivolumab versus single agent chemotherapy, an overall survival benefit was observed (Gillison et al, AACR Proceedings 2016), indicating the possibility of a new FDA approval for this type of therapy in the near future. Anti-PD-1 mAb are also being tested in various novel combinations in the phase I setting, such as nivolumab plus agonistic anti-CD137 mAb (urelumab, NCT02253992), nivolumab plus anti-LAG-3 mAb (NCT01968109), as well as cetuximab plus urelumab (NCT02110082).

An additional class of immunomodulatory receptors includes the checkpoint receptors such as LAG-3 or the killer-cell immunoglobulin-like receptors (KIRs). These receptors interact with MHC I molecules to regulate immune response, and in general suppress cytotoxicity, particularly "turning off" NK cells when HLA is present on tumor cells. Anti-KIR mAb thus might remove the major inhibitory signal on NK cells. Ongoing pharmaceutical-sponsored trials include the investigation of an anti-KIR mAb in combination with the anti-CTLA-4 mAb ipilumumab (NCT01750580) or anti-PD-1 mAb nivolumab (NCT01714739).

In addition to blocking negative regulatory receptors on effector lymphocytes, another strategy has emerged to enhance and trigger positive, co-stimulatory signals using agonistic mAbs and small molecules. So far, the investigation of TNFR-targeting mAb in clinical trials for HNSCC is in phase I trials. Because of the important costimulatory pathways for immune cell activation, substances like CP-870,893 (Pfizer), an IgG2 CD40 agonist, OX40 mAb (AZ/Medimmune), an IgG2 OX40 agonist or urelumab (Bristol-Myers Squibb), an IgG4 CD137 agonist, have been investigated with cetuximab or with nivolumab in clinical trials (33), which are currently enrolling HNSCC patients. These studies are hampered by a limited ability to

define what constitutes an "agonistic" effect in preclinical models. Toll-like receptor (TLR) agonists induce the maturation and cross-priming of dendritic cells (DC) and have been shown to induce NK cell dependent lysis of tumor cells in combination with TA-targeted mAb such as anti-EGFR cetuximab (34). The TLR8 agonist, motolimod, is under investigation in combination with cetuximab-based therapy in HNSCC (NCT02124850 and NCT01334177).

Role of Immunity in Response to Chemoradiotherapy

Cytotoxic cancer therapies alone are aimed at tumor eradication through direct killing of cancer cells. However, full and sustained clinical remission is elusive for many patients receiving standard-of-care treatments. Striking clinical observations in recent years indicated that patients harboring certain malignancies achieved higher clinical benefit with immunotherapy if previously treated with certain anticancer therapies. These observations are now supported by accumulating evidence demonstrating that conventional and emerging anticancer therapies modulate the tumor to induce a more immunostimulatory milieu (35, 36).

Immunogenic Cell Death and Immunogenic Modulation by Chemoradiotherapy

Cancer therapeutic regimens trigger cancer cell death while stimulating endogenous immune responses against the tumor, termed 'immunogenic cell death'. (36, 37) The cardinal signs of immunogenic cell death are (a) calreticulin exposure on the surface of dying cells, (b) the release of HMGB1, (c) the release of ATP, which acts on dendritic cells (DCs) to facilitate the presentation of TAs to the immune system. Tumor cells that survive therapy have been shown to alter their biology to render them more sensitive to immune mediated killing, termed 'immunogenic modulation'. (35, 38) Immunogenic modulation encompasses a spectrum of

molecular alterations in the biology of the cancer cell that independently or collectively make the tumor more amenable to cytotoxic T lymphocyte (CTL) –mediated destruction. These include: (a) downregulation of antiapoptotic/survival genes, (b) modulation of antigen processing machinery (APM) components, and (c) calreticulin translocation to the cell surface of the tumor. One can envision that these immunogenic consequences of anticancer therapy, ranging from immunogenic cell death to immunogenic modulation, can be harnessed to achieve synergy with immunotherapy regimens, therefore maximizing the clinical benefit for patients with HNSCC receiving combination therapy.

If immunotherapies are to be used early in the disease process, they would most likely need to be used in combination with chemotherapeutic agents. While counterintuitive, it has recently been shown that immunotherapy may not only be compatible with chemotherapy, but also may actually be synergistic (39). Various chemotherapy agents have been shown to induce immunogenic modulation in tumors of diverse origin by upregulating immune-relevant proteins on the surface of cancer cells, including TAs, calreticulin, adhesion molecules such as ICAM-1, and major histocompatibility complex (MHC) Class I proteins. These phenotypic changes translated into increased murine and/or human tumor sensitivity to CTL mediated lysis *in vitro* after exposure to sublethal doses of chemotherapy with cisplatin (40), taxanes (41), or cisplatin plus vinorelbine (42). These preclinical findings and others have translated into various hypothesis-generating clinical trials. Several things are important in considering the use of chemotherapy with immunotherapy: (a) the combined use of immunotherapy and chemotherapy early in the disease process should not be confused with the use of immunotherapy following multiple regimens of different chemotherapeutic agents in the advanced disease setting, where the immune system would most likely be impaired; (b) not all chemotherapeutic agents will be

synergistic with immunotherapy; and (c) dose and scheduling of immunotherapy when used with chemotherapy may be extremely important, and following immune function may guide trial optimization.

Checkpoint Inhibitors and Radiation Therapy

RT can induce a continuum of immunogenic alterations in dying and/or surviving tumor cells. Lethal irradiation has been reported to induce immunogenic cell death. Although immune responses in cancer patients receiving radiation therapy (RT) alone are often weak and rarely translate into protective immunity, the immunogenic effects of RT can be exploited to promote synergistic clinical benefit for patients receiving combination regimens with immunotherapy (43, 44). It has been demonstrated that the use of relatively low doses of external beam radiation, insufficient to kill tumors, induces immunogenic modulation, altering those tumor cells to render them more susceptible to T-cell–mediated lysis. Cell surface expression of MHC class I molecules and calreticulin on tumors was increased in a radiation dose-dependent manner as a consequence of several factors; initially, enhanced degradation of existing proteins occurred which resulted in an increased intracellular peptide pool, ultimately rendering the cells more susceptible to T cell-mediated killing (35, 45). RT combined with a cancer immunotherapy elicits greater TA-specific $CD8^+$ T-cell responses and/or reduction in tumor burden than either modality alone. Importantly, in the case of vaccines, the induction of $CD8^+$ T-cells specific for multiple TAs not encoded by the vaccine has been observed after combination therapy (epitope spreading). This polyclonal T-cell response functionally mediated the regression of TA-negative metastases at distal subcutaneous or pulmonary sites (46). These findings have translated into promising clinical benefits for HNSCC patients receiving RT plus immunotherapy. Of importance, it has been shown specifically with an *in-vitro* model of HNSCC that treatment with

RT and cisplatin chemotherapy can lead to synergistic sensitivity to antigen-specific T cell killing (47).

In addition to direct cytotoxic effects, RT may induce an immune effect important to tumor cell death (48). Preclinical data support synergy between checkpoint inhibitors and RT. Mouse models of poorly immunogenic tumors have demonstrated that concomitant administration of anti-CTLA-4 antibodies and RT results in antitumor T cell responses both in the radiation field as well as outside of it (an abscopal effect) (48, 49). PD-1 blockade after completion of RT also has been shown to induce rejection of persistent tumors in mouse models (50). Combination PD-1 blockade and CD137 stimulation increased response to RT in a mouse model of triple negative breast cancer (51), and PD-L1 blockade concomitant with RT improved survival in comparison to either therapy alone in mouse models of glioma (52). In human subjects, case-reports support the existence of a clinically significant abscopal effect for patients with melanoma who have received ipilumumab prior to RT (53, 54). These data support a hypothesis that checkpoint inhibitors administered prior to or concomitant with RT can induce clinically significant anti-tumor immune responses by "vaccination" to TAs exposed during radiation-induced cell death (55). Such a phenomenon may be particularly relevant to viral-induced tumors, such as HPV-positive HNSCC containing unique non-self antigens, and to highly genetically unstable tumors such as HPV-negative HNSCC demonstrating a high proportion of nonsynonymous mutations (56).

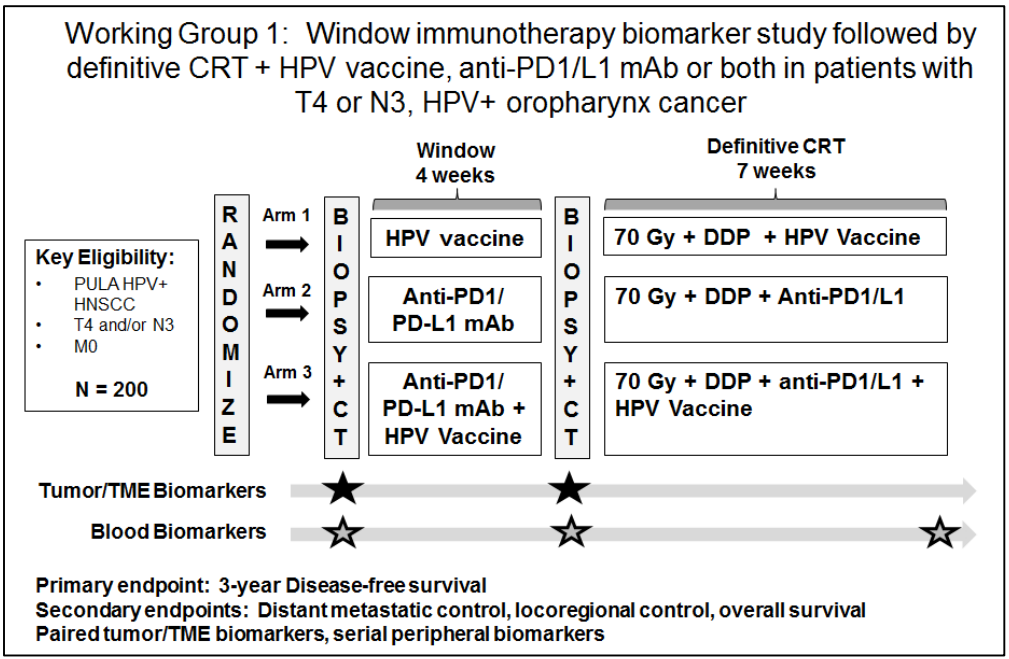
Integration of Immunotherapy into Clinically Defined Patient Groups

The NCI funded a Clinical Trials Planning Meeting (CTPM) to facilitate rational design of combinations of immunotherapies for phase II and III randomized trials in HNSCC. The

meeting was organized around 4 break-out groups. Three groups were focused upon specific biologic subsets of HNSCC: HPV-positive previously-untreated, locally advanced (PULA) disease, HPV-negative PULA disease, and recurrent/metastatic HNSCC. A fourth group of scientists focused on correlative tissue and imaging biomarkers. After developing harmonized recommendations for biomarker and imaging correlatives, this fourth group's proposed correlative studies and assays were integrated into the discussions and trial designs emanating from the 3 therapeutic cohort groups.

Previously-Untreated, Locally Advanced HPV-positive HNSCC

Despite histologic convergence upon squamous cell carcinoma, HNSCC is no longer considered a single disease due to etiologic divergence. In addition to the classic risk factors of tobacco and alcohol, HPV type 16 now represents a primary cause of HNSCC in North America and Europe (57, 58). HPV status and pack-years of tobacco exposure are the major determinants of survival in HNSCC, followed by nodal stage.(59) Based upon these three prognostic factors, patients with HNSCC can be classified into three risk groups having low, intermediate, or high risk of death. This clinical risk classification has framed national clinical trial priorities in PULA HNSCC. Specifically, de-intensification strategies are being tested in patients with low-risk HPV-positive HNSCC whereas intensification strategies represent the major unmet need for high-risk HPV-negative and intermediate-risk HPV-positive disease.(60-62)



For patients with HPV-positive PULA HNSCC, Working group #1 identified

two priorities: more targeted HPV-specific therapy taking advantage of unique non-self, viral TAs present within HPV-positive tumors, and to determine the sequencing and optimal chemoradiotherapy (CRT) regimens that do not inhibit immunotherapeutic efficacy. Currently, immunotherapeutic trials open or in development include eliminating systemic cytotoxic chemotherapy by combining IMRT with cetuximab and anti-CTLA-4 mAb (ipilumumab, NCT01935921), in which the overlap of ipilumumab exposure begins at week 5 of cetuximab-RT. Additionally, "intermediate risk" HPV-positive and "high-risk" HPV-negative patients will be treated with concurrent, weekly cisplatin CRT with anti-PD-1 mAb, a natural "add-on" strategy that is in development (RTOG Foundation trial #3504) and will open to enrollment in the near future.

First-generation "de-intensification" clinical trials for HPV-positive PULA disease enrolled both good and intermediate risk patients, with the goal to reduce chemotherapy and/or RT doses (fields) (ECOG 1308, RTOG 1016). As clinical risk stratification evolves, second generation de-intensification trials are selecting only good risk patients (HN002). New trials are needed for intermediate risk, worse prognosis HPV-positive disease. The proposed trial aims to harnesses novel systemic immunotherapy and utilize the unique viral antigens (the oncogenes E6 and E7) expressed in HPV-positive HNSCC, to improve disease-free survival (DFS) as well as make an impact on the burden of uncommon, though lethal distant metastatic disease for intermediate risk HPV-positive PULA disease (T3/4, N2c/N3, >10 pk-yr smokers HPV-positive patients, ref. (62)). The proposed concept (see schema, Figure XXX) would compare anti-PD-1 plus cisplatin CRT to the combination of anti-PD-1/CRT plus HPV-specific E6/E7 vaccination. Several vaccines are available and tested in phase I trials for cervical and other HPV-positive cancers, and include peptide plus adjuvant, DNA- or Listerolysin O based vectors. Collaboration

between a cooperative group and 1-2 pharmaceutical company sponsors is likely to be necessary. A neoadjuvant (pre-CRT) phase of 1-2 doses of vaccine ± anti-PD-1 mAb was strongly considered, since the timing and sequence of HPV-specific T cell expansion vis-à-vis cytotoxic CRT, which may inhibit lymphocyte expansion, is undetermined. This approach, though more cumbersome, would also permit correlation of dynamic tumor and peripheral immune biomarkers with clinical outcomes.

Previously-Untreated, Locally Advanced HPV-negative HNSCC

Eighty percent of HNSCC diagnoses worldwide remain secondary to environmental carcinogens, including tobacco and alcohol. Recent improvements in 5-year OS for the HNSCC population as a whole are largely attributable to the epidemic of good risk, HPV-positive HNSCC, which involves younger and lower-risk populations (58). OS for patients with high risk, PULA HPV-negative HNSCC has improved only marginally in the last two decades due to the incorporation of concurrent cisplatin in curative-intent paradigms. The current standard for the nonsurgical management of PULA HPV-negative HNSCC is concurrent cisplatin CRT, which improved OS, DFS, and locoregional control (LRC) compared with RT alone in the sentinel Intergroup 0126 trial – a trial that was populated prior to the HPV epidemic (63, 64). Standards for the adjuvant management of PULA HPV-negative HNSCC are determined by pathologic risk. Specifically, for patients who demonstrated one or more high risk pathologic features, including a positive surgical margin or extracapsular nodal extension, concurrent cisplatin-RT appeared to provide a clinical benefit compared with RT alone in the landmark Phase III EORTC 22931 and RTOG 9501 trials (65, 66). Despite this advance, patients with high risk HPV-negative disease have a 3-year DFS of only 30-50% (65-67). Although LRC and OS are

improved with concurrent cisplatin-RT, a meta-analysis indicated disappointing local and distant failure rates of 50% and 15% respectively, and an absolute survival benefit of only 6.5% compared to RT alone (68). Poor outcomes persist despite intensification with altered fractionation (69), multi-drug induction (70), or EGFR-targeted mAb (71).

For HPV-negative patients, new intensification approaches represent a major unmet clinical need. The PULA HPV-negative working group initially discussed two clinical trial paradigms for patients with high risk disease: the integration of immunotherapy into definitive cisplatin CRT and the integration of immunotherapy into trimodality therapy for high-risk patients **(Figure X).** Ultimately, the recommended focus on the trimodality model capitalized on three opportunities: 1) *Accessibility of tumor and TME for serial assessment.* The natural anatomy of HNSCC presents specific accessibility of the primary tumor and TME for serial biopsy. In the proposed trial, the incorporation of primary surgery permits a "window of opportunity" for exposure to a specific immunotherapy between diagnostic biopsy and planned surgery, facilitating pharmacodynamic evaluation of tumor and TME responses in paired specimens. 2) *The integration of immunotherapy with RT*. Ionizing RT induces adaptive immune responses via three broad mechanisms that could be synergistic with immunotherapy, including release of TAs for processing and presentation, upregulation of stimulatory chemokines within the TME, and increased tumoral expression of TA and MHC (72). 3) *The integration of immunotherapy with cisplatin*. Although cytotoxic chemotherapy is conventionally viewed as immunosuppressive, cisplatin also demonstrates stimulatory effects including upregulation of MHC, recruitment and proliferation of effector cells, enhanced cytolytic activity of effector cells, and downregulation of MDSCs and T_{reg} cells (40).

The immune checkpoint inhibitors, antagonizing the CTLA-4 or PD-1 pathways, were considered of greatest priority for development in the HPV-negative PULA population. First, environmentally induced HNSCC demonstrates a high mutational burden (73, 74). Mutational load, as well as the presence of highly immunogenic neoantigens, has been correlated with response to immune checkpoint inhibitors in other solid tumors (75, 76). Second, RT dynamically upregulates PD-L1 on both tumor and myeloid-derived suppressor cells (MDSC), reducing the adaptive response and theoretically facilitating future relapse. In two syngeneic preclinical models, concurrent PD-L1 blockade and RT were synergistic in controlling tumor growth, and generated prolonged protective T cell immunity as demonstrated by subsequent abscopal effect (77).

The central hypothesis test of the proposed randomized phase II trial considers whether adding immunotherapy to adjuvant cisplatin CRT increases DFS in patients with high risk, resected PULA HPV-negative HNSCC. In this trial design, the window of monotherapeutic exposure prior to definitive surgery creates a unique opportunity to study placebo-controlled, pre- and post-treatment tumor and blood specimens to isolate immune mechanisms, and to correlate baseline and pharmacodynamic biomarkers with 2-year DFS. We propose to evaluate baseline and changes in immune-inflammatory biomarkers in both tumor and TME, and to correlate these biomarkers with 2-year DFS. Markers will include: IHC or immunofluorescence for CD3, CD8, CD45RO, CD4/FOXP3, PD-L1, Ki-67; flow cytometry for TIL and MDSC subsets; T cell activation panel and memory subsets; changes in TCR clonality; and whole exome sequencing for peptide-encoding tumor neoantigens.

Recurrent/Metastatic HNSCC

Recurrent/Metastatic HNSCC

Patients with recurrent or metastatic (R/M) HNSCC have a particularly poor prognosis, with a median OS of approximately 10 months. Akin to what is observed in the setting of primary disease, patients with HPV-positive R/M tumors enjoy improved outcomes, with a 2-year OS of approximately 55% vs. 28% for their HPV-negative counterparts (78). For nearly three decades, the cornerstone of first line palliative systemic therapy has been cisplatin (79), frequently combined with 5-fluorouracil or a taxane due to increased response rates (albeit with no conclusive evidence of superior OS compared to cisplatin monotherapy (80). In 2006, cetuximab became the first FDA-approved TA-targeted mAb in HNSCC. When combined with platinum and fluorouracil, cetuximab increased both progression-free and OS in R/M disease (the so-called "EXTREME" regimen) (81). Cetuximab is also indicated as monotherapy in patients with R/M, platinum-refractory HNSCC (82). Unfortunately, these treatments are generally not curative and no established therapies exist for the cetuximab-refractory population -- an area of profound unmet need.

In response to this therapeutic void, there has been a proliferation of clinical trials testing immunotherapeutic mAbs in patients with R/M disease (Table 2). For example, a phase Ib clinical trial investigated the anti-PD-1 mAb pembrolizumab (MK-3475, Merck) and yielded response rates (PR/CR) of approximately 20%. Importantly, and contrary to existing data with standard chemotherapeutics, response rates were similar in both HPV-positive and HPV-negative cohorts. These early efficacy data were substantiated in the recent phase III trial, CheckMate 141, that compared single agent nivolumab with investigator's choice single-agent therapy. This trial closed early when an overall survival benefit was shown (NCT02105636, n=360 patients) and results will be reported in the near future (Gillison, et al *AACR Proceedings,* 2016).

Importantly, these promising results are not limited to anti-PD-1, as anti-PD-L1 has also shown comparable efficacy in a phase I trial (Fury, ESMO 2014). The success of this initial stud prompted the design of a phase III trial evaluating MEDI4736 alone or in combination with the anti-CTLA-4 mAb, tremelimumab, as compared to standard of care, second-line agents (NCT02369874, n=720 patients). Stratification by PD-L1 expression status is planned.

Based on positive outcomes associated with the use of checkpoint inhibition following first line failure, a recently initiated phase III trial will now move PD-1 targeting forward into the first line R/M setting. Specifically, this trial will compare the anti-PD-1 mAb pembrolizumab alone or in combination with platinum/5-FU, vs the EXTREME regimen (NCT02358031, n=600 patients). Despite the excitement generated by evaluation of checkpoint inhibition as first line therapy in the R/M setting, the uncomfortable reality is that a large number of these treated patients will likely continue to die of their disease. Indeed, it was this reality which prompted the formation of the R/M disease working group, charged with the development of clinical trials to meet the needs of patients with who are refractory to existing therapy.

While many trials were proposed for development, the clinical trial eventually adopted by the recurrent metastatic working group was the brainchild of the late Dr. Holbrook Kohrt. This trial design was premised on two fundamental considerations: 1.) That defined co-signaling pathways can be induced on Fc γ receptor (FcγR) bearing immune effector cells through FcγR engagement by the aggregated Fc fragments of immobilized antibodies (83), and 2.) That blockade of select immunologic checkpoints e.g. PD-1/PD-L1 (29, 84), in combination with stimulation of defined co signaling molecules e.g. CD137 (4-1BB), have synergistic anti-tumor activity. Data laboratory first demonstrated that engagement of CD16 on the surface of NK cells induced high levels of CD137 expression (85). Subsequent studies demonstrated that CD137

could be induced on NK cells by the Fc fragments of antibodies bound to the tumor cell surface and that engagement of CD137 on these NK cells (86, 87) or by (DC, ref. (21) by agonistic antibodies could potentiate their antitumor activity

Based on these data, the working group proposed a prospective randomized clinical trial design with three-arms. In this schema, all groups would receive cetuximab "induction" on day 1, followed by additional doses on days 8 and 15. Importantly, the purpose of cetuximab administration in this setting was not simply to mediate killing of EGFR expressing tumors, but was also to induce CD137 expression on the surface of infiltrating NK cells. On study day 2, group 1 would receive an agonistic mAb against CD137, group 2 would receive anti PD-1 or anti PD-L1 and group 3 would receive a combination of anti-CD137/PD-1 or PD-L1. Each cycle was designed to last 21 days and response to treatment would be assessed at the end of 12 cycles. The two primary endpoints were safety and 6-month progression free survival. Successful completion of the study, would enable determination of :1. The ability of Cetuximab to induce CD137 on circulating NK cells in patients with SCCHN, and 2. The ability of anti-CD137/PD-1 or PD-L1 to improve survival in comparison to either agent alone. A limitation of the design might be the inability to include a cetuximab only cohort, based on feasibility considerations, as well the lack of toxicity or efficacy data for combinations with urelumab (agonistic anti-CD137).

Immunotherapy Trial Biomarkers and Unique HNSCC Patient Specimen Considerations

The CTPM leadership observed that similarities between the different clinical settings presented an opportunity to develop biologic sample collection and biomarkers for all of them simultaneously. From tumor samples, immunohistochemical (IHC)/immunofluorescence (IF) detection of immune markers provide a measure of baseline immune cell infiltration, phenotype, localization and "inflammation," sometimes referred to as an "immuno-score" since this has

been shown to have prognostic and predictive capacity for immunotherapy in other diseases including colorectal cancer (11, 88-90). These markers include CD3, CD8, CD45RO, CD4/FOXP3, and perhaps PDL-1 (on tumors vs. myeloid cells). The Biomarkers working group recommended combining these basic stains for infiltrate with the specific targets in proposed trial (PD-1, CTLA-4, OX-40, TIM-3, LAG-3, CD40, etc.). Multiplexed IF makes testing multiple parameters more feasible (Stack, E. et al., JITC 2016, in press).

From fresh frozen tissue the following genomic or signaling assays were recommended:

1. RNA Seq (to include inhibitory/costimulation/exhaustion molecules targeted)
2. TCR diversity (as a measure of TCR skewing and clonality of the infiltrated T cell response)
3. Any trial-specific pathways (e.g. phospho-SMAD in the setting of a TGFβ inhibitor study proposed at the CTPM)

The above assessments would be performed on all biopsies taken, including the "window" (neoadjuvant) trials taking advantage of paired pre- and post-treatment tumor specimens in the HPV-negative and the HPV-positive PULA trials. A new biopsy would be needed for the recurrent/metastatic study (not on primary tumor banked earlier). Some technologies can utilize FFPE tissue, which is more easily obtained (e.g. Nanostring).

From peripheral blood samples (i.e. ficoll-gradient separated peripheral blood mononuclear cells, PBMC) flow cytometry should accomplish the following: relative quantification of circulating suppressive MDSC and T_{reg} cells; T cell activation panels (e.g. ICOS in CTLA-4 trials; CD69 for general activation); lymphocyte memory subsets (CD45RO, CCR7 central trafficking); NK cells; PD-1, CTLA-4 and/or any trial design-related co-stimulatory/co-inhibitory molecules. Also specific intracellular molecules (TGFβ: phospho-STAT) would be

measured. It was recognized at the CTPM that the cooperative groups within the NCTN that design HNSCC studies (ECOG/ACRIN and NRG Oncology) are not currently collecting and processing fresh PBMC for functional and phenotypic studies, and that processes, infrastructure, and funding support would need to be developed for real-time shipping, processing and storage, to take advantage of the great opportunities in different immunotherapeutic strategies being employed, in order to maximize predictive, prognostic as well as mechanism of action biomarker analyses.

Antigen-specific cytokine flow cytometry is possible using MHC: peptide multimers or non-HLA-restricted overlapping peptide pools: For HPV-positive tumors: E6 and E7 peptide pools (including testing for surface CD4 and CD8, and polyfunctional intracellular cytokines and effector molecules (IFNγ, TNFα, IL-2, granzymes); For non-HPV tumors: shared tumor antigen peptide pools can be pursued (e.g. p53, survivin) with surface CD4 and CD8, polyfunctional intracellular cytokines and effector molecules. Control antigen peptide pools can be utilized to document and monitor memory recall responses. Additional cellular blood assays were also considered, including genomic SNP analysis for possible predictive genomic biomarkers from PBMC germline DNA. Similarly, transcriptional signatures have been identified from peripheral blood mRNA that may be unbiased and hypothesis generating (REF).

From serum, recommended assays include multiplex cytokine analysis (for trial of comparison of agents only); inflammatory molecules (especially for cytokines) as potential mediators of toxicity (baseline IL-17 and CTLA-4 toxicity, CRP). Currently, 30-60 different analytes are tested in each small sample.

Imaging biomarkers are an important correlate in novel prospective trials but this field was felt to be underdeveloped as a whole in immunotherapy, given several factors. These include

occasional “delayed” or atypical/mixed responses, which are reflected in immune response (ir) RECIST for R/M disease (91). For short term, anti-PD-1 “window” neoadjuvant studies, PET-FDG/CT pre and after 4 week induction may be a predictor of early response via SUV measurements, since anatomic shrinkage may not be seen in the short term. However, infiltrating immune cells may be metabolically active, confounding interpretation of increased FDG avidity in the TME. The imaging biomarker experts noted that there is no current technology for assessment of immune activity and infiltration via imaging, which represents a major unmet clinical need.

Potential pitfalls and additional considerations exist in these immune biomarker assessments. For instance, there are unanswered technical questions regarding the feasibility of tumor analysis. For blood, given some limitations in volumes and yields, prioritization is needed for the different assays. It is assumed that absolute lymphocyte counts (ALC) are serially obtained pre/during/post in clinical labs, which is a candidate biomarker for some checkpoint blockade therapies (particularly CTLA-4). Lastly, stool samples and oral swabs could be considered for future microbiome studies.

Conclusion

Cancer immunology is a rapidly evolving field and only recently have we begun to understand the complex interaction between cancer and the host immune system. Tumor cells demonstrate several methods to exploit the immune system to help promote angiogenesis, derive pro-survival and proliferative signals, and induce metastasis and tumor progression. At the same time, cancers are able to cloak themselves from the immune system by self-modification and by immunosuppression of the host. Recent results from clinical trials show evidence for effective anticancer immunotherapies. Because of the manifold tumor evasion strategies and hence

different response rates for treatments, combinational therapies will be helpful to develop for cancer treatment.

The HNSCC Immunotherapy CTPM was designed to harness these insights and to generate better understanding of several promising immunotherapeutic agents that are currently in clinical use as well as others in development. Four clinical trial concepts emerged during this important and productive meeting. Great enthusiasm and collaborative effort will lead to the "hand-off" of these concepts to the head and neck committees of ECOG/ACRIN and NRG Oncology for submission and review by NCI CTEP and the Head and Neck Steering committee processes. Success will likely depend on development of industry collaborations and support. The integration of industry into the open, educational portion of the meeting was intended to facilitate and enhance these interactions and relationships. Given the unique features of HNSCC, including tumor accessibility for serial biopsies and the balance between carcinogen and virally induced cancer subsets, these trials should provide important information for the field of immunotherapy as a whole.

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Table 1: Potential Therapeutic Targets in HNSCC

Drug (Company)	Target	IgG class	HNSCC development stage	Proposed Mechanism of Action
Tumor Antigen Targeted Monoclonal Antibodies				
Cetuximab (Bristol-Myers Squibb, Eli Lilly)	EGFR antagonist	IgG1	Phase III/IV	Tumor growth inhibition, cellular immunity
Panitumumab (Amgen)	EGFR antagonist	IgG2	Phase II/III	Tumor growth inhibition
AV-203 (Aveo)	HER3 antagonist	IgG1	Phase I (monotherapy; cetuximab combination)	Tumor growth inhibition
Cixutumumab (Eli Lilly)	IGFR antagonist	IgG1	Phase 0-II (neoadjuvant monotherapy; cetuximab combination)	Tumor growth inhibition
Cytokine Targeted Monoclonal Antibodies				
Bevacizumab (Genentech)	VEGF neutralizing Ab	IgG1	Phase III (platinum chemotherapy +/−)	Inhibition of Angiogenesis, impairment of VEGF-induced immunosuppression
Ficlatuzumab (Aveo)	HGF neutralizing Ab	IgG1	Phase I (cetuximab combination; cisplatin-RT combination)	Tumor growth inhibition
TNF Receptor Targeted Monoclonal Antibodies				
MEDI0562 (Astra-Zeneca/Medimmune)	OX40 agonist	IgG2	Phase Ib	Stimulation of cellular immunity
Urelumab (Bristol-Myers Squibb)	CD137 agonist	IgG4	Phase I	Stimulation of cellular immunity
PF-05082566 (Pfizer)	CD137 agonist	IgG2	Phase I	Stimulation of cellular immunity
Immune Checkpoint Targeted Monoclonal Antibodies				
Ipilimumab (Bristol-Myers Squibb)	CTLA4	IgG1	Phase I (cetuximab-RT combination)	Blockade/depletion of T_{reg}, enhancement of CTL
Tremelimumab (AZ/Medimmune)	CTLA4	IgG2	Phase I	Blockade/depletion of T_{reg}, enhancement of CTL activity
MEDI4736 (AZ/Medimmune)	PD-L1	IgG1	Phase II	Enhancement of CTL activity
Pembrolizumab (MK-3475, Merck)	PD-1	IgG4	Phase I	Enhancement of CTL activity
Nivolumab (Bristol-Myers Squibb)	PD-1	IgG4	Phase III	Enhancement of CTL activity

Table 2.

Drug	Mechanism
Enhancing ADCC	
IL-12 (NCI)	Cytokine agonist of NK cell activation
IL-15 (NCI)	Cytokine agonist of NK cell activation
VTX-2337	TLR 8 agonist; enhanced DC activation and IL-12 secretion
Lirilumab (BMS)	Anti-Killer Inhibitor Receptor (KIR) mAb
1-7F9 (Innate)	Anti-KIR mAb
Targeting Immunosuppressive Cytokines	
Siltuximab	Anti-IL-6 mAb
CAT-192	Anti-TGF-β mAb
T cell Co-stimulatory Agonists	
CP-870,893 (Pfizer)	CD40 agonist mAb
OX40 mAb (AgonOx, Providence Health)	OX40 agonist mAb
Urelumab (BMS)	CD137 agonist mAb
PF-05082566 (Pfizer)	CD137 agonist mAb
IMP321 (Immutep)	Recombinant soluble dimeric LAG3
T cell Immune Checkpoint Inhibitors	
Ipilimumab (BMS)	Anti-CTLA4 mAb
Tremelimumab (AZ/Medimmune)	Anti-CTLA4 mAb
Nivolumab (BMS)	Anti-PD1 mAb
Pembrolizumab (Merck)	Anti-PD1 mAb
Durvalumab (MEDI-4736 (AZ/Medimmune)	Anti-PDL1 mAb
MPDL3280A (Genentech)	Anti-PDL1 mAb
MSB0010718C (EMD Serono)	Anti-PDL1 mAb
AUNP12 (peptide) (Pierre Fabre/Aurigene)	Anti-PDL1 peptide
BMS-986016 (BMS)	Anti-LAG3 mAb
INCB024360 (Incyte)	Orally available inhibitor of indoleamine 2,3-dioxygenase (IDO1)

Table 3

Tumor	PBMC	Serum	Imaging	Future
Infiltrate: CD3, CD8, CD45RO, CD4/FOXP3, PDL-1; frequency, location IHC, IF	Suppressors : T_{reg}, MDSC	Multiplexed circulating cytokines, chemokines, growth factors	PET-FDG/CT pre and after 4 week induction	Stool/oral swabs for microbiome
Major checkpoints/costim. (PD-1, CTLA-4, TIM-3, LAG-3, OX-40, CD40)	Effector activation (ICOS, CD69), effector/memory, cytotoxicity	Circulating antibodies		Imaging immune response
NK cells	NK cells			
Ki67	Trial specific pathways			
RNAseq	HPV-positive: virus peptide pools			
TCR diversity	HPV-negative: shared tumor antigen peptide pools			
Trial specific pathways	ALC as SOC			