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Immunotherapy of Head and Neck Cancer: Emerging Clinical Trials From a National Cancer Institute Head and Neck Cancer Steering Committee Planning Meeting

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Abstract

Recent advances have permitted successful therapeutic targeting of the immune system in 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 to address the issue of how to further investigate the use of immunotherapy in patients with HNSCC. The goals of the meeting were to consider phase 2 or 3 trial designs primarily in 3 different patient populations: those with

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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 Clinical Trials Planning Meeting from his extensive experience in the field.

previously untreated, human papillomavirus-initiated oropharyngeal cancers; those with previously untreated, human papillomavirus-negative HNSCC; and those with 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 immune components and 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 National Cancer Institute-funded National Clinical Trials Network. This article summarizes the proceedings of this Clinical Trials Planning Meeting, the purpose of which was to facilitate the rigorous development and design of randomized phase 2 and 3 immunotherapeutic trials in patients with HNSCC. Although reviews usually are published immediately after the meeting is held, this report is unique because there are now tangible clinical trial designs that have been funded and put into practice and the studies are being activated to accrual.

Keywords

checkpoint inhibitors; clinical trials; head and neck cancer; human papillomavirus; immunotherapy

INTRODUCTION

The objective of cancer immunotherapy is to reactivate the immune system to target malignant cells, and it has been demonstrating recent clinical efficacy in many cancer types.[1] Derangements in the immune system or alterations in the transformed cells may allow immune escape, which then enables the cancer to manifest. Immunomodulatory therapies that overcome immune suppressive signals in patients with Head and Neck Squamous Cell Carcinoma (HNSCC) have therapeutic promise.[2] The recent clinical efficacy of US Food and Drug Administration (FDA)-approved monoclonal antibodies (MoAbs) targeting immune checkpoint receptors, including anticytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) and anti-programmed cell death protein 1 (anti-PD-1), provided further potential for patient benefit as positive clinical data emerge. This led to the approval by the National Cancer Institute (NCI) of a proposal to convene a group of experts focused on developing immunotherapies rationally and integrating this novel modality into conventional radiotherapy (RT), chemotherapy, and surgical oncologic therapies.

The meeting (which was held November 9–10, 2014 at the NCI Clinical Center in Bethesda, MD) 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 and lung and renal cell carcinoma. The concept was established that to establish effective immunotherapies, understanding the different pathways of tumor immune evasion is necessary. The profound although 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-positive regulatory T cells (T_{reg}), macrophages, and myeloid-derived suppressor cells (MDSCs).

MoAb-Based Immunotherapy for HNSCC

Today, the most widely used form of cancer immunotherapy is MoAb therapy,[3] including tumor antigen (TA)-targeted MoAbs, cytokine-targeted MoAbs, tumor necrosis factor receptor (TNFR) family costimulatory targeted MoAbs, and immune checkpoint-targeted MoAbs (Table 1). To our knowledge, the best studied FDA-approved agent for HNSCC is cetuximab, a mouse-human chimeric immunoglobulin (Ig) G1 antiepidermal growth factor receptor (EGFR) MoAb.[4,5] Anti-EGFR MoAbs can mediate antigen-specific immune responses through direct killing via natural killer (NK) cell or monocytes lysis or tumor phagocytosis and subsequent antigen processing. In addition to extensive clinical and correlative immune response data using cetuximab, MEHD7945A, an antihuman epidermal growth factor receptor 3 (HER3)/EGFR human MoAb targeting HER3 and EGFR, is currently being tested in a phase 1/2 clinical trials for HNSCC (ClinicalTrials.gov identifiers NCT01577173 and NCT01911598). Enhancing the secondary immune response to TA-targeted MoAbs by combination with other immune-targeted therapies is a particularly appealing approach for patients with HNSCC, given that cetuximab is a standard, FDA-approved agent in those with locally advanced or recurrent/metastatic (R/M) disease.

Immune checkpoints and costimulatory receptors in HNSCC—Costimulatory molecules modify T-cell activation, and the duration and extent of immune responses is regulated by coinhibitory pathways (called “immune checkpoints”) that prevent excessive autoimmunity. Immune checkpoints can be manipulated as a mechanism of tumor immune evasion.[6] Examples include CTLA-4 and its ligands CD80 and CD86 and PD-1 and its ligands PD-L1 and PD-L2. Blocking anti-CTLA-4 MoAb therapy results in the rejection of syngeneic murine cancers.[7] An anti-CTLA-4 MoAb, ipilimumab, demonstrated clinical benefit and was approved by the FDA in 2011 for patients with metastatic melanoma.[8] Tremelimumab also targets CTLA-4 and currently is under investigation in patients with HNSCC. More recently, anti-PD-1 or PD-L1 MoAbs have demonstrated clinical efficacy, either alone[9–11] or in combination with ipilimumab,[12] including in patients with HNSCC.[13,14]

Evasion of antitumor immunity by HNSCC occurs by high tumor expression of PD-L1 and/or tumor immune infiltration by PD-1-positive T lymphocytes.[15] PD-L1 is expressed in 50% to 60% of HNSCC,[16] and tumor infiltration by PD-1-positive regulatory T cells (T_{reg}) may be more common for patients with human papillomavirus (HPV)-positive compared with HPV-negative HNSCC.[17] Indeed, membrane and/or intracytoplasmic PD-L1 expression is common in both types of HNSCC.[18] Importantly, these studies also demonstrated that expression of PD-L1 can be induced by interferon gamma (IFN-γ), suggesting that the tumor microenvironment (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.[19] Badoual et al reported tumor infiltration by PD-1-positive, CD8-positive, and PD-1-positive and CD4-positive lymphocytes was more common among patients with HPV-positive than HPV-negative HNSCC. In 33 of 64 cases of HNSCC (52%), high levels of PD-L1 expression were observed, but there was no association between PD-L1 expression and tumor HPV status.[17] A higher expression of immune checkpoint receptors (CTLA-4 and PD-1) in intratumoral T_{reg} cells compared with on matched peripheral blood samples has

been observed among patients with HNSCC.[20] These data strongly support a role for PD-1 inhibition in the treatment of patients with HNSCC. Seiwert et al recently reported promising preliminary efficacy associated with the anti-PD-1 MoAb pembrolizumab in a large (>130 patients) phase 1b cohort with refractory, R/M HNSCC, as measured by response rate and overall survival (OS).[14] In a CheckMate 141 study, a randomized phase 3 trial of nivolumab versus single-agent chemotherapy, an OS benefit was observed,[21] with a 30% improvement in OS and a doubling of patients alive at 1 year, indicating that FDA approval for this agent is imminent. Pembrolizumab was approved by the FDA in August 2016 for the treatment of HNSCC. Anti-PD-1 MoAbs also are being tested in various novel combinations in the phase 1 setting, such as nivolumab plus an agonistic anti-CD137 MoAb (urelumab; ClinicalTrials.gov identifier NCT02253992) and nivolumab plus an anti-lymphocyte-activation protein 3 (LAG-3) MoAb (ClinicalTrials.gov identifier NCT01968109), as well as cetuximab plus urelumab (ClinicalTrials.gov identifier NCT02110082).

Other checkpoint receptors (Table 2) such as LAG-3 or the killer-cell immunoglobulin-like receptors, which interact with major histocompatibility complex (MHC) molecules to regulate immune responses, currently are being investigated in combination with anti-PD-1. Ongoing pharmaceutical-sponsored trials include the investigation of an anti-killer-cell immunoglobulin-like receptor MoAb in combination with the anti-CTLA-4 MoAb ipilimumab (ClinicalTrials.gov identifier NCT01750580) or the anti-PD-1 MoAb nivolumab (ClinicalTrials.gov identifier NCT01714739).

In addition to blocking negative regulatory receptors on effector lymphocytes, another strategy has emerged to enhance and trigger positive, costimulatory signals using agonistic MoAbs and small molecules. To our knowledge to date, the investigation of TNFR-targeting MoAbs in clinical trials for HNSCC currently is in phase 1. Because of the important costimulatory pathways for immune cell activation, substances such as CP-870,893 (Pfizer), an IgG2 CD40 agonist; OX40 MoAb (AstraZeneca/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.[22] Toll-like receptor agonists induce the maturation and cross-priming of dendritic cells (DCs), and have been shown to induce NK cell-dependent lysis of tumor cells in combination with TA-targeted MoAbs such as the anti-EGFR MoAb cetuximab.[23] The toll-like receptor 8 -agonist motolimod currently is under investigation in combination with cetuximab-based therapy in patients with HNSCC (ClinicalTrials.gov NCT02124850 and NCT01334177).

Integration of Immunotherapy into Clinically Defined Patient Groups

The NCI-funded Clinical Trials Planning Meeting facilitated the rational design of combinations of immunotherapies for phase 2 and 3 randomized trials in patients with HNSCC. The meeting was organized around 4 breakout groups. Three groups were focused on specific biologic subsets of HNSCC: HPV-positive, previously untreated, locally advanced (PULA) disease; HPV-negative PULA disease; and R/M 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.

Recurrent/metastatic HNSCC—Patients with 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 rate of approximately 55% versus 28% for their HPV-negative counterparts.[24] For nearly 3 decades, the cornerstone of first-line palliative systemic therapy has been cisplatin,[25] frequently combined with 5-fluorouracil or a taxane due to increased response rates (albeit with no conclusive evidence of superior OS compared with cisplatin monotherapy).[26] In 2006, cetuximab became the first FDA-approved, TA-targeted MoAb for patients with HNSCC. When combined with platinum and 5-fluorouracil, cetuximab increased both progression-free survival and OS in patients with R/M disease (the so-called "EXTREME" regimen).[27] Cetuximab also is indicated as monotherapy in patients with R/M, platinum-refractory HNSCC.[28] Unfortunately, these treatments generally are not curative and to the best of our knowledge no established therapies exist for the cetuximab-refractory population, which is an area of profound unmet need.

In response to this therapeutic void, there has been a proliferation of clinical trials testing immunotherapeutic MoAbs in patients with R/M disease (Table 2). For example, a phase 1b clinical trial investigated the anti-PD-1 MoAb pembrolizumab (MK-3475; Merck) and yielded response rates (partial response/complete response) of approximately 20%. Importantly, and contrary to existing data with standard chemotherapeutics, response rates were found to be similar in both HPV-positive and HPV-negative cohorts. These early efficacy data were substantiated in the recent phase 3 trial, CheckMate 141, which compared single-agent nivolumab with investigator's choice single-agent therapy. This trial closed early when an OS benefit was shown (360 patients) (ClinicalTrials.gov identifier NCT02105636), and the results will be reported in the near future.[21]

Importantly, these promising results are not limited to anti-PD-1, as anti-PD-L1 also has demonstrated comparable efficacy in a phase 1 trial. The success of this initial study prompted the design of a phase 3 trial evaluating MEDI4736 alone or in combination with the anti-CTLA-4 MoAb tremelimumab compared with standard of care, second-line agents (720 patients) (ClinicalTrials.gov identifier NCT02369874). Stratification by PD-L1 expression status is planned.

Based on the positive outcomes found to be associated with the use of checkpoint inhibition after first-line failure, a recently initiated phase 3 trial will now move PD-1 targeting forward into the first-line setting for patients with R/M disease. Specifically, this trial will compare the anti-PD-1 MoAb pembrolizumab alone or in combination with platinum/5-fluorouracil versus the EXTREME regimen (600 patients) (ClinicalTrials.gov identifier NCT02358031). Despite the excitement generated by the 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 that prompted the formation of the R/M disease working group, which was charged with the development of clinical trials to meet the needs of patients whose disease is refractory to existing therapy.

Although 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 2 fundamental considerations: first, that defined cosignaling pathways can be induced on Fc γ receptor (FcγR)-bearing immune effector cells through FcγR engagement by the aggregated Fc fragments of immobilized antibodies[29]; and second, that blockade of select immunologic checkpoints (eg, PD-1/PD-L1[15,30] in combination with the stimulation of defined cosignaling molecules (eg, CD137 [4-1BB]) have synergistic antitumor activity. His laboratory and that of one of the coauthors (R.L.F.) have demonstrated that engagement of CD16 on the surface of NK cells induced high levels of CD137 expression.[31,32] 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[33,34] or by DC[35] by agonistic antibodies could potentiate their antitumor activity.[32]

Based on these data, the working group proposed a prospective randomized clinical trial design with 3 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 also to induce CD137 expression on the surface of infiltrating NK cells. On study day 2, patients in group 1 would receive an agonistic MoAb against CD137, patients in group 2 would receive anti-PD-1 or anti-PD-L1, and patients in 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 2 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 HNSCC; and 2) the ability of anti-CD137/ PD-1 or PD-L1 to improve survival in comparison with either agent alone. A limitation of the design might be the inability to include a cetuximabonly cohort, based on feasibility considerations, as well the lack of toxicity or efficacy data for combinations with urelumab (agonistic anti-CD137).

Role of immunity in response to chemoradiotherapy—Cytotoxic cancer therapies alone are aimed at tumor eradication through the 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 have 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.[36,37]

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.”[37,38] The cardinal signs of immunogenic cell death are 1) calreticulin exposure on the surface of dying cells; 2) the release of HMGB1; and 3) the release of ATP, which acts on 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.”[36,39] 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: 1) downregulation of antiapoptotic/survival genes; 2) modulation of antigen-processing machinery components; and 3) 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. Although counterintuitive, it has recently been shown that immunotherapy may not only be compatible with chemotherapy, but also actually may be synergistic. 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 intercellular adhesion molecule 1 (ICAM-1), and 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 points are important when considering the use of chemotherapy with immunotherapy: 1) the combined use of immunotherapy and chemotherapy early in the disease process should not be confused with the use of immunotherapy after multiple regimens of different chemotherapeutic agents in the advanced disease setting, in which the immune system would most likely be impaired; 2) not all chemotherapeutic agents will be synergistic with immunotherapy; and 3) the dose and scheduling of immunotherapy when used with chemotherapy may be extremely important, and after immune function may guide trial optimization.

Checkpoint inhibitors and radiotherapy—Radiotherapy (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 patients with cancer undergoing 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, thereby altering those tumor cells to render them more susceptible to T cell–mediated lysis.[36,45] These findings have translated into promising clinical benefits for patients with HNSCC who are 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.[46]

Previously untreated, locally advanced HNSCC

Previously untreated, locally advanced, HPV-positive oropharyngeal cancer: 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.[47,48] HPV status and pack-years of tobacco exposure are the major determinants of survival among patients with HNSCC, followed by lymph node stage.[49] Based on these 3 prognostic factors, patients with HNSCC can be classified into 3 risk groups having a low, intermediate, or high risk of death. This clinical risk classification has framed national clinical trial priorities in PULA HNSCC. Specifically, deintensification strategies currently are being tested in patients with low-risk, HPV-positive HNSCC whereas intensification strategies represent the major unmet need for individuals with high-risk HPV-negative and intermediate-risk HPV-positive disease.[50–52]

For patients with HPV-positive, PULA HNSCC, working group 1 (Fig. 1) identified 2 priorities: 1) more targeted HPV-specific therapy taking advantage of unique non-self, viral TAs present within HPV-positive tumors; and 2) to determine the sequencing and optimal chemoradiotherapy (CRT) regimens that do not inhibit immunotherapeutic efficacy (Fig. 1). Currently, immunotherapeutic trials open or currently in development include eliminating systemic cytotoxic chemotherapy by combining intensity-modulated radiotherapy with cetuximab and the anti-CTLA-4 MoAb ipilimumab (ClinicalTrials.gov identifier NCT01935921), in which the overlap of ipilimumab exposure begins at week 5 of treatment with cetuximab and RT. In addition, patients with “intermediate-risk,” HPV-positive and “high-risk,” HPV-negative disease will be treated with concurrent, weekly CRT with cisplatin with an anti-PD-1 MoAb, a natural “add-on” strategy that currently is in development (Radiation Therapy Oncology Group [RTOG] Foundation trial 3504) and will open to enrollment in the near future.

First-generation “deintensification” clinical trials for patients with HPV-positive PULA disease enrolled patients with both good and intermediate risk, with the goal of reducing chemotherapy and/or RT doses (fields) (Eastern Cooperative Oncology Group [ECOG] 1308, RTOG 1016). As clinical risk stratification evolves, second-generation deintensification trials are selecting only good-risk patients (HN002). New trials are needed for patients with intermediate-risk, worse-prognosis, HPV-positive disease. The proposed trial aims to harness novel systemic immunotherapy and use 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, although lethal, distant metastatic disease for patients with intermediate-risk, HPV-positive PULA disease (those with T3/4 disease, those with N2c/N3 disease, > 10 pack-year smokers, and HPV-positive patients[52]. The proposed concept (Fig. 1) would compare anti-PD-1 plus cisplatin CRT with the combination of anti-PD-1/CRT plus HPV-specific E6/E7 vaccination. Several vaccines currently are available and have been tested in phase 1 trials for cervical and other HPV-positive cancers, and include peptide plus adjuvant, DNA-based or Listerolysin O-based vectors. Collaboration between a cooperative group and 1 or 2 pharmaceutical company sponsors is likely to be necessary. A neoadjuvant (pre-CRT) phase of 1 to 2 doses of vaccine with or without an anti-PD-1 MoAb was strongly considered because the timing and sequence of HPV-specific T-cell expansion vis-a-vis cytotoxic CRT, which may inhibit

lymphocyte expansion, is undetermined. This approach, although more cumbersome, also would permit the correlation of dynamic tumor and peripheral immune biomarkers with clinical outcomes.

PULA HPV-negative HNSCC—Approximately 80% 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.[48] The OS for patients with high-risk, PULA, HPV-negative HNSCC has improved only marginally within the last 20 years due to the incorporation of concurrent cisplatin in curative-intent paradigms. The current standard for the nonsurgical management of patients with PULA, HPV-negative, HNSCC is concurrent cisplatin and CRT, which improved OS, DFS, and locoregional control compared with RT alone in the sentinel Intergroup 0126 trial, a trial that was populated before the HPV epidemic.[53,54] Standards for the adjuvant management of patients with PULA, HPV-negative, HNSCC are determined by pathologic risk. Specifically, for patients who demonstrated ≥1 high-risk pathologic features, including a positive surgical margin or extracapsular lymph node extension, concurrent cisplatin and RT appeared to provide a clinical benefit compared with RT alone in the landmark phase 3 European Organisation for Research and Treatment of Cancer (EORTC) 22931 and RTOG 9501 trials.[55,56] Despite this advance, patients with high-risk, HPV-negative disease have a 3-year DFS rate of only 30% to 50%.[55–57] Although locoregional control and OS are improved with concurrent cisplatin and 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 with RT alone.[58] Poor outcomes persist despite intensification with altered fractionation,[59] multidrug induction,[60] or EGFR-targeted MoAbs.[61]

For HPV-negative patients, new intensification approaches represent a major unmet clinical need. The PULA HPV-negative working group initially discussed 2 clinical trial paradigms for patients with high-risk disease: 1) the integration of immunotherapy into definitive cisplatin CRT; and 2) the integration of immunotherapy into trimodality therapy for high-risk patients (Fig. 2). Ultimately, the recommended focus on the trimodality model capitalized on 3 opportunities. First, the accessibility of the 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, thereby facilitating pharmacodynamic evaluation of the tumor and TME responses in paired specimens. The second opportunity is the integration of immunotherapy with RT (Figure 3). Ionizing RT induces adaptive immune responses via 3 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.[62] The third opportunity is 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 to be of greatest priority for development in the HPV-negative PULA population. First, environmentally induced HNSCC demonstrates a high mutational burden.[63,64] Mutational load, as well as the presence of highly immunogenic neoantigens, has been correlated with response to immune checkpoint inhibitors in other solid tumors.[65,66] Second, RT dynamically upregulates PD-L1 on both tumor and MDSCs, thereby reducing the adaptive response and theoretically facilitating future disease recurrence. In 2 syngeneic preclinical models, concurrent PD-L1 blockade and RT were found to be synergistic in controlling tumor growth, and generated prolonged protective T-cell immunity, as demonstrated by subsequent abscopal effect.[67]

The central hypothesis of the proposed randomized phase 2 trial considers whether adding immunotherapy to CRT with adjuvant cisplatin increases the DFS in patients with high-risk, resected, PULA, HPV-negative HNSCC. In this trial design, the window of monotherapeutic exposure before definitive surgery creates a unique opportunity with which to study placebo-controlled, pretreatment and posttreatment 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 the TME, and to correlate these biomarkers with the 2-year DFS. Markers will include immunohistochemistry (IHC) or immunofluorescence (IF) for CD3, CD8, CD45RO, CD4/forkhead box P3 (FOXP3), PD-L1, and Ki-67; flow cytometry for tumor-infiltrating lymphocytes and MDSC subsets; T-cell activation panel and memory subsets; changes in T-cell receptor (TCR) clonality; and whole exome sequencing for peptide-encoding tumor neoantigens.

Immunotherapy Trial Biomarkers and Unique HNSCC Patient Specimen Considerations

From tumor samples, IHC/IF detection of immune markers provide a measure of baseline immune cell infiltration, phenotype, localization, and “inflammation,” sometimes referred to as an “immunoscore” because this has been shown to have prognostic and predictive capacity for immunotherapy in other diseases, including colorectal cancer.[68–71] 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 a proposed trial (PD-1, CTLA-4, OX-40, TIM-3, LAG-3, CD40, etc). Multiplexed IF makes testing multiple parameters more feasible.[72]

From fresh frozen tissue, the following genomic or signaling assays were recommended (Table 3): 1) RNA analysis to determine the IFN-γ gene signature; 2) PD-L1 and PD-L2 IHC staining on tumor and infiltration myeloid inflammatory cells; 3) RNA sequencing (to include the inhibitory/costimulation/exhaustion molecules targeted); 4) TCR diversity (as a measure of TCR skewing and clonality of the infiltrated T-cell response); and 5) any trial-specific pathways (eg, phospho-SMAD in the setting of a transforming growth factor β [TGF-β] 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 pretreatment and posttreatment tumor specimens in the HPV-negative and the HPV-positive PULA trials. A new biopsy would be needed for the R/M study (not on primary tumor banked earlier). Some technologies can use formalin-fixed, paraffin-embedded tissue, which is more easily obtained.

From peripheral blood samples (ie, ficoll-gradient separated peripheral blood mononuclear cells [PBMCs]), flow cytometry should accomplish the following: relative quantification of circulating suppressive MDSCs and T_{reg} cells; T-cell activation panels (eg, inducible costimulator in CTLA-4 trials and CD69 for general activation); lymphocyte memory subsets (CD45RO, CCR7 central trafficking); NK cells; and PD-1, CTLA-4, and/or any trial design-related costimulatory/coinhibitory molecules. Specific intracellular molecules (TGF-β: phospho-STAT) also would be measured. To the best of our knowledge, ECOG/American College of Radiology Imaging Network and NRG Oncology are not currently collecting and processing fresh PBMCs for functional and phenotypic studies, and 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 used and to maximize the predictive and prognostic as well as mechanism of action biomarker analyses.

Antigen-specific cytokine flow cytometry is possible using MHC: peptide multimers or non-human leukocyte antigen-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 (IFN-γ, TNF-α, interleukin 2, and granzymes). For non-HPV tumors, shared tumor antigen peptide pools can be pursued (eg, p53, survivin) with surface CD4 and CD8, polyfunctional intracellular cytokines, and effector molecules. Control antigen peptide pools can be used to document and monitor memory recall responses. Additional cellular blood assays also were considered, including genomic single-nucleotide polymorphism analysis for possible predictive genomic biomarkers from PBMC germline DNA. Similarly, transcriptional signatures have been identified from peripheral blood messenger RNA that may be unbiased and hypothesis-generating.

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

Imaging biomarkers are an important correlate in novel prospective trials but this field was believed 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 Response Evaluation Criteria In Solid Tumors (RECIST) for R/M disease.[73] For the short term, anti-PD-1 "window" neoadjuvant studies, [^{18}F]fludeoxyglucose-positron emission tomography/computed tomography before and after 4-week induction may be a predictor of early response via standardized uptake value measurements, because anatomic shrinkage may not be observed in the short term. However, infiltrating immune cells may be

metabolically active, confounding interpretation of increased [^{18}F]fludeoxyglucose avidity in the TME. The imaging biomarker experts noted that there is no current technology for the 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 example, 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, which are a candidate biomarker for some checkpoint blockade therapies (particularly CTLA-4), are serially obtained before/during/after in clinical laboratories. Last, stool samples and oral swabs could be considered for future microbiome studies.

Conclusions

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 with which 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 provide evidence for effective anticancer immunotherapies. Because of the manifold tumor evasion strategies and hence different response rates for treatments, combination therapies will be helpful in developing cancer treatments.

The HNSCC Immunotherapy CTPM was designed to harness these insights and to generate a better under-standing of several promising immunotherapeutic agents that currently are in clinical use as well as others currently 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/American College of Radiology Imaging Network and NRG Oncology for submission and review by NCI Cancer Therapy Evaluation Program and the Head and Neck Steering committee processes. Success will likely depend on the 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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Design in HPV (+) PULA Disease: Bryan Bell, Anthony Cmelak, Dimitrios Colevas, Adam Dicker, Avraham Eisbruch, Young-Jun Kim, Loren Mell, Harry Quon, Ralph Weichselbaum, Gregory Wolf, and Edward Zhang. Group 2-Best Agent(s) and Trial Designs in HPV (−) PULA Disease: Walter Lee, Mitchel Machtay, Judith Manola, Renato Martins, Michael Spiotto, Erich Sturgis, John Sunwoo, Ravindra Uppaluri, and Carter van Waes. Group 3-Best Agent(s) and Trial Design in Recurrent/Metastatic Disease: Laura Chow, Christine Chung, Marka Crittenden, Antonio Jimeno, Holbrook Kohrt, John Lee, Brian Nussenbaum, Tanguy Seiwert, Andrew Sikora, Jeremy Taylor, and Stuart Wong. Group 4-Monitoring for Efficacy in Immunotherapeutic Trials-Specimen Analyses, Imaging, Correlative Immune Monitoring: Jorge Carrasquillo, Francesco Marincola, Jeffrey Moyer, Sara Pai, Elad Sharon, James Stapleton, Howard Streicher, Rathan Subramaniam, and Xiao-Jing Wang.

CONFLICT OF INTEREST DISCLOSURES

Julie E. Bauman has acted as a paid scientific consultant for Lilly, Merck, Incyte, and EMD-Serono and has received a clinical trial research grant from Merck for work performed outside of the current study. Ezra Cohen has received fees from Bristol-Myers Squibb, Pfizer, and AstraZeneca for work performed outside of the current study. Robert L. Ferris has received grants from and acted as a paid member of the Advisory Board for AstraZeneca/MedImmune, Bristol-Myers Squibb, and Merck; acted as a paid member of the Advisory Board for Lilly and Pfizer; and has received a grant from VentiRx Pharmaceuticals for work performed outside of the current study. Barbara A. Burtness has received a grant and personal fees from Merck, a grant from Advaxis, and personal fees from AstraZeneca and Amgen for work performed outside of the current study. Mary L. Disis has received grants from VentiRx, EMD-Serono, Celgene, Janssen, and Seattle Genetics for work performed outside of the current study, and is a stockholder in VentiRx and Epithany. Bernard A. Fox has received grants from Bristol-Myers Squibb and AstraZeneca/Medimmune for work performed as part of the current study. Dr. Fox is also founder and owner of UbiVac; has received personal fees from Argos, Dendreon, 3M, and Ventana/ Roche; has received nonfinancial support from Aduro and Definiens; has received grants and personal fees from Janssen/Johnson & Johnson; has received personal fees and nonfinancial support from PerkinElmer; and has received a grant from Viralytics for work performed outside of the current study. In addition, Dr. Fox has a patent (US 20140112977 A1) licensed to UbiVac. Thomas F. Gajewski has received a grant from and acted as a paid member of the Advisory Board for Merck; acted as a paid member of the Advisory Board for Incyte, Roche, Bayer, AbbVie, Forma, and Jounce; has received grants from Bristol-Myers Squibb, Seattle Genetics, Genentech/Roche, and Ono; and has patents from Evelo and Aduro. Maura L. Gillison has received a clinical trial contract from Bristol-Myers Squibb to Ohio State University and personal fees from Bristol-Myers Squibb for consulting and lectures for work performed as part of the current study and has received travel costs from Merck, Lilly, Amgen, AstraZeneca, Bristol-Myers Squibb, and Celgene and a clinical trial contract to Ohio State University from AstraZeneca, Merck, and Bristol-Myers Squibb for work performed outside of the current study. David Raben has received personal fees from AstraZeneca and Merck for work performed outside of the current study. Scott E. Strome has stock in, is a cofounder of, has received research support from, and acted as a paid consultant for Gliknik; has received research support from Pfizer; has received payment from the Mayo Clinic College of Medicine for IP licensed to third parties regarding the clinical application of B7-H1; and has had 3 patents issued and licensed and receives royalties from them through the Mayo Clinic.

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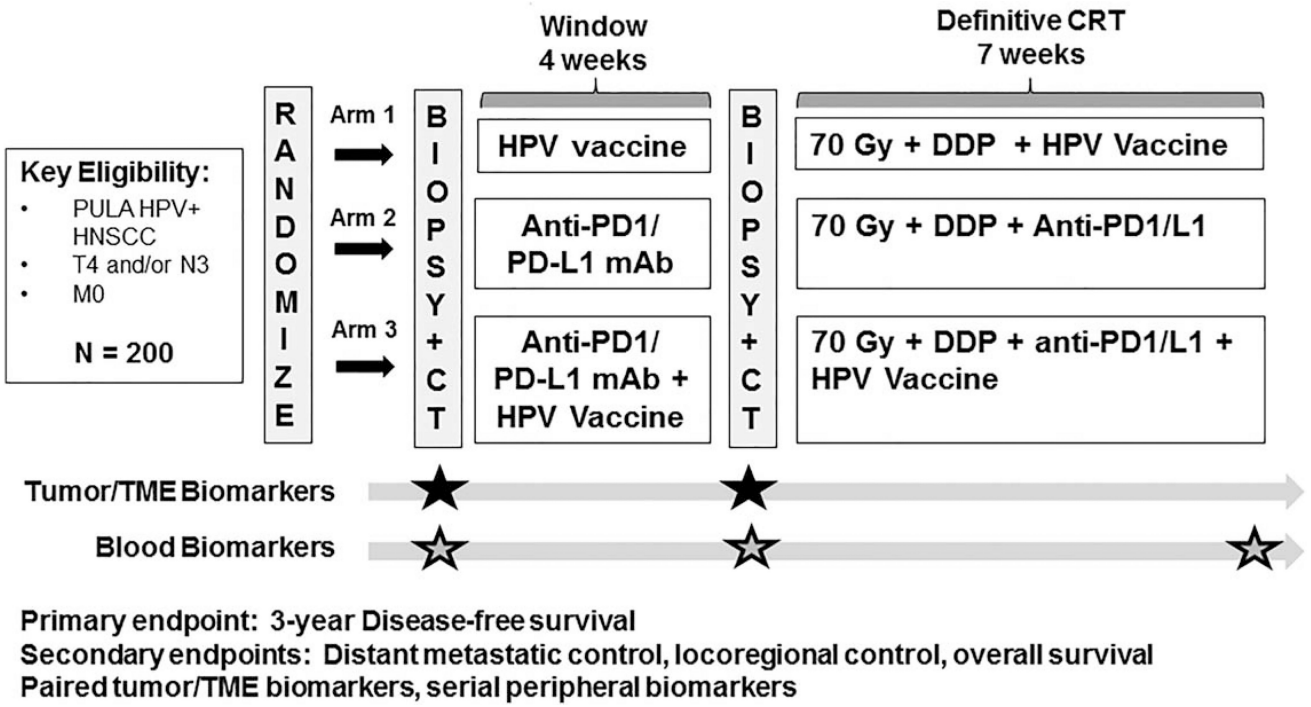

Figure 1.

Working Group 1. Window immunotherapy biomarker study followed by definitive chemoradiotherapy (CRT) plus human papillomavirus (HPV) vaccine, anti-programmed death 1 (PD-1)/PD-ligand 1 axis (PD1/PL1) monoclonal antibody (mAb), or both in patients with T4 or N3, HPV-positive (+) oropharynx cancer. CT indicates computed tomography; DDP, cisplatin; Gy, grays; HNSCC, head and neck squamous cell carcinoma; PD-L1, programmed death-ligand 1; PULA, previously untreated, locally advanced; TME, tumor microenvironment.

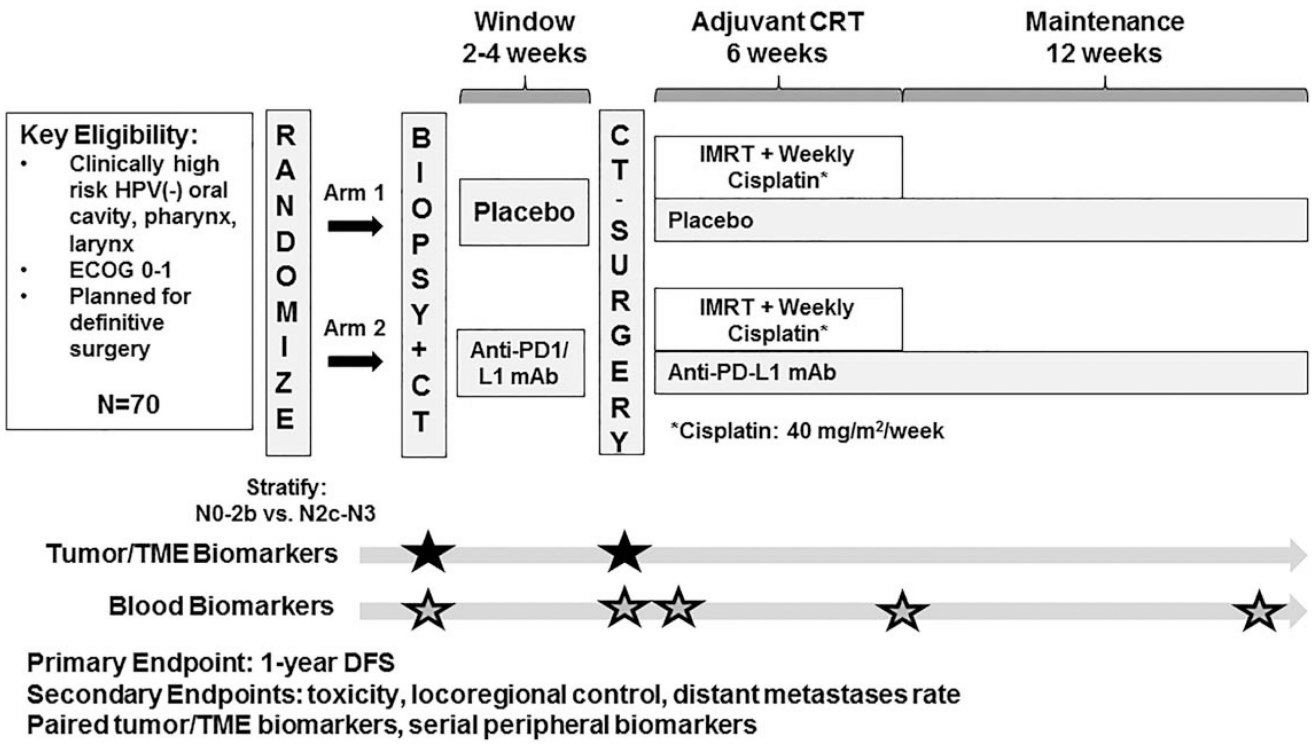

Figure 2.

Working Group 2. Randomized, phase 2 study of adjuvant cisplatin and radiotherapy with or without antiprogrammed death-ligand 1 (PD-L1) monoclonal antibody (mAb) in patients with high-risk, human papillomavirus (HPV)-negative (−) head and neck cancer with window correlatives. CRT indicates chemoradiotherapy; CT, computed tomography; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; IMRT, intensity-modulated radiotherapy; PD1/L1, programmed death 1 (PD-1)/PD-ligand 1 axis; TME, tumor microenvironment.

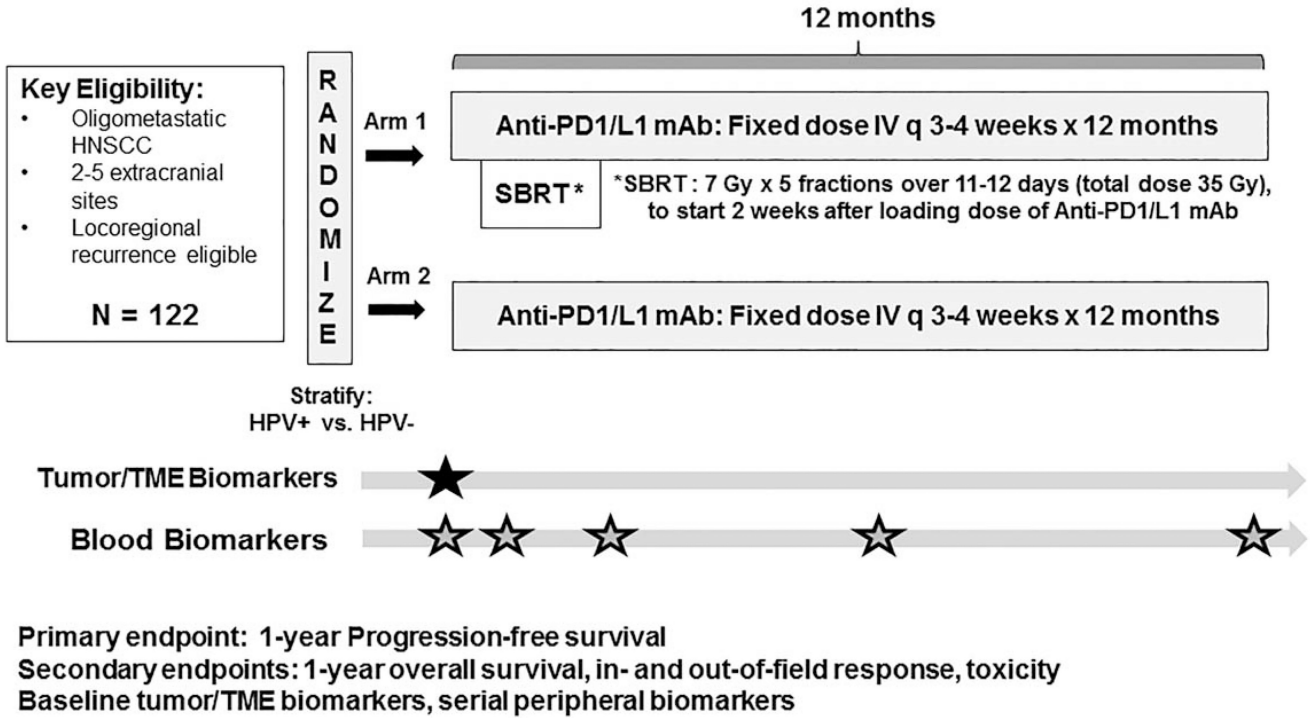

Figure 3.

Working Group 3. A randomized phase 2 study of stereotactic body radiosurgery (SBRT) plus antiprogrammed death 1 (PD-1)/PD-ligand 1 axis (PD1/L1) monoclonal antibody (mAb) versus antiprogrammed death-ligand 1 (PD1/L1) mAb alone for oligometastatic head and neck cancer. Gy indicates grays; HNSCC, head and neck squamous cell carcinoma; IV, intravenously; q, every; TME, tumor microenvironment.

TABLE 1

Potential Therapeutic Targets in Head & Neck Squamous Cell Carcinoma (HNSCC)

Drug (Company)	Target	IgG Class	HNSCC Development Stage	Proposed Mechanism of Action
Tumor antigen-targeted MoAbs				
Cetuximab (Bristol-Myers Squibb, Eli Lilly)	EGFR antagonist	IgG1	Phase 3/4	Tumor growth inhibition, cellular immunity
Panitumumab (Amgen)	EGFR antagonist	IgG2	Phase 2/3	Tumor growth inhibition
AV-203 (Aveo)	HER3 antagonist	IgG1	Phase I (monotherapy; cetuximab combination)	Tumor growth inhibition
Cixutumumab (Eli Lilly)	IGFR antagonist	IgG1	Phase 0–2 (neoadjuvant monotherapy; cetuximab combination)	Tumor growth inhibition
Cytokine -targeted MoAbs				
Bevacizumab (Genentech)	VEGF neutralizing Ab	IgG1	Phase 3 (platinum chemotherapy+/−)	Inhibition of angiogenesis, impairment of VEGF-induced immunosuppression
Ficlatuzumab (Aveo)	HGF neutralizing Ab	IgG1	Phase 1 (cetuximab combination; cisplatin-RT combination)	Tumor growth inhibition
TNF receptor-targeted MoAbs				
MEDI0562 (AstraZeneca/Medimmune)	OX40 agonist	IgG2	Phase 1b	Stimulation of cellular immunity
Urelumab (Bristol-Myers Squibb)	CD137 agonist	IgG4	Phase 1	Stimulation of cellular immunity
PF-05082566 (Pfizer)	CD137 agonist	IgG2	Phase 1	Stimulation of cellular immunity
Immune checkpoint targeted MoAbs				
Ipilimumab (Bristol-Myers Squibb)	CTLA-4	IgG1	Phase 1 (cetuximab-RT combination)	Blockade/depletion of T_{reg}, enhancement of CTL
Tremelimumab (AstraZeneca/Medimmune)	CTLA-4	IgG2	Phase 1	Blockade/depletion of T_{reg}, enhancement of CTL activity
MEDI4736 (AstraZeneca/Medimmune)	PD-L1	IgG1	Phase 2	Enhancement of CTL activity
Pembrolizumab (MK-3475; Merck)	PD-1	IgG4	Phase 1	Enhancement of CTL activity
Nivolumab (Bristol-Myers Squibb)	PD-1	IgG4	Phase 3	Enhancement of CTL activity

Abbreviations: CTL, cytotoxic T-lymphocyte; CTLA-4, cytotoxic T-lymphocyte antigen 4; EGFR, epidermal growth factor receptor; HER3, human epidermal growth factor receptor 3; HGF, hepatocyte growth factor; HNSCC, head and neck squamous cell carcinoma; IGFR, insulin-like growth factor receptor; IgG, immunoglobulin G; MoAb, monoclonal antibodies; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; RT, radiotherapy; T_{reg}, regulatory T cell; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

TABLE 2

Immunotherapeutic Agents

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 andh IL-12 secretion
Lirilumab (Bristol-Myers Squibb)	Anti-KIR MoAb
1-7F9 (Innate)	Anti-KIR MoAb
Targeting immunosuppressive cytokines	
Siltuximab	Anti-IL-6 MoAb
CAT-192	Anti-TGF-β MoAb
T-cell costimulatory agonists	
CP-870,893 (Pfizer)	CD40 agonist MoAb
OX40 MoAb (AgonOx; Providence Health)	OX40 agonist MoAb
Urelumab (Bristol-Myers Squibb)	CD137 agonist MoAb
PF-05082566 (Pfizer)	CD137 agonist MoAb
IMP321 (Immutep)	Recombinant soluble dimeric LAG-3
T-cell immune checkpoint inhibitors	
Ipilimumab (Bristol-Myers Squibb)	Anti-CTLA-4 MoAb
Tremelimumab (AstraZeneca/Medimmune)	Anti-CTLA-4 MoAb
Nivolumab (Bristol-Myers Squibb)	Anti-PD-1 MoAb
Pembrolizumab (Merck)	Anti-PD-1 MoAb
Durvalumab (MEDI-4736 (AstraZeneca/Medimmune)	Anti-PD-L1 MoAb
MPDL3280A (Genentech)	Anti-PD-L1 MoAb
MSB0010718C (EMD-Serono)	Anti-PD-L1 MoAb
AUNP12 (peptide) (Pierre Fabre/Aurigene)	Anti-PD-L1 peptide
BMS-986016 (Bristol-Myers Squibb)	Anti-LAG-3 MoAb
INCB024360 (Incyte)	Orally available inhibitor of indoleamine 2,3-dioxygenase (IDO1)

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CTLA-4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cells; IL, interleukin; KIR, killer inhibitor receptor; LAG-3, lymphocyte-activation protein 3; MoAb, monoclonal antibody; NCI, National Cancer Institute; NK, natural killer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TGF-β, tumor growth factor-β; TLR, toll-like receptor.

TABLE 3

Correlative Biomarkers for Cancer Immunotherapy

Tumor	PBMC	Serum	Imaging	Future
Infiltrate: CD3, CD8, CD45RO, CD4/FOXP3, PD-L1; frequency, location IHC, IF	Suppressors: T_{reg}, MDSC	Multiplexed circulating cytokines, chemokines, growth factors	FDG-PET/CT before and after 4-wk induction	Stool/oral swabs for microbiome
Major checkpoints/ costimulatory (PD-1, CTLA-4, TIM-3, LAG-3, OX-40, and CD40)	Effector activation (ICOS, CD69), effector/memory, cytotoxicity	Circulating antibodies		Imaging immune response
NK cells	NK cells			
Ki-67	Trial-specific pathways			
RNA Seq	HPV-positive: virus peptide pools			
TCR diversity	HPV-negative: shared tumor antigen peptide pools			
Trial-specific pathways	ALC as SOC			

Abbreviations: ALC, acetyl-l-carnitine; CT, computed tomography; CTLA-4, cytotoxic T-lymphocyte antigen 4; FDG-PET, [^{18}F]fludeoxyglucose-positron emission tomography; FOXP3, forkhead box P3; HPV, human papillomavirus; ICOS, inducible costimulator; IF, immunofluorescence; IHC, immunohistochemistry; LAG-3, lymphocyte-activation protein 3; MDSC, myeloid-derived suppressor cells; NK, natural killer; PBMC, peripheral blood mononuclear cells; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SOC, standard of care; T_{reg}, regulatory T cells; TCR, T-cell receptor; TIM-3, T-cell immunoglobulin 3.