Ethics Approval The study was approved by WIRB, Study Number 1188591 and IRB Tracking Number: 20180297.

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435 A PHASE II TRIAL OF NIVOLUMAB PLUS AXITINIB IN PATIENTS WITH ANTI-PD1 REFRACTORY ADVANCED MELANOMA

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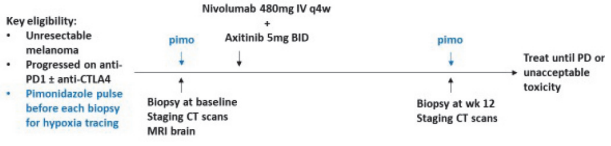
Background Immunotherapy has changed the treatment landscape for melanoma, although many patients (pts) do not respond to treatment. While there are likely multiple mechanisms of resistance at play, one key mechanism is the generation of an immunosuppressive and metabolically harsh tumor microenvironment (TME).[1] This is likely the result of an altered angiogenic pattern along with dysregulated metabolism of the tumor itself, which leads to hypoxia.[2] CD8+ tumor infiltrating lymphocytes (TIL) isolated from tumors with high oxidative metabolism have an exhausted phenotype and decreased functionality (decreased IFN-γ and TNF-α production).[3] Thus, TIL may be blunted due to failure to meet their metabolic needs. Vascular endothelial growth factor (VEGF) is a critical mediator of angiogenesis and is overexpressed in many solid tumors, including melanoma. Axitinib has high inhibitory activity for VEGF receptors1, 2, and 3. In a preclinical B16 melanoma model, we found that anti-PD1 plus axitinib provided an improved and durable response compared to monotherapy with either agent. We hypothesize that by modulating angiogenesis, axitinib will reduce intra-tumoral hypoxia and resultant T cell dysfunction, which will re-sensitize melanoma to anti-PD1 therapy.

Methods This is an investigator-initiated, phase II trial of nivolumab plus axitinib for pts with unresectable stage III or IV melanoma who have progressed on prior anti-PD1 therapy with or without concomitant anti-CTLA4. Prior treatment with BRAF/MEK inhibitors is permitted. Pts with brain metastases are permitted if they are asymptomatic and have stable disease 2 weeks after CNS-directed treatment. Pts will receive nivolumab 480 mg IV every 4 weeks and axitinib PO 5 mg twice daily for up to two years or until progression or unacceptable toxicity. Timing of biopsies is reported in figure 1, with an optional biopsy at progression. Pts will receive an oral dose of pimonidazole 0.5 mg/m^2 before each biopsy to permit in vivo evaluation of intra-tumoral hypoxia. Primary endpoint: overall response rate (ORR). Secondary endpoints: safety, progression-free survival, overall survival, and correlative analyses (evaluation of hypoxia in the TME, TIL function, immune phenotype, and tumor cell metabolism). Statistical analysis includes Simon's minimax two-stage design. The null hypothesis is that the true ORR is 10%, tested against a one-sided alternative of 25% or higher. N=31 patients with a type I error rate of 0.08 and power 0.81 when the true response rate is 0.25.



Abstract 435 Figure 1 Study schema

Results N/A

Conclusions N/A

Trial Registration NCT04493203

Ethics Approval The study was approved by the University of Pittsburgh Institutional Review Board, approval number HCC 20-101.

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Combination immunotherapies

436 RATIONAL SEQUENCING OF IMMUNE-ONCOLOGY THERAPIES ACHIEVES DURABLE RESPONSE AND IMMUNOLOGIC MEMORY

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Background Oncologically-sound standard of care therapy often indicates ablation of draining lymphatic basins to eradicate repositories of metastatic disease. However, emerging cancer immunotherapies often necessitate intact secondary lymphoid organs to achieve maximum effect. Therefore, multimodal immune-oncology (IO) therapeutic approaches introduce an inherent paradox into the clinical management of the cancer patient: how to reconcile the clinical benefit of lymphatic ablation with the destruction of an indispensable immune organ.

Methods Here, we leverage a novel preclinical model of tobacco-signature head and neck squamous cell carcinoma (HNSCC) to examine the impact of lymphatic ablation on the efficacy of immunotherapy and to identify sequences of therapy that maximize durable response without compromising oncologically-sound standard of care therapy.

Results We show that cervical lymphatic ablation in tumor bearing animals abolishes the response to CTLA- 4 blockade by eradicating lymph-node associated conventional dendritic cells and restricting CD8 T cell priming and subsequent tumor infiltration. By modelling recurrent HNSCC, we find that upfront, elective cervical lymphatic ablation eliminates the tumor response to adjuvant CTLA-4 blockade in contrast to a lymphatic-sparing approach, which preserves sensitivity to CTLA-4 blockade. In the neoadjuvant setting, we show that delayed, but not early, cervical lymphatic ablation leads to durable response after CTLA-4 blockade. Lastly, we demonstrate that a successful tumor response to CTLA-4 blockade begets long-lasting immunologic memory, resistant to delayed cervical lymphatic ablation.

Conclusions Collectively, this work addresses an inherent paradox in the delivery of combination IO therapy, informs

optimal sequencing of multimodal therapy and affords a premise for the introduction of CTLA-4 blockade into the clinical management of HNSCC.

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437 ADENOVIRUS IL-12 AND DOCETAXEL IN COMBINATION WITH ANTI-PD1 AS AN EFFECTIVE TREATMENT STRATEGY FOR TNBC

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Background In 2020, over 42,000 women in the US are expected to die from Breast Cancer (BC). Triple Negative Breast Cancer (TNBC), a subtype defined by lack of estrogen receptor (ER), progesterone receptor (PR) and HER2 amplification, account for 15–20% of all BC. TNBC is more prevalent in pre-menopausal African-American and Hispanic women. Currently, chemotherapy is the standard of care for TNBC. Unfortunately, despise the high rate of initial response to neo-adjuvant chemotherapy, TNBC have higher rates of distant recurrence, and few (less than 30%) of the patients survive more than 5 years. Even though this subtype express high levels of PD-L1, the response to checkpoint inhibitor therapy have been modest. We hypothesized that the induction of cell death (Docetaxel) coupled with an immuno-activated milieu (locally injected adv.IL-12) would prime the tumor to respond to Anti-PD1 therapy. In this study, we investigated the effects of initially treating TNBC with a single dose of Docetaxel and adv.IL-12, followed by Anti-PD1 in syngeneic models.

Methods Syngeneic E0771 and 4T1 cell lines were injected in the mammary fat pad of C57BL/6, and Balb/c mice respectively. On day 0, mice in the Triple Combo group received a single dose (20 mg/kg) of Docetaxel and an intratumoral injection (1.25 × 109) of mAdv.IL-12 (a replication defective adenoviral vector containing mouse IL-12 cDNA under the transcriptional control of Rous sarcoma virus long terminal repeat) (provided by Dr. Chen), followed by IP injection Anti-PD1 (InVivoMab anti-mouse PD-1 CD279) on days 3,5,7,10,12, and 14. The other groups, received single therapy following the same procedure. On day 19, Tumor Infiltrating Lymphocytes (TILS) were isolated by Ficoll gradient and submitted for immuno-phenotyping by CyTOF analysis to the HMRI ImmunoMonitoring Core, in addition, tumor lysates were used to measure cytokine expression using Millipore Sigma's Milliplex MAP Mouse Cytokine/Chemokine Magnetic Beads panel (cat: MCYTMAG-70K). Survival status over time, as well as tumor volume (measured every 3 days) were monitored in both models.

Results Triple combination inhibited tumor growth in the 4T1 model while significantly delaying E0771 tumor progression. Triple Combo (TC) group had significantly higher number of TILS in both models, while the phenotype and cytokine expression significantly differed. In 4T1, TC increase the infiltration of both CD8 and CD4 effector cells, while significantly decreasing neutrophils. The levels of G-CSF, Rantes were significantly upregulated in this model, while pro-tumorigenic cytokines such as IL-6, LIF, IL-1b, and anti-inflammatory cytokines such as IL-9 and IL-10 were downregulated. In E0771, only effector, and IFN-g producing CD8 levels were increased in TC group. Although TC treated animals survived an average of 18 days more than single Doc treated animals, levels of IL-6, IL-1b, LIF, KC, TNFa and VEGF levels were higher at the end of the study.

Conclusions Ad.IL-12 plus Docetaxel followed by Anti-PD1 therapy appears to only be beneficial to a specific subgroup of TNBC. We are actively studying the molecular difference between the two models used in this study, as well as investigating the clinical relevance of these markers using our extensive repertoire of PDXs in a humanized mice model.

Ethics Approval The study was approved by the Houston Methodist Research Hospital IACUC committee AUP: 0320-0023

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438 SYNERGY BETWEEN SEA-CD40 AND CHEMOTHERAPEUTICS DRIVES CURATIVE ANTITUMOR ACTIVITY IN PRECLINICAL MODELS

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Background CD40 is a co-stimulatory receptor of the TNF receptor superfamily expressed on antigen presenting cells (APCs). Antibodies targeting CD40 may have antitumor therapeutic benefit by driving innate immune cell activation that supports generation of antigen-specific T cell responses. Multiple CD40-directed antibodies are in clinical development in both solid and hematologic indications and differ according to immunoglobulin isotype, affinity to CD40, and differential FcγR-binding. SEA-CD40 is an agonistic nonfucosylated, humanized IgG1 monoclonal antibody directed against CD40. SEA-CD40 is distinct from other CD40 targeted agents in clinical development as it binds with increased affinity to FcγRIIIa resulting in enhanced effector function and CD40 agonism. This unique composition of SEA-CD40 could amplify immune stimulation and antitumor activity relative to other CD40-directed therapeutics.

Methods Effective immunity requires the presence of diverse antigens to drive generation of distinct antigen-specific memory T cells. SEA-CD40 in many ways works like a vaccine as it can increase active acquired immunity against endogenous tumor antigens. A potential limiting factor for maximal SEA-CD40 antitumor activity across multiple tumor types may be the limited level and diversity of tumor-associated antigens within the tumor microenvironment (TME). Chemotherapeutic agents drive tumor cell death resulting in the release and increase of tumor antigens locally within the TME. Combining chemotherapeutic agents with SEA-CD40 could facilitate robust antigen release and amplified presentation of those antigens to CD8+ T cells. Antitumor activity and immune cell changes of SEA-CD40 in combination with chemotherapeutic agents was evaluated in vitro and in vivo using human CD40 transgenic mice.

Results In preclinical mouse models, SEA-CD40 combined with chemotherapeutic agents to drive robust anti-tumor activity. The nature of the chemotherapeutic agent influenced immune cell activation within the tumor microenvironment (TME) and extent of combinability with SEA-CD40. Preclinical assessment indicates that chemotherapeutics which induce immunogenic cell death (ICD) combine with SEA-CD40 to increase curative activity compared to non-ICD-inducing chemotherapeutics. The preferred partnership of SEA-CD40 with ICD-inducing agents, such as a monomethyl auristatin E (MMAE) antibody-drug conjugate, increased curative antitumor