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Purpose/Objective(s): The expansion of peripheral blood myeloid cells, including myeloid-derived suppressor cells (MDSC), contributes to immune dysfunction seen in patients with glioblastoma (GBM). There is limited understanding of how standard adjuvant therapy, six weeks of temozolomide (TMZ)/radiation therapy (RT), affects these cells. The purpose of our study is to describe systemic immune dynamics during standard TMZ/RT, by measuring myeloid cell subsets through multi-time point *ex vivo* peripheral blood studies in patients with GBM. We hypothesize that MDSC populations expand during standard course adjuvant TMZ/RT and this may adversely affect tumor control and potential for effectiveness of immune checkpoint inhibitor therapies.

Materials/Methods: We are prospectively enrolling patients with newly diagnosed GBM and collecting peripheral blood at seven time points (TP1-7): prior to initiation of TMZ/RT, weekly during TMZ/RT, and one month after completion of TMZ/RT. We evaluate immunophenotype and activation status of fresh peripheral blood mononuclear cells through multi-parametric cell surface and intracellular flow cytometry. In available cases, fresh tumor tissue is evaluated with a matching peripheral flow cytometry panel. Protein expression in fixed surgical resection tissue is explored with digital spatial profiling technology. Analytes within plasma will be assessed by multiplexing. Clinical outcome will be evaluated with imaging at six months and will be related to immunological data. Causal inference modeling will explore relationships between individual immune factors and outcome.

Results: To date, 16 subjects have enrolled: one died, one declined blood draws, and three are receiving alternative therapies. Based on available data from two subjects that have completed all time points, MDSC ($CD33^{+}HLA\text{-}DR^{-}$) percent frequency (%fx) increases above baseline during the course of TMZ/RT (A: TP1 32.4% to max 36.2% (TP3); B: TP1 10.9% to max TP5 of 31.5%). Functionally, the %fx of myeloid cells producing the tumor-promoting cytokine, TGFβ, increases from baseline (A: 1.25%; B: 1.59%), peaks during TP6, the last time point during TMZ/RT (A: 67.2%; B: 18.1%), and decreases one month post-TMZ/RT (A: 4.36%; B: 5.79%).

Conclusion: Available results identify an expansion of MDSC and change in activation status of myeloid cells during adjuvant TMZ/RT. Updated data including additional patients will be presented. Our study may aid in understanding the immunologic impact of standard of care TMZ/RT. As RT regimens for GBM were developed with the focus of tumor cell kill, our data may lead to future studies to determine the efficacy of therapy directed at immunologic endpoints, and personalized immunologic RT, in the treatment of GBM and other tumors.

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NBTXR3 Activated By Radiotherapy Generates an Anti-Tumor Immune Response

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Purpose/Objective(s): Hafnium oxide nanoparticles (NBTXR3) activated by radiotherapy (RT) increase radiation dose deposit within cancer cells compared to RT alone. Currently 7 clinical trials are underway to evaluate NBTXR3+RT. To date, no dose limiting toxicities (DLTs) have been observed. Given that RT can prime an anti-tumor immune response we hypothesized that this response could be enhanced by NBTXR3+RT in both animals and humans.

Materials/Methods: Immunocompetent mice were injected in both flanks with CT26 cells. An intratumoral injection of NBTXR3 (or vehicle) was performed in right flank tumors, followed by RT (3x4Gy). Tumor growth was followed, and animals sacrificed when tumors reached 800mm3. Alternatively, tumors were collected 3 days after last RT fraction and immune cell infiltrates analyzed by immunohistochemistry (IHC). Pts with locally advanced soft tissue sarcoma (STS) [NCT02379845] received either NBTXR3+RT or RT alone. Pre- and post-treatment tumor tissues (biopsy and tumor resection respectively) from pts were analyzed by IHC and Digital Pathology for immune biomarkers (>16 pts per arm).

Results: Animal studies demonstrated that NBTXR3+RT can induce an immune response which was not observed with RT alone. IHC analyses showed that significantly more CD8+ cells were present in NBTXR3+RT treated and untreated tumors, compared to tumors from mice treated with RT alone. Similarly, increased CD8+ T cell infiltration pre- vs post-treatment was observed in tumor tissues from STS pts treated with NBTXR3+RT. An increase in biomarkers, including CD8 and PD1, following NBTXR3 +RT was also observed by IHC in tumor samples from STS pts compared to RT alone.

Conclusion: These results demonstrate that NBTXR3+RT induces a specific adaptive immune profile in both mice and STS pts. As such, it may convert immunologically "cold" tumors into "hot" tumors, opening the potential for combination with immunotherapeutic agents. We have therefore sought to investigate the safety and systemic effect of NBTXR3 activated by stereotactic ablative radiotherapy (SABR) in combination with anti-PD-1 antibody in pts with locoregionally recurrent or metastatic (to lung or liver) head and neck squamous cell carcinoma (HNSCC), as well as in metastatic non-small cell lung cancer (NSCLC) and liver metastasis patients [NCT03589339].

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Immunogenic Cell Death Induced by Tumor Treating Fields (TTFields) Enhances Efficacy When Combined with Anti-PD-1 Therapy in Lung and Colon Cancer Animal Models

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Purpose/Objective(s): Tumor Treating Fields (TTFields) therapy is an FDA approved anti-neoplastic treatment modality delivered via noninvasive application of low-intensity, intermediate-frequency, non-ionizing alternating electric fields. Previously it was shown that TTFields induce immunogenic cell death. In this study we evaluated whether TTFields-induced cell death can be further enhanced by the combination with anti-PD-1 therapy in murine models.
Materials/Methods: Bone marrow derived dendritic cells (DCs) were co-incubated with TTFields treated cells and phagocytosis by DCs and DCs maturation were evaluated. The combination of TTFields and anti-PD-1 was evaluated in short duration treatment protocol in orthotopic lung cancer model and long duration treatment protocol in heterotopic subcutaneous colon cancer model. Tumor volume was monitored and analysis was performed for phenotypic characterization of infiltrating immune cells.
Results: We demonstrate that TTFields treated cells promote phagocytosis by DCs, DCs maturation in vitro, and promote immune cells recruitment in vivo. We also show that the combined treatment of lung tumor-bearing mice with TTFields plus anti-PD-1 led to a significant decrease in tumor volume compared to the other treatment groups. Significant increases in CD45+ tumor infiltrating cells were observed in the TTFields plus anti-PD-1 group in both models. In the orthotopic lung tumors, the CD45+ infiltrating cells, specifically macrophages and DCs, demonstrated upregulation of surface PD-L1 expression following short treatment duration. Correspondingly, cytotoxic T-cells isolated from these tumors have shown higher levels of IFN-γ production relative to untreated mice. In the heterotopic subcutaneous colon cancer tumors, significant increases in T-cell infiltration was observed following long treatment duration of heterotopic subcutaneous colon cancer tumors with TTFields plus anti-PD-1.
Conclusion: These results demonstrate robust efficacy with concurrent application of TTFields and anti PD-1 therapy in mouse models of lung and colon cancers. These data suggest that combining TTFields with anti-PD-1 may achieve tumor control by further enhancing antitumor immunity.
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Indoximod Strongly Enhances Effects of Combined Hrt and PD1/PD-L1 Checkpoint Blockade

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Purpose/Objective(s): Indoleamine-2,3-dioxygenease (IDO) degrades tryptophan to the immunosuppressive metabolite kynurenine. IDO plays critical roles in peripheral immune tolerance and anergy. A recent phase 3 trial evaluating an IDO inhibitor + anti-PD1 antibody (aPD1) in metastatic melanoma patients missed its primary endpoint of improving progression-free survival compared to aPD1 monotherapy. However, IDO is induced by IFNg, and hypofractionated radiotherapy (hRT) alone or in combination with immune checkpoint blockade can induce IFNg-secreting, tumor-specific T cells. We therefore studied, in mouse models, whether the triple combination of hRT, aPD1, and the IDO pathway inhibitor (IDOi) indoximod is superior to the respective double combinations.
Materials/Methods: We compared the triple treatment with hRT (12 Gy × 2) + aPD1 + IDOi with the double combinations of hRT + aPD1, hRT + IDOi, and aPD1 + IDOi, and with the respective monotherapies in mice bearing large (300–500 mm3) B16 melanoma or 4T1 breast carcinoma tumors. Tumor growth and survival of the mice were determined. The dependence of the therapeutic effects on CD8+ T cells and NK cells was studied using depleting antibodies. Frequencies and functionality (differentiation and exhaustion state) of tumor-specific CD8+ T cells in tumor tissue and draining lymph nodes were determined flow cytometrically by using MHC tetramers and various antibodies.
Results: In our models with relatively large tumors, the tumors did not regress following treatment with hRT + aPD1. The aPD1/IDOi double combination was not effective at all. In contrast, the triple treatment induced marked tumor regressions in both tumor models. The survival benefits were highly significant compared to hRT + aPD1 (B16 melanoma model $p = 0.002$; 4T1 model $p = 0.0001$). CD8+ T cell depletion strongly, and NK cell depletion partly, abrogated tumor control and survival benefits. Flow-cytometric analyses showed significant increases in numbers and functionality of tumor-specific CD8+ T cells and NK cells for the triple combination group compared to the other groups.
Conclusion: Our data in two aggressive tumor models show that the triple therapy with hRT, aPD1, and an IDOi can induce much stronger tumor regression than double combinations or monotherapy with the individual components. Our findings may serve as a rationale for the clinical evaluation of this triple combination therapy in patients with high-risk locally advanced or oligometastatic tumors.
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Characterization of Radiation-Induced Lung Fibrosis and Mode of Cell Death Using Single and Multi-Pulsed Proton Flash Irradiation

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Purpose/Objective(s): Radiation induced toxicity is the primary limiter to dose escalation in radiation therapy. Recent preclinical studies indicate a reduction of normal tissue toxicity using ultra-high dose-rate (FLASH) radiation on the order of 40 Gy/sec or higher, with comparable tumor control. The resulting therapeutic window enhancement has exciting implications for cancer care, but the results thus far are limited to electrons in an energy range with minimal utility for human treatment. Some clinical proton therapy systems are capable of FLASH dose rates at depths which would provide access to most tumor sites. Therefore, demonstrating therapeutic window enhancement for proton FLASH would have significant implications on clinical translation of this novel therapy paradigm. This study presents a comparison of various biological endpoints comparing three modes of irradiation, conventional, FLASH, and so-called pulsed-FLASH.
Materials/Methods: The thorax region of age and sex matched C57/BL6 mice were irradiated to doses of 15, 17.5, 20 Gy on a clinical pencil-beam-