achieved by depleting monoclonal antibodies and confirmed by flow cytometry. T-cell receptor deficient (TCR KO) mice were used to confirm findings in T-cell depletion experiments. 9464D-GD2 parental cells have low MHC-I expression; subclones with low and moderate MHC Class I expression were obtained by flow cytometry sorting and the impact of MHC class I expression on immune cell infiltrate and survival was assessed.

Results The effectiveness of RT and combination immunotherapy was not significantly reduced by NK or T cell depletion, and TCR KO mice had similar tumor growth and survival to mice that underwent T-cell depletion. Moderate MHC class I expression did not slow tumor growth or improve survival in mice bearing 9464D-GD2 tumors (over those with low MHC-I) following treatment. Moderate MHC class I expression also did not alter individual immune cell subsets in treated tumors (figure 1). Overall, increased infiltration of CD8 T-cells, CD4 T-cells, and depletion of T regulatory cells was observed in all treated tumors ($p < 0.05$).

Conclusions Treatment with RT and combination immunotherapy (IT-IC, anti-CTLA4, anti-CD40, CpG) may act through mechanisms that are MHC class I, NK-cell and T-cell independent. Further investigation of the role of innate immunity and myeloid subsets in this scenario is warranted.

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NK CELLS ACTIVATION AND RECRUITMENT TO IRRADIATED TUMORS IS INCREASED IN THE PRESENCE OF IL-15

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Background Focal radiotherapy (RT) promotes tumor infiltration by conventional dendritic cells type 1 (cDC1), an effect dependent on radiation's ability to induce type I interferon (IFN-I) secretion. We recently demonstrated that peritumoral s.c. IL-15, while ineffective by itself, synergized with RT, inducing complete regression of the irradiated tumor and long-term protective memory in two murine carcinomas models (TSA, MCA-38) (1). These responses were abrogated in the absence of CD8 T cells or cDC1. Detailed investigations in the TSA model showed that, whereas IL-15 alone had no effects on cDC1, it did significantly increase intratumoral cDC1 numbers and expression of costimulatory molecules CD80 and CD86 induced by RT (1). In addition to CD8 T cells, IL-15 activates NK cells, which have also been implicated in cDC1 tumor recruitment (2). Thus, we hypothesized that NK cells may play a role in the synergy between radiation therapy and IL-15.

Methods To test this hypothesis, BALB/c mice were injected with TSA mammary carcinoma cells and treated with RT (8Gy X3) and daily subcutaneous injections of IL-15 (5µg). Tumors were excised at day 18 and analyzed by immunostaining for NKp46+ cells on tumor sections and flow cytometry after tumor dissociation.

Results The number of intra-tumoral NKp46+ NK cells was significantly higher ($p < 0.005$) in mice treated with IL-15 as compared to control. Whereas RT itself had no effect, it further increased NK cell numbers above what was achieved by IL-15 alone ($p < 0.05$). In addition, tumor infiltrating NK cells expressed higher levels of CD137/4-1 BB, an effect largely driven by IL-15. Finally, NK cells depletion by anti-asialo GM1 before initiation of the treatment abrogated the enhanced cDC1 infiltration in tumors of mice treated with RT + IL-15, and the therapeutic effect of the combination.

Conclusions Our results strongly suggest a role for NK cells in the anti-tumor immune response induced by the combination of RT and IL-15. We are currently working to confirm the role of NK cells by using a complementary approach of engineering TSA cells to overexpress CLEC2D/Clr-b, the ligand for the inhibitory NKR-P1 NK receptor (3,4). Data obtained will improve current knowledge about the interaction of RT with IL-15 and support a rationale strategy for translation to the clinic.

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THE IMMUNO-METABOLIC ENZYME FASN PREVENTS ANTI-TUMOR IMMUNE RESPONSES IN IRRADIATED GLIOBLASTOMA

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Background Immunotherapy (IT) has evolved as an essential pillar against cancer due to unprecedented successes in several malignancies. However, only 10% of glioblastoma (GBM) patients respond to IT, presumably due to the paucity of tumor-infiltrating lymphocytes. Radiotherapy (RT) can promote T cells infiltration to generate anti-tumor immune responses, but can also exacerbate potent immune inhibitory mechanism to facilitate immune evasion. Among which, metabolic reprogramming of irradiated GBM represents an emerging mechanism of immune resistance. Notably, increased lipogenesis by the fatty acid synthase (FASN) is a hallmark of GBM that was shown to mediate radioresistance and immunosuppression in other cancer types. Therefore, we hypothesize that de novo lipid biosynthesis mediated by FASN represents an innate immune evasion mechanism in irradiated GBM.

Methods We first defined metabolic changes 24hrs after RT (10 gray - Gy) by seahorse assay and metabolomics in the

syngeneic murine GBM model, GL261. To confirm alterations in the lipogenesis pathway, we measured the expression FASN by western blot and the cell lipid content by BODIPY staining and flow cytometry. Finally, GL261 cells were engineered to express an inducible shRNA silencing FASN (GL261shFASN) or its non-silencing control (GL261shNS) and orthotopically implanted on day 0. On day 6, knockdown of FASN was induced by feeding the mice with doxycycline. On day 11, mice received 10Gy irradiation selectively to the tumor. Evaluation of the immune contexture was determined by in situ immunofluorescence on day 19 (n=3/group). Remaining mice were followed for survival (n=7/group).

Results Mitochondrial respiration and glycolysis were significantly enhanced in RT-GL261 cells in vitro. Metabolomic profiling of RT-GL261 cells showed a strong increase in pathways related to nucleotide, amino acids and lipid metabolism. Consistent with this last observation, we found upregulation of FASN and lipids accumulation in RT-GL261 cells as compared to non-RT GL261 cells. In vivo, GL261shFASN tumors presented increased infiltration of CD11c+ and CD8+ T cells as compared to GL261shNS tumors; an observation that was amplified in RT-GL261shFASN tumors. Consistent with a recruitment of CD11c+ and CD8+ T cells, 43% of mice bearing GL261shFASN tumor survived for at least 60 days without tumor regrowth vs. 35 days in GL261shNS tumor bearing mice.

Conclusions Altogether our data suggest that RT is inducing a metabolic reprogramming of GBM by promoting FASN-mediated lipid synthesis to foster immunosuppression. While much work remains to be done, our data propose FASN as a novel therapeutic target to overcome immunosuppression and sensitize irradiated GBM to ITs.

Ethics Approval All mice experiments were approved by the Institutional Animal Care and Use Committee, protocol number 2019-0042.

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IMPROVING SPECIFIC TARGETING OF TUMORS THROUGH BISPECIFIC SNIPER ANTIBODIES

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Background Disialoganglioside 2 (GD2) is expressed on neuroblastomas as well as melanomas, small cell lung cancers, and sarcomas. Anti-GD2 mAb (Dinutuximab) can be used to treat these cancers and is part of the standard care for neuroblastoma. While GD2 is expressed minimally on most normal tissues, it is expressed on some nerve cells, and anti-GD2 treatment can cause neuropathic pain. A separate tumor-antigen, B7H3, is overexpressed on multiple tumor types, including those listed above, with minimal expression on most normal cells and no expression on nerve cells. We developed a bispecific SNIPER antibody, INV721, to simultaneously target these 2 tumor antigens, with one arm specific to GD2 and the other arm to B7H3. The individual Fab arms targeting GD2 and B7H3 are each low to moderate affinity, such that INV721 will only bind with high affinity when both arms bind to their antigens on the same cell, resulting in high-specificity of the SNIPER to tumor cells.

Methods INV721 binding to GD2/B7H3-expressing tumors was confirmed by flow cytometry, as well as in tumor-bearing mice injected with ⁸⁹Zr-labeled to monitor in vivo biodistribution via positron emission tomography imaging. Antibody-dependent cellular cytotoxicity (ADCC) testing of INV721 was performed on human neuroblastoma and melanoma cell lines with an Incucyte spheroid-killing-assay. In vivo efficacy studies were carried out in mice bearing GD2/B7H3-expressing melanoma tumors to test our in situ vaccine (ISV) regimen, which included testing combinations of external beam radiation therapy (RT, 12Gy) ± INV721 (40 ug/dose) ± IL2 (75K U/dose).

Results INV721 showed binding by flow cytometry to tumors that express both GD2 and B7H3 but minimal binding to cells that don't express both antigens. ⁸⁹Zr-INV721 showed elevated and persistent accumulation in the tumor with minimal uptake in normal tissues. Incucyte spheroid-killing assays revealed that INV721 was capable of ADCC. The ISV combination of RT+INV721+IL2 was capable of curing mice bearing ~57 mm³ melanoma tumors (12/12 mice tumor free), with >70% of these mice exhibiting long-term immune memory.

Conclusions INV721 binds to cells that express both GD2 and B7H3, and these preliminary studies show that INV721 is effective in our ISV regimen at curing mice bearing tumors that express these antigens. We are continuing our efforts to determine if INV721 is associated with less pain than Dinutuximab. The goal of this SNIPER-antibody is to enhance the tumor-specific delivery of therapeutic mAbs, which may decrease toxicity and improve efficacy for cancers expressing both GD2 and B7H3.

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SINGLE AGENT IMMUNOTHERAPY RESPONSE IN PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA WITH PRIOR HISTORY OF RADIATION THERAPY

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Background Locally advanced head and neck squamous cell carcinomas (HNSCC) are treated with multidisciplinary approach which includes radiation therapy. Immunotherapy with nivolumab or pembrolizumab is used for platinum refractory disease. We analyzed the association of radiation treatment patterns and immunotherapy responses HNSCC.

Methods We performed a retrospective analysis at University of New Mexico Comprehensive Cancer Center for patients with diagnosis of HNSCC treated at our institution between 2011 and 2020 with immunotherapy agent's nivolumab and pembrolizumab. Our cohort included 21 patients with previous history of definitive radiation therapy for HNSCC who received immunotherapy for recurrent disease, as part of adjuvant treatment as either front-line or second line therapy. In terms of response, patients were divided into responders (R) and non-responders (NR). Responders were defined as the presence of partial remission in initial imaging or stable disease for a period of six months or longer.

Results Of our 21 patients, 10 patients were R and 11 patients were NR. p16 positivity was 6 (60%) in R vs 3 (27%) in NR. 8 patients in R group (80%) and 10 patients in NR group (91%) had prior platinum based chemotherapy concurrent with radiation or for recurrence as salvage