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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461 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 mm^3 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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462 SINGLE AGENT IMMUNOTHERAPY RESPONSE IN PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA WITH PRIOR HISTORY OF RADIATION THERAPY

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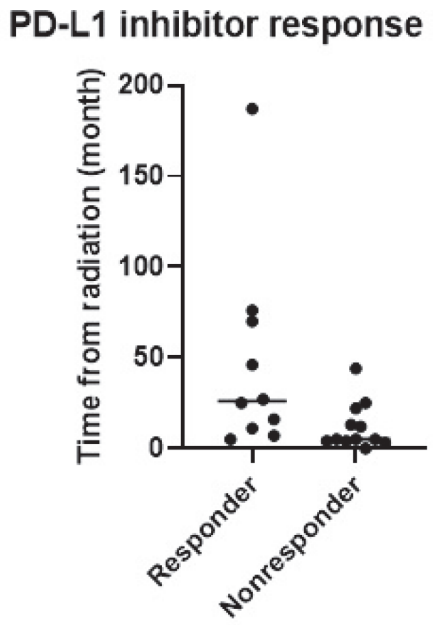
Background Locally advanced head and neck squamous cell carcinomas (HNSCC) are treated with multidisciplinary approach which includes radiation therapy. Immunotherapy with nivolumab or pembrolizumab is used for platinum refractory disease. We analyzed the association of radiation treatment patterns and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



Abstract 462 Figure 1 Time from last day of radiation treatment to start of immunotherapy

chemotherapy.All patients had radiation therapy prior to immunotherapy for adjuvant or for definitive treatment. Time from last day of radiation treatment to start of immunotherapy was 47 months in R group while it was 9 months in NR group (P<0.05). (Figure 1) There was no difference in time from radiation to immunotherapy depending on the P16 status. Immunotherapy was stopped after completing 2 years of immunotherapy for 3 patients. 2 of those patients resumed immunotherapy due to progression, and continue to have response after resuming treatment. One of these patients received SBRT to lung nodule after resuming immunotherapy.

Conclusions Immunotherapy with single agent PD-L1 inhibitor is used for platinum refractory disease in HNSCC, however response rates are low. Our study shows that the patients who had early recurrence and received immunotherapy closer to definitive radiation therapy had lower response rate. Therefore we need further studies to investigate changes in immune microenvironment with radiation therapy for better immune targeting of patients with early recurrence after radiation treatment.

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463 METASTATIC GASTRIC CANCER PATIENT BENEFITING FROM COMBINED RADIO-IMMUNOTHERAPY TREATMENT DISPLAYED SUSTAINED ANTI-NY-ESO-1 SPECIFIC T CELLS AND EXPRESSED IMPORTANT IMMUNO-MODULATORY MARKERS

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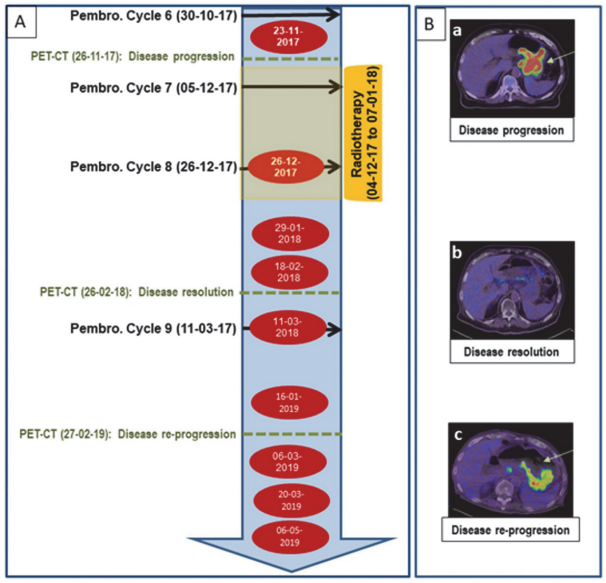
Background Combined radio-immunotherapy is currently being investigated to treat cancer patients. Anti-PD-1 immunotherapy offers the prospect of long-term disease control in solid tumors. Radiotherapy has the ability to promote immunogenic cell death leading to the release of tumor antigens, increasing infiltration and activation of T cells. NY-ESO-1 is a cancer-testis antigen expressed in 20% of advanced gastric cancers and known to induce humoral and cellular immune responses in cancer patients. We report on the dynamic immune response to the NY-ESO-1 antigen and important immune-related biomarkers in a metastatic gastric cancer patient treated with radiotherapy combined with anti-PD-1 pembrolizumab antibody.

Methods Our patient was an 81-year-old male diagnosed with locally advanced unresectable MMR-deficient gastric cancer having progressed to a metastatic state under a second line of systemic treatment consisting of an anti-PD-1 pembrolizumab antibody. The patient was subsequently treated by local radiotherapy administered concomitantly with anti-PD-1, with a complete response on follow-up radiologic assessment. Disease control was sustained with no further therapy for a period of 12 months before relapse (figure 1).

Results We have identified an NY-ESO-1-specific IFN-? secretion from the patients T cells that was significantly increased at response (****p?0.0001) (figure 2). A novel promiscuous immunogenic NY-ESO-1 peptide P39 (P153-167) restricted to the 4 patient's HLA-DQ and HLA-DP alleles was identified. Interestingly, this peptide contained the known NY-ESO-1-derived HLA-A2-02:01(P157-165) immunogenic epitope. We have also identified a CD107+ cytotoxic T cells subset within a specific CD8+/HLA-A2-NY-ESO-1 T cell population that was low at disease-progression, markedly increased at disease-resolution and significantly decreased again at disease-re-progression (figure 3). Finally, we identified 2 groups of cytokines/chemokines. Group 1 contains 5 cytokines (IFN-?, TNF-a, IL-2, IL-5 and IL-6) that were present at disease progression, significantly downregulated at disease resolution and dramatically upregulated again at disease re-progression. Group 2 contains 4 biomarkers (Perforin, sFAS, MIP-3a and CXCL-11/ITAC) that were present at disease progression, significantly upregulated at disease resolution and dramatically downregulated again at disease re-progression (figure 4).



Abstract 463 Figure 1 Clinical course of disease and time-points for collection of blood samples, for pembrolizumab (Pembro) cycles, radiotherapy sessions, and PET-CT imaging