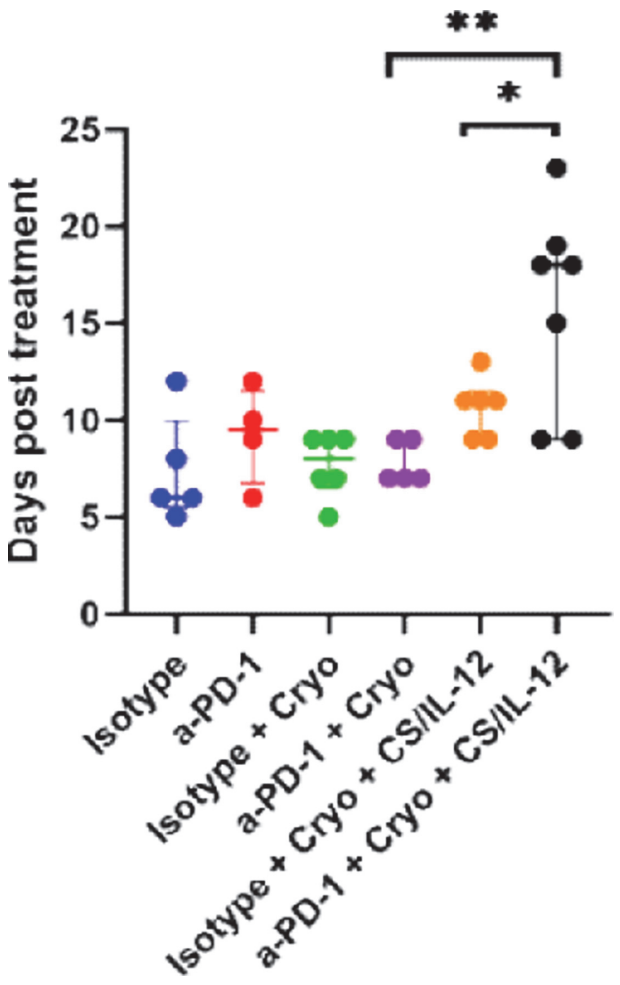

Abstract 457 Figure 2 Days p.t. for untreated MC38 to reach 500 mm^3

Days post treatment to reach 500 mm^3 was determined as the day on which the untreated tumor volume measurement exceeded 500 mm^3 for the first time and growth continued to increase afterward for at least two days. Statistics: One-way ANOVA * p<0.05, ** p<0.01

were protected (5/5) compared to only 43% protected of the cryo + CS/IL-12 group (3/7).

Conclusions Conclusions: While cryoablation in combination with immunotherapy has the potential to treat advanced, unresectable primary tumors and distant untreated tumors, the addition of a single injection of IL-12 is not enough to induce a strong abscopal effect. Furthermore, it may actually worsen the establishment of effector memory cells. The addition of anti-PD-1 only slows abscopal tumor growth. Future work is needed to understand the mechanism of T cell priming in the context of the post-ablative tumor.

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458 **ANTITUMOR MECHANISMS OF LOCAL RADIATION AND COMBINATION IMMUNOTHERAPY IN AN IMMUNOLOGICALLY COLD MODEL OF NEUROBLASTOMA**

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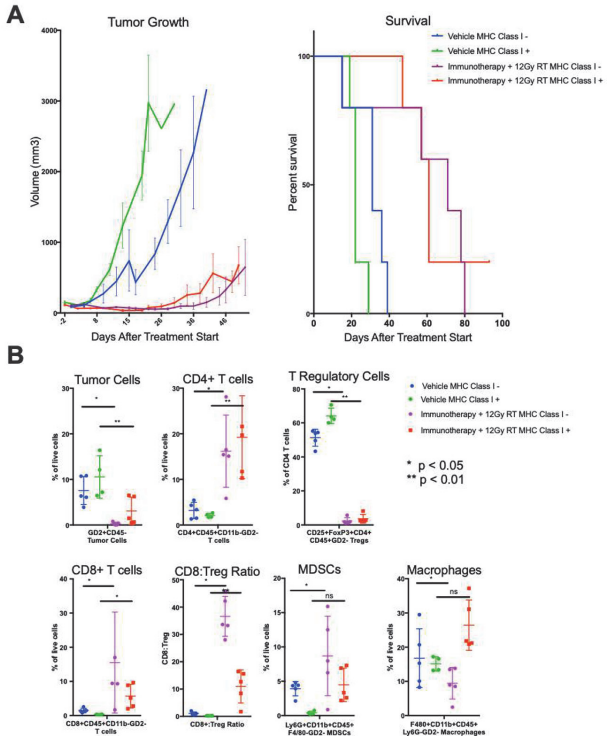
Background The standard treatment for high-risk neuroblastoma includes a combination immunotherapeutic approach consisting of IL-2, GM-CSF, and monoclonal antibodies directed against GD2, a disialoganglioside preferentially expressed in neuroblastoma and melanoma.[1] We recently described an effective a preclinical in-situ vaccination strategy combining local radiation therapy (RT), IL-2-linked to anti-GD2 monoclonal antibody (intratumoral immunocytokine, IT-IC), checkpoint inhibition (anti-CTLA4), and drivers of innate immunity (anti-CD40 and CpG).[2] This strategy is effective in curing mice with immunologically-cold neuroblastoma. We sought to better characterize the anti-tumor mechanisms that mediate this effect.

Methods Mice bearing GD2-expressing, immunologically-cold neuroblastoma tumors (9464D-GD2) were treated with 12Gy RT and combination immunotherapy (IT-IC, anti-CTLA-4, CpG, anti-CD40) over 12 days as previously described.[2] Depletion of individual immune cell sets during treatment was



Abstract 458 Figure 1 Effect of MHC class I expression on response to RT and combination immunotherapy (IT-IC, anti-CTLA4, anti-CD40, CpG). A) Increased MHC class I expression in 9464D-GD2 derived tumors did not alter tumor growth or survival following treatment. B) Increased MHC class I expression did not alter immune subsets following treatment of 9464D-GD tumors with radiation and combination immunotherapy. Increased numbers of CD8+ and CD4+ T-cells was observed with both moderate and absent MHC class I expression. T regulatory cells were also effectively depleted in both treated groups

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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459 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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460 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