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