Abstract # 1358

Fatty acid metabolism limits type I interferon responses in irradiated glioblastoma

Mara De Martino[1*], Camille Daviaud[1], María Cecilia Lira[1], Matthew H. Ingalls[2], Fabian Schneider[3], Regan Baird[3], James Mansfield[3], J. Spencer Schwarz[2], Oliver Braubach[2] and Claire Vanpouille-Box[1,4]

[1]Department of Radiation Oncology, Weill Cornell Medicine, New York, NY, USA. [2]Bruker Spatial Biology, Saint Louis, MO, USA. [3]Visiopharm A/S, Horsholm, Denmark. [4]Sandra and Edward Meyer Cancer Center, New York, NY, USA.

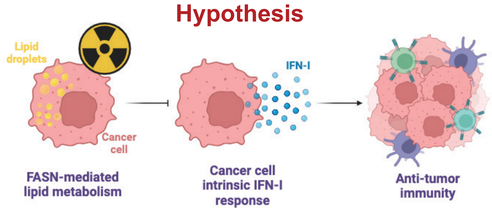
*mde4002@med.cornell.edu

Society for Immunotherapy of Cancer sitc2024 NOV. 6-10 | HOUSTON

Background

Radiation therapy (RT) induces nucleic acid sensing followed by cancer cell-intrinsic interferon (IFN-I) responses in various cancers. However, combining RT with immunotherapy in glioblastoma has failed to improve patient survival, suggesting robust immunosuppression. Our previous findings showed that RT induces a metabolic shift towards fatty acid synthase (FASN)-mediated lipid synthesis, supporting glioblastoma survival (De Martino, et al. Cancer Lett. 2023). Since fatty acid metabolism hampers IFN-I responses in viral immunity, we **hypothesized that FASN might similarly inhibit IFN-I in irradiated glioblastoma, acting as an immune evasion mechanism.**

Hypothesis



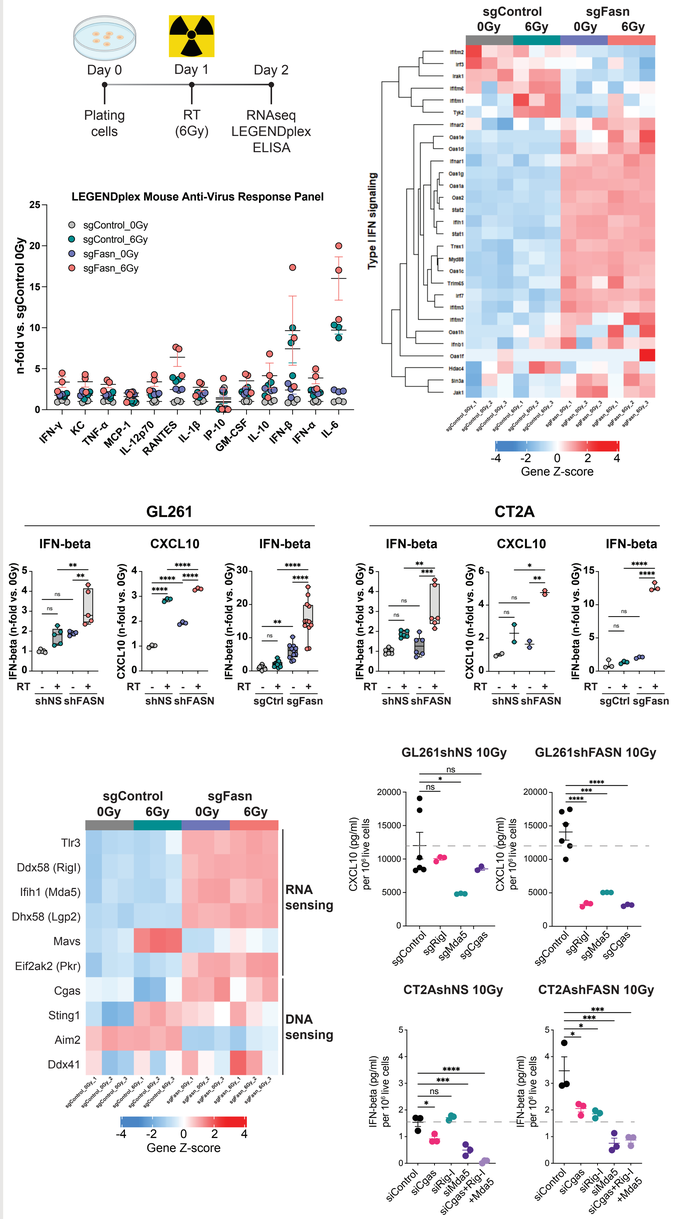
Methodology

- *In vitro*, FASN was targeted using a doxycycline-inducible shRNA, CRISPR/Cas9, and FASN inhibitors (FASNi) in murine glioblastoma cells (GL261 and CT2A). Nucleic acid sensors (cGAS, RIG-I, and MDA5) were blocked using CRISPR/Cas9. Glioblastoma cells derivatives were irradiated with 6Gy and collected 24hrs post RT to assess IFN-I by RNAseq, LEGENDplex assay, and ELISA.
- *In vivo*, CT2AshNS and CT2AshFASN cells were intracranially injected and selective irradiation (10Gy) was administered on day 10. Anti-IFNAR antibody was injected on days 6, 10 and 14. To evaluate the translation potential, mice bearing glioblastoma tumors received systemic FASNi (daily, i.p., days 9-18) and clinically relevant radiation (5 fractions of 6Gy). The immune landscape was evaluated by flow cytometry, immunofluorescence, and multiplex imaging with CellScape ChipCytometry (Bruker Spatial Biology) followed by analysis using Phenoplex (Visiopharm) (n=3-5/group).
- All statistical analyses were performed using GraphPad Prism 10 and are considered significant when $p<0.05$. Differences between multiple conditions were determined using a oneway ANOVA test. If the ANOVA residuals were normally distributed (Shapiro–Wilk test), a Tukey follow-up test was then performed for multiple comparison.

Summary

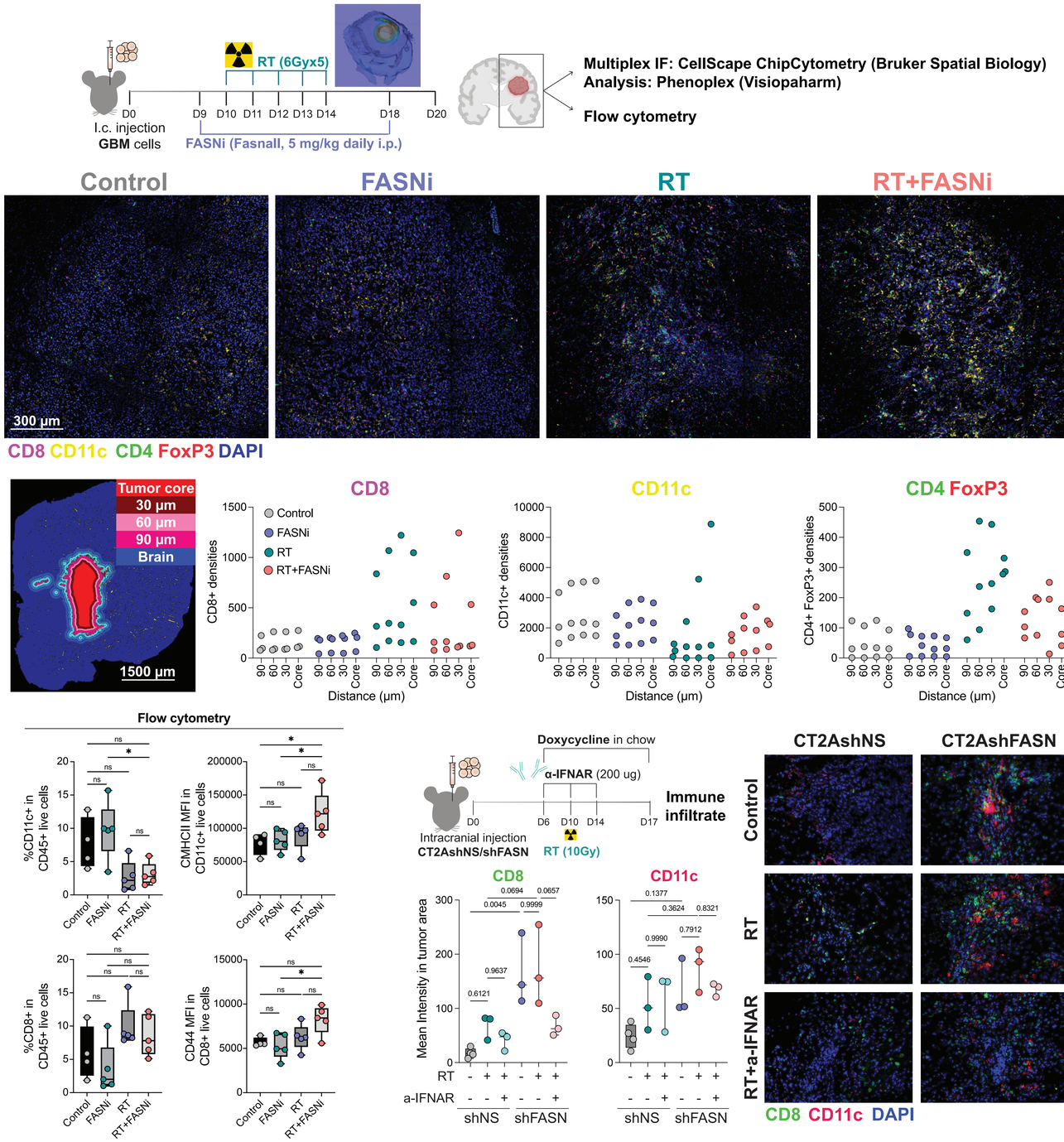
Our data suggest that fatty acid metabolism regulates the immunogenicity of irradiated GBM and highlights **FASN as a key driver of immune evasion and a potential target to enhance the immunostimulatory effects of RT in GBM.**

I- RT-induced FASN prevents IFN-I production in GBM



To investigate FASN-mediated lipid synthesis in IFN-I responses in irradiated GBM, FASN-blocked GBM cells were subjected to RT *in vitro*. FASN blockade synergized with RT to increase IFN-I. Blocking cGAS, RIG-I, or MDA5 significantly reduced IFN-beta and CXCL10 levels, with triple knockdown of cGAS+RIG-I+MDA5 further suppressing IFN-beta production. These findings suggest that both dsDNA and dsRNA sensing pathways drive IFN-I induction in irradiated FASN-deficient GBM cells. Thus, **RT-induced FASN inhibits nucleic acid sensing to limit IFN-I responses in irradiated GBM.**

II- Targeting FASN promotes immune infiltration in irradiated GBM



To study the role of FASN in immune evasion within GBM, spatial multiplexing and flow cytometry were performed on irradiated GL261 tumors treated with systemic pharmacological inhibition of FASN. Density assessments in the tumor core and margins revealed that FASN inhibition, in combination with RT, enhances infiltration of CD8+ T cells and CD11c+ cells while reducing Treg infiltration. Flow cytometry analysis showed activation of CD8+ and CD11c+ cells. Immunofluorescence confirmed increased presence of CD8+ and CD11c+ cells in CT2AshFASN tumors (n=3). This effect was lost when the IFN-I pathway was blocked with anti-IFNAR, suggesting that **targeting FASN promotes immune cell infiltration in irradiated GBM via IFN-I signaling.**