INDUCING IMMUNE RESPONSE WITH FLASH AND CONVENTIONAL RADIATION IN DIFFUSE MIDLINE GLIOMA (DMG)

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Background Diffuse Midline Glioma (DMG) is a fatal inoperable pediatric brain tumor associated with H3K27M mutation and median survival of 9–15 months. Radiation therapy (RT) is the primary treatment extending life by three months. Conventional dose-rate RT (2Gy/min, CONV) can induce an immune response with type 1 interferon (IFN1) in multiple cancers including adult brain tumors, but IFN1 response in DMG is unknown. Ultra-fast dose-rate RT (90Gy/second, FLASH) has comparable tumor control to CONV with decreased toxicity, however, evaluation of immune response is limited. In this study, we compared immune response in murine DMG post CONV and FLASH RT using single-cell RNA sequencing (scRNA-seq) and flow cytometry.

Methods We treated an orthotopic, syngeneic murine model of brainstem DMG (4423) with 15Gy CONV or FLASH RT. Four days post-RT, we isolated CD45+ cells and performed scRNA-seq to compare CONV, FLASH, unirradiated tumor and normal brainstem. We also performed flow cytometry at both day 4 and day 10 post-RT. Single-sample gene set enrichment analysis (ssGSEA) was performed and scores compared using Mann-Whitney U test. Flow cytometry proportions were evaluated using Mann-Whitney U test or Kruskal-Wallis corrected for multiple comparisons.

Results ScRNA-seq reveals 33,308 immune cells in 17 unique clusters (figure 1A). Four distinct microglia subtypes are found representing a spectrum from homeostatic to activated with more activated microglia in RT groups. ssGSEA for IFN1 pathway in microglia shows no difference in FLASH and CONV compared to tumor (p=0.5994). Flow at day 4 shows no significant difference in proportion of immune cells comparing FLASH, CONV and tumor. We find increased IFN1 pathway scores for CONV compared to FLASH in MACs (p<0.0001, figure 1B) and DCs (p<0.0001, figure 1D). We find a similar trend by flow cytometry at day 4 with IFNAR on MACs (p=0.066, figure 1C) and DCs (p=0.0079, figure 1E). Flow at day 10 shows increased IFNAR in MACs in both CONV (p=0.0058) and FLASH (p=0.0109) compared to tumor while IFNAR in DCs is similar.

Conclusions In summary, while immune proportions are similar, we find differential IFN1 enrichment in CONV compared to FLASH at day 4 in MACs and DCs, while this response at day 10 is similar comparing dose rates, possibly due to a later IFN1 response in FLASH. Our work is the first to study immune alterations and IFN1 comparing different dose-rates of RT in DMG, highlighting the potential for combining radiation and immunotherapy in these tumors.

Abstract 53 Figure 1 Clustering of CD45+ immune cells and differential production of IFN1 response. a) Clustering of 33,308 CD45+ immune cells by scRNA-seq. b) IFN1 pathway enrichment scores of cells in each group in the macrophage cluster by scRNA-seq (****=p<0.0001, **=p<0.01). c) IFNAR+ expression of macrophages in each group by flow cytometry at day 4 and day 10. d) IFN1 pathway enrichment scores of cells in each group in the dendritic cells cluster by scRNA-seq (****=p<0.0001). e) IFNAR+ expression of dendritic cells in each group by flow cytometry at day 4 and day 10.

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