TERTIARY LYMPHOID STRUCTURES IN MELANOMAS HARBOUR INCREASED PROPORTIONS OF STEM-LIKE EFFECTOR T-CELLS COMPARED TO TUMOR-INFILTRATING LYMPHOCYTES

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Background Tertiary lymphoid structures (TLS) in melanoma are associated with improved survival and response to checkpoint blockade therapy (CBT). However, whether and how TLS contribute to this response is unknown. If TLS contribute to the responses, they may be expected to contain higher proportions of T-cells with therapeutic potential, than tumor infiltrating lymphocytes outside of TLS. TCF1/TCF7 has been identified as a marker for a stem cell-like T-cell that mediates the proliferative response post-CBT in tumor infiltrating cells. We hypothesized that TLS would contain higher fractions of stem-like TCF7+ and TCF7+TOX+ T-cells poised to respond post-CBT. To address this hypothesis, we evaluated TLS and tumor sites of both desmoplastic and non-desmoplastic melanomas, for markers of stem like T-cells (TCF7, TOX).

Methods Primary desmoplastic melanomas (PDM) and non-desmoplastic metastatic melanomas (NDMM) were evaluated for TLS presence (yes/no) and immune markers, by 7-color multiplex Immunofluorescence histology. Specimens were stained with the following two panels: TLS panel: CD20, CD3, CD8, PNAd, DC-Lamp, Ki67, and DAPI; T cell activity panel: CD3, CD8, CD20, Granzyme-b, TOX, TCF7, and DAPI. Lymphoid aggregates were identified as TLS if possessing organized T-cell and B-cell regions in addition to high endothelial venule-like vasculature (PNAd+). Cells were enumerated from TLS and tumor regions of TLS+ tumor specimens from PDM, and NDMM, with Halo software (Indica Labs). CD3+CD8+ (CD8+) cells and CD3+CD8- (CD4+) cells were evaluated for activation markers, and the proportions of cells expressing activation markers was calculated. Statistical comparisons were done with unpaired Wilcoxon tests in RStudio.

Results TLS were identified in 25 out of 39 evaluated (64%) PDM, and 37 of 111 (33%) NDMM. Comparing TLS to tumor outside the TLS, we found that PDM TLS contain significantly elevated proportions of CD8+TCF7+ cells and CD4+TCF7+ cells compared to tumor (table 1). NDMM TLS also contain elevated proportions of CD8+TCF7+ cells and CD4+TCF7+ cells relative to tumor (table 1). In addition, TLS in PDM and NDMM contain elevated proportions of CD8+TOX+TCF7+ cells and CD4+TOX+TCF7+ cells relative to their respective tumor (table 1).

Conclusions The elevated proportion of TCF7+ T-cells, and TCF7+TOX+ T-cells in TLS from PDM and NDMM relative to their tumor suggests that stem-like T-cells are enriched in TLS, and that T-cell regeneration may be occurring there. Additionally, TLS may be helping in the maintenance or generation of TCF7+ T-cells in the tumor microenvironment.