

CD68+ macrophages were found to be closely associated with tumor cells, PD-L1+ macrophages were found to have the closest interaction with tumor cells. The potential of these cell phenotypes to generate a strongly immunosuppressive microenvironment need to be explored in additional cases.

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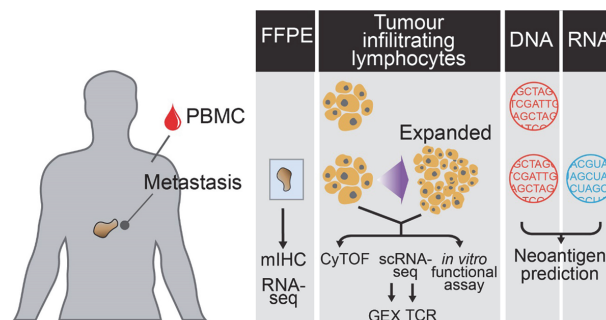
548 **CD8+ TISSUE-RESIDENT MEMORY T CELLS ARE TUMOUR REACTIVE AND INCREASE AFTER IMMUNOTHERAPY IN A CASE OF METASTATIC MUCOSAL MELANOMA**

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Background Mucosal melanoma is a rare subtype of melanoma originating from mucosal tissues,¹ metastases are very aggressive and respond poorly to therapy, including immune checkpoint inhibitors (ICI) such as anti-CTLA4 and anti-PD1 antibodies.^{2–5} CD8+ T cells constitute the most abundant immune infiltrate in metastatic melanoma, of which the Tissue Resident Memory subset (TRM) is of particular interest.⁶ CD8+ TRM cells express the highest levels of immune checkpoint receptors, proliferate in response to ICI and correlate with longer disease-free and overall survival.^{6–8} The immune landscape in mucosal melanoma remains poorly characterized. We aimed to: 1) phenotype CD8+ T cells and TRM infiltrating metastatic mucosal melanoma, 2) characterize the clonality of TRM in relation to other CD8+ T cell subsets and 3) define the capacity of CD8+ T cells and TRM to respond to melanoma cells and to in vivo and in vitro anti-PD1 treatment.

Methods We investigated the CD8+ T and TRM cells infiltrating two temporally- and spatially-distant subcutaneous metastases, these originated from a primary vaginal mucosal melanoma. One metastasis was excised prior to anti-PD1 treatment and one was anti-PD1 refractory, having progressed on treatment. We used mass cytometry and single-cell RNA and TCR sequencing to characterise the phenotype and clonality of the T cells, multiplex immunohistochemistry to define their spatial relationship with tumour cells and other T cells, and functional assays to determine TRM response to tumour cells (figure 1).

Results CD8+ TRM frequency increased with time and anti-PD1 treatment, forming clusters at the tumour margin. T cells in the anti-PD1 refractory lesion were more activated than T cells in the first tumour and were bound by anti-PD1 antibody in vivo. T cells could not be stimulated by anti-PD1 directly ex vivo. Both metastatic lesions shared common T cell clusters including TRM. Furthermore, TRM in each tumour shared T cell clones, suggesting the presence of common antigens between metastatic sites. Indeed, the two metastases had a similar mutational profile. In vitro expanded tumour infiltrating lymphocytes from both lesions recognized tumour cells from both lesions and the same neoantigen generated from a single point mutation in the gene CDKN1C. Finally, tumour cells stimulated TRM cells more robustly than other T cell subsets.



Abstract 548 Figure 1 Graphical depiction of the methods used to characterise T cells in mucosal metastatic melanoma

Conclusions In this patient with vaginal mucosal melanoma, subsequent melanoma metastases of clonal origin attracted CD8+ T cells of similar specificity, among which TRM cells responded more vigorously to tumour cells than other T cell subsets.

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Ethics Approval Patients diagnosed with stage 3 or 4 metastatic melanoma and undergoing clinically indicated surgery were enrolled in prospective studies approved by the Peter MacCallum Cancer Centre human ethics research committee (13/141). All experimental protocols have been approved and clinical data has been collected prospectively.

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549 **CHARACTERIZING DOUBLE POSITIVE T CELLS IN THE TUMOR MICROENVIRONMENT: A TALE OF PROMISCUOUS CELL FATES**

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Background CD4 and CD8 T cells are genetically and functionally distinct cell subsets of the adaptive immune system that play pivotal roles in immune surveillance and disease control. During development in the thymus, transcription factors ThPOK and Runx3 regulate the differentiation and maturation of these two lineages into single positive T cells that enter the periphery with mutually exclusive expression of either the CD4 or CD8 co-receptor.^{1–2} Despite our expectation that these two cell fates are fixed, mature CD4+CD8+ double positive (DP) T cells have been described in the context of numerous immunological responses, including cancer, but their molecular and functional properties and therapeutic relevance remain controversial and largely unknown.^{3–5}

Methods Our lab has identified and characterized a heterogeneous DP T cell population in murine and human melanoma tumors comprised of CD4 and CD8 T cells re-expressing the opposite co-receptor and a parallel uptake in the opposite cell type's phenotype and function. Using CD4 (Trp1) and CD8 (Pmel) transgenic TCR T cells specific to B16 melanoma antigens gp75 and gp100 respectively, we demonstrate the re-expression of the opposite co-receptor following adoptive T cell transfer in B16 melanoma tumor bearing mice.

Results Specifically, up to 50% of transferred CD4 Trp1 T cells will re-express CD8 to become a DP T cell in the tumor microenvironment. Further, these CD4 derived DP T cells upregulate CD8 lineage regulator Runx3 and cytolytic genes Gzmb, Gzmk, and Prf1 to become potent cytotoxic T cells. Alternatively, a subset of CD8 Pmel T cells differentiate into DP T cells characterized by the increased expression of CD4, ThPOK, and regulatory marker FoxP3 (figure 1). In addition, we utilized 10x single cell and ATAC sequencing to further characterize these divergent DP T cell populations among open repertoire T cells isolated from murine and human melanoma tumors.

Conclusions Our findings highlight the capability of single positive T cells to differentiate in response to antigen and local stimuli into novel T cell subsets with polyfunctional characteristics. The resulting cell subsets will potentially affect the tumor microenvironment in distinct ways. Our studies may inform therapeutic approaches to identify antigen specific T cells as well as innovative signaling pathways to target when genetically engineering T cells to optimize cytotoxic function in the setting of adoptive cell therapy.

Ethics Approval The human biospecimen analyses were approved by Memorial Sloan Kettering Cancer Center IRB #06-107

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Immune-stimulants and immune modulators

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AN AXL-TARGETING MONOCLONAL ANTIBODY THAT INHIBITS AXL ACTIVITY AND POTENTLY STIMULATES THE INNATE IMMUNE RESPONSE

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Background Axl is a member of the TAM (Tyro3/Axl/MerTK) family of receptor tyrosine kinases and a negative regulator of innate immunity. Activation of Axl through its ligand Gas6 leads to suppression of myeloid cell activity, while its activation in tumor cells drives tumor growth, metastasis, and is associated with acquired resistance to targeted therapies, radiotherapy and chemotherapy.

Methods Purified monoclonal antibodies and variants thereof were tested in human cancer lines and primary human myeloid cells for effects on Axl signaling and immune activation, respectively.

Results We describe a humanized IgG1 Axl-targeting monoclonal antibody (mAb), CDX-0168, that binds to the ligand-binding domain of Axl with sub-nanomolar affinity and potently inhibits Gas6 binding. In tumor cells, CDX-0168 inhibits Gas6-dependent Axl phosphorylation and signaling and elicits tumor cell killing via ADCC in vitro and in vivo. In primary human immune cells, CDX-0168 treatment induces potent release of pro-inflammatory cytokines and chemokines from dendritic cells, monocytes and macrophages through an Fc receptor-dependent mechanism and enhanced T cell activation in mixed lymphocyte reactions. Axl inhibition may further enhance antitumor activity associated with PD-(L)1 blockade. To this end, we generated a tetravalent bispecific Axl x PD-L1 antibody combining CDX-0168 with a potent anti-PD-L1 mAb (9H9) using an IgG-scFv format. The bispecific antibody elicits greater cytokine release and T cell activation in vitro than the combination of the parental antibodies, while maintaining robust Axl and PD-L1 blockade.

Conclusions Additional studies investigating simultaneous blockade of the Axl and PD-L1 pathways with other agents may further exploit the potential for this novel anti-cancer therapeutic approach.

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SUMOYLATION INHIBITOR TAK-981 ACTIVATES NK CELLS AND MACROPHAGES VIA TYPE I INTERFERON SIGNALING AND SHOWS SYNERGISTIC ACTIVITY IN COMBINATION WITH RITUXIMAB AND DARATUMUMAB IN PRECLINICAL MODELS

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Background TAK-981 is a first-in-class small molecule inhibitor of the SUMO activating enzyme in Phase 1 clinical trials. SUMOylation has previously been implicated in the