384 AN INVESTIGATION INTO THE ROLE OF CD4+ TUMOR-INFILTRATING LYMPHOCYTES (TIL) IN METASTATIC MELANOMA PATIENTS WITH A COMPLETE RESPONSE TO ADOPTIVE CELL THERAPY

[1]MacLean Hall*, [1]Holly Branthoover, [1]Patrick Innamarato, [1]Amy Hall, [1]Alex Alfaro, [1]Allison Richards, [1]Jeani Rich, [1]Jonathan Hensel, [2]Jim Bender, [2]Jake Ceccarelli, [2]TJ Langer, [1]Matthew Beatty, [1]John Mullinax, [1]Jamie Teer, [1]Amod Sarnaik, [1]Shari Pilon-Thomas. *[1]Moffitt Cancer Center, Tampa, FL, USA; [2]Turnstone Biologics, New York, NY, USA*

Background Immunotherapy for cancer has long been focused on the generation of CD8+ cytotoxic T lymphocyte responses, independent of their dynamic CD4+ T cell counterpart. One promising approach, adoptive cell transfer (ACT) of tumor-infiltrating lymphocytes (TIL), has yielded response rates ranging from 28–55%.[1–2] Investigation into the role of CD4+ TIL in this setting remains critically underexplored as an opportunity to improve upon these successes.

Methods Two metastatic melanoma patients (PT1 and PT2) were treated with TIL on a completed clinical trial at Moffitt Cancer Center (NCT01005745). Tumor recognition by TIL was assessed via co-culture with tumor. Whole exome (WES) and RNA Sequencing were performed on cryopreserved tumor sections and mutant peptide-MHC binding was predicted. TIL were stimulated with antigen presenting cells (APCs) loaded with neoantigen-derived 25mer peptides and sorted based on 41BB/OX40 upregulation, followed by functional immunologic assays. TCR sequencing was conducted on patient peripheral blood as well as isolated neoantigen-specific TIL clones to determine persistence in vivo and cognate peptide-MHC targets were determined empirically.

Results PT1, infused with predominantly CD4+ TIL (88%), achieved a complete response (CR) despite lack of IFNγ detection with conventional in vitro tumor co-culture methods. Infusion product TIL were sorted by upregulation of OX40 and 41BB upon stimulation with APCs loaded with the mutant peptide pool. Neoantigen reactivity arose from a single peptide sequence, which conferred recognition by a CD4+ TIL clone, which comprised 17% of the infusion product and enriched to greater than 80% after sorting via FACS. These CD4+ TIL produced IFNγ, TNFα, and granzyme B in response to peptide-loaded APCs in an HLA-DR dependent manner. TCRβ overlap revealed this CD4+ clone peaked at two weeks post-infusion (40%) and persisted after infusion for at least six weeks. PT2 was infused with highly reactive, primarily CD8+ (88%) TIL and also achieved a CR. Isolated CD4+ TIL were also responsive to tumor antigens in the context of MHC Class II in vitro. Tumor-reactive CD4+ TIL were enriched by IFNγ capture and delayed xenograft growth in vivo ($p<0.01$). Neoantigen peptides stimulated predominantly CD4+ TIL to upregulate OX40/41BB and produce IFNγ, TNFα, and granzyme B.

Conclusions Investigation of these case studies demonstrated evidence of CD4+ TIL involvement in complete clinical responses after ACT. Ongoing studies will define the precise role of tumor-reactive CD4+ T cells in the anti-tumor immune response and provide the framework for future investigation into their function and therapeutic efficacy.

Trial Registration NCT01005745

REFERENCES

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Ethics Approval Approved by USF IRB approval number Ame5_107905. All participants gave informed consent before taking part.

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