A case study investigation into the role of CD4+ tumor-infiltrating lymphocytes in a metastatic melanoma patient with a complete response to adoptive cell therapy

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ABSTRACT

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%. Although lasting and complete responses have been achieved, there is substantial opportunity for improvement. Investigation into the role of CD4+ TIL in this setting remains critically underexplored. CD4+ T cells recognize tumor antigen presented on MHC Class II either directly on tumor cells or indirectly through antigen presenting cells (APCs) and are able to elicit potent anti-tumor responses under the appropriate conditions. Here, we present a case study of a metastatic melanoma patient who received adoptive transfer of a predominantly (88%) CD4+ TIL product. This patient demonstrated a complete response (CR) to therapy despite a lack of detection of IFNg in the infusion product *in vitro* when these TIL were co-cultured with autologous tumor prior to ACT. Tumor recognition was also absent when CD8+ TIL were isolated and stimulated directly with HLA-matched tumor lines, indicating a lack of recognition of shared melanoma antigens presented on MHC Class I. Longitudinal analysis of the peripheral blood of this patient confirmed that the infused CD4+ TIL persisted after therapy for at least six weeks. Whole exome sequencing (WES) performed on the TIL surgical specimen discovered 88 non-synonymous single nucleotide variants (SNVs) as candidate neoantigens. Predicted binding of the resulting mutant peptides to autologous HLA molecules generated a predominantly MHC Class II restricted profile, with 81.8% of variants capable of MHC Class II presentation and greater than half exclusive to MHC Class II only. CD4+ TIL were screened for tumor antigen recognition by upregulation of OX40 and 41BB after stimulation with autologous APCs loaded with mutant peptides. Nearly half (49.2%) of CD4+ TIL responded to tumor-derived peptides. These CD4+ TIL were then sorted into tumor-reactive and non-reactive subsets for further clonal analysis of phenotype and transcriptional profile (scRNASeq) of these T cells in order to characterize the nature of the CD4+ TIL response to tumor antigen. Overall, thorough interrogation of this patient's case study demonstrated evidence of CD4+ TIL involvement in a complete clinical response 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.

Fig 1. Adoptive Cell Therapy with TIL

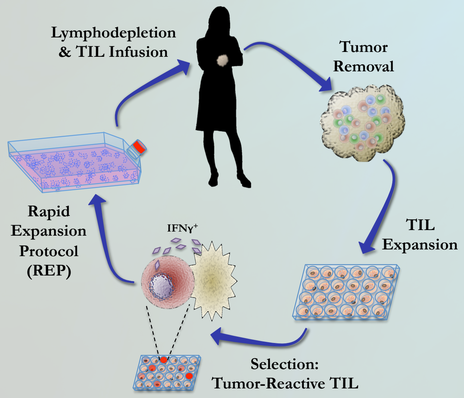
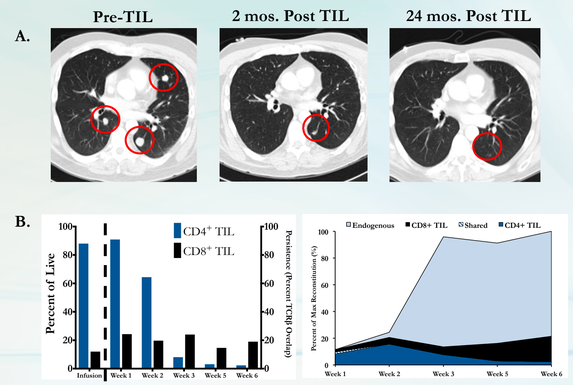
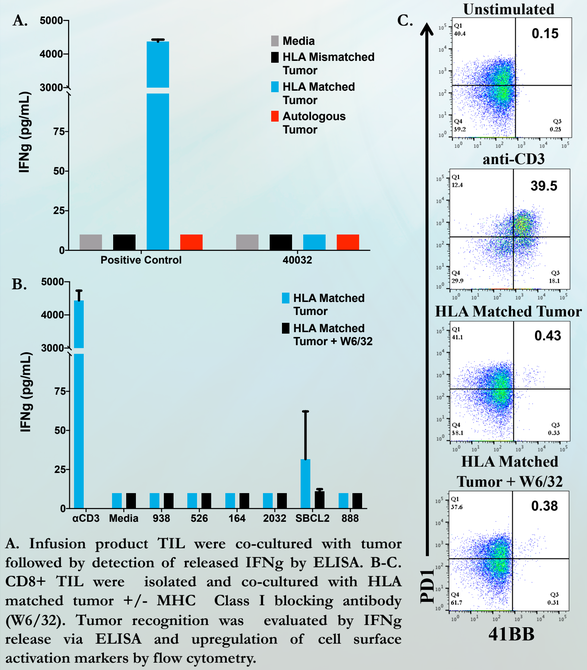


Fig 2. CD4+ TIL persist *in vivo*



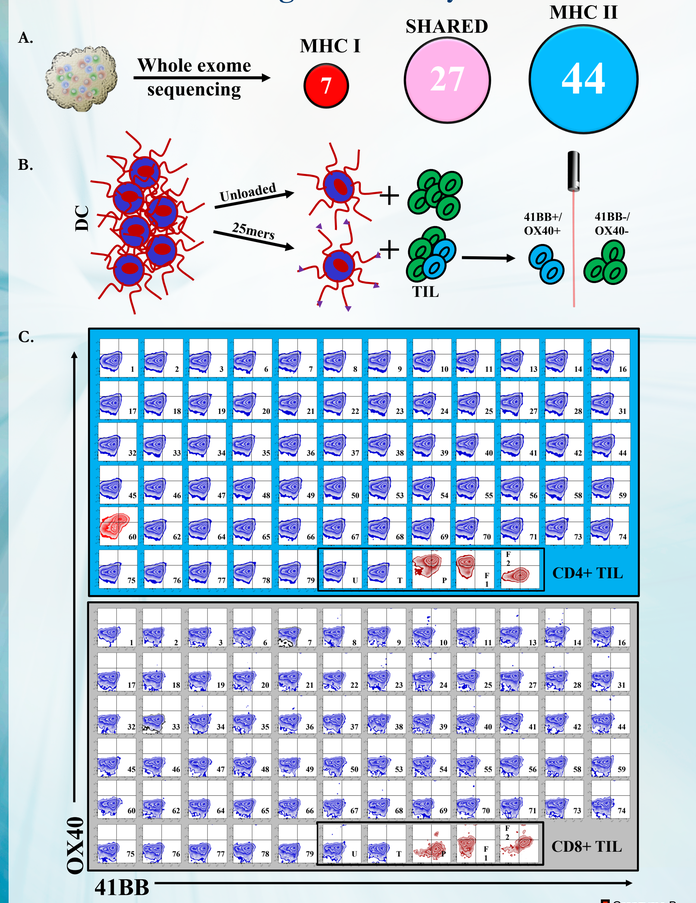
A. CT scans of lung metastases from complete responder (CR) infused with predominantly CD4+ TIL [1]. B. Persistence measured by TCRβ overlap between CD4+ and CD8+ TIL and weekly PBMC repertoire.

Fig 3. CD8+ TIL fail to recognize tumor



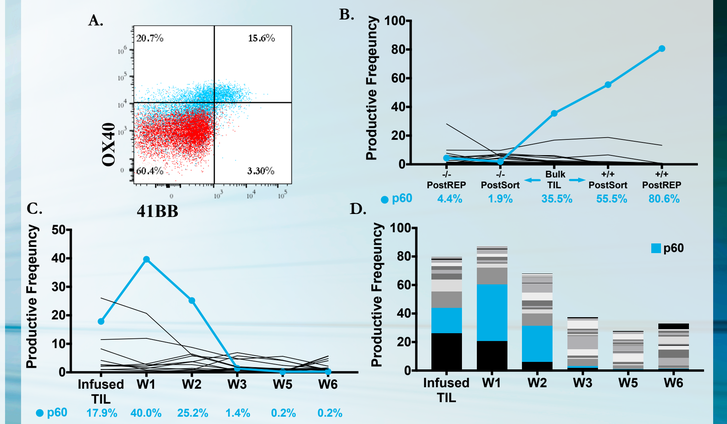
A. Infusion product TIL were co-cultured with tumor followed by detection of released IFNg by ELISA. B-C. CD8+ TIL were isolated and co-cultured with HLA matched tumor +/- MHC Class I blocking antibody (W6/32). Tumor recognition was evaluated by IFNg release via ELISA and upregulation of cell surface activation markers by flow cytometry.

Fig 4. Polyfunctional neoantigen-specific CD4+ TIL detected via immunogenomic analysis



A. Mutant 25mers were predicted for MHC binding from whole exome sequencing. B-D. TIL were stimulated with peptides loaded on dendritic cells (DC) and assessed for increased cell surface expression of OX40 and 41BB by flow cytometry and effector molecule production by ELLA.

Fig 5. Clonal tracing demonstrates *in vitro* enrichment and *in vivo* persistence



A. Neoantigen-specific TIL were sorted on OX40/41BB expression (+/+ vs. -/-) in response to peptide. B-D. Sorted and expanded (REP) TIL populations were sequenced at the TCRβ locus and tracked from infusion product across weekly patient peripheral blood samples for clonal persistence.

CONCLUSIONS & FUTURE DIRECTIONS

- Patient achieved a complete response (CR) after ACT with predominantly CD4+ TIL
- CD4+ TIL were highly functional in response to autologous tumor peptides
- Neoantigen-specific CD4+ TIL were enriched by OX40/41BB selection, expanded and demonstrated strong persistence within the patient

- Identify TCRαβ and mutant peptide cognate pair
- Determine the transcriptional profile of these neoantigen CD4+ TIL
- Further elucidate the function of these CD4+ TIL *in vitro* and *in vivo*
- Expand analysis to additional patient samples to understand importance of CD4+ TIL in ACT

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Moffitt Cancer Center has licensed Intellectual Property related to the proliferation and expansion of bulk tumor-infiltrating lymphocytes (TIL) to Iovance Biotherapeutics. MH, AAS, and SPT are inventors on such Intellectual Property.

REFERENCES

1. Pilon-Thomas S, *et al. J Immunother.* 2012; **35**: 615-20.

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