364 ANTIGEN ABUNDANCE AND TCR AVIDITY IMPACT T CELL-MEDIATED TUMOR RECOGNITION IN NOVEL B16F10 ACT MODEL

[1]Jenna Newman*, [1]John Finnigan, [2]Andrew Ishizuka, [2]Geoffrey Lynn, [3]Alexander Rubinsteyn, [1]Timothy O'Donnell, [4]Jeffrey Hammerbacher, [1]Nina Bhardwaj. *[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA; [2]Avidea Technologies, Washington, USA; [3]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [4]Medical University of South Carolina, Charleston, SC, USA*

Background Adoptive cell transfer (ACT) of neoantigen-reactive $CD8^+$ T cells has had some success in the clinic; however, mouse models recapitulating neoantigen-reactive $CD8^+$ T cell ACT have been limited, especially in poorly immunogenic models such as the murine melanoma model B16F10. Further, direct comparison of neoantigen-reactive $CD8^+$ T cell ACT versus ACT utilizing T cells reactive against overexpressed-self or heteroclitic tumor-associated antigen (TAA) peptides has been lacking. To address these gaps, we developed a model system to study neoantigen- and TAA-reactive $CD8^+$ T cell ACT in parallel.

Methods Whole exome sequencing and RNA sequencing were employed to predict neoantigens present in B16F10. C57BL/6 mice were then administered charge-modified TLR7/8 conjugate vaccines targeting neoantigenic peptides predicted to elicit T cell responses. Vaccination against neoepitopes and previously characterized TAA epitopes elicited neoantigen- or TAA-reactive $CD8^+$ T cells and modest tumor growth control; T cell receptors were isolated from neoantigen- and TAA-reactive $CD8^+$ T cell clones. To develop an ACT model, we conducted CRISPR/Cas9-mediated knockdown of endogenous TCR and subsequent transduction (g-retrovirus encoding neoantigen- or TAA-reactive TCRs) in murine $CD8^+$ T cells. T cells were expanded *in vitro* for use in downstream *in vitro* and *in vivo* applications.

Results Peptide stimulation *in vitro* of neoantigen- and TAA-reactive T cells revealed wide ranges of 1) specificity (vs. cross-reactivity to wild type peptide), and 2) avidity for cognate peptide. Neoantigen- and TAA-reactive T cells were able to recognize B16F10 cells *in vitro*, with the most robust recognition (readout:% T cells $IFNg^+$) when target antigen is highly expressed by tumor cells. Ability of neoantigen- or TAA-reactive T cells to kill B16F10 *in vitro* was strongly dependent upon both tumor antigen expression and T cells' TCR avidity. Similarly, reduction of tumor growth *in vivo* required both high tumor antigen expression and transfer of high avidity neoantigen- or TAA-reactive $CD8^+$ T cells.

Conclusions To conclude, we have created a novel model of neoantigen- and TAA-reactive ACT in immunotherapy-refractory B16F10 melanoma. Our data suggest that antigen abundance and TCR avidity are parameters that influence ACT efficacy; future research will be conducted to dissect the individual and summative contributions of these parameters and translate this knowledge towards improving ACT design in the clinic.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0364