Defining T-Cell Responses to Mutant and Non-Mutant Antigens in Mouse Melanoma During Anti-CTLA-4 Immune Checkpoint Therapy

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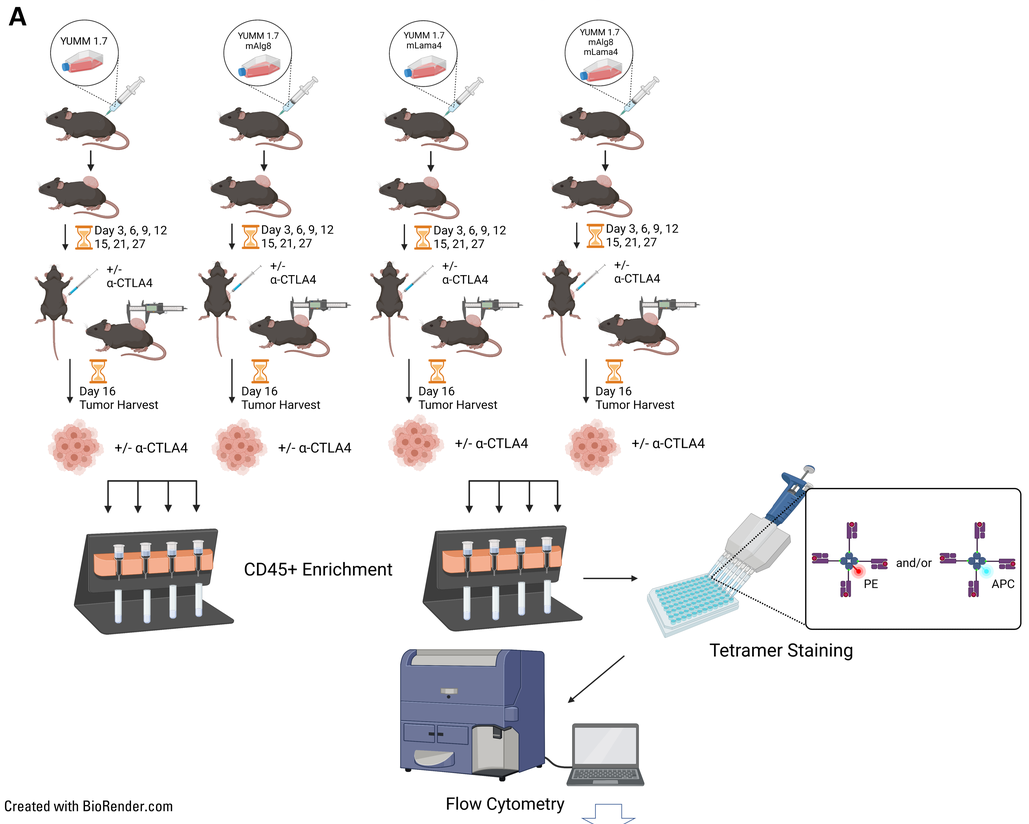
INTRODUCTION

The immune system affects cancer pathology in two distinct ways: (1) by inhibiting tumor outgrowth and (2) influencing tumor immunogenicity thereby increasing cancer cell fitness in an immunocompetent host. T-cell recognition of tumor antigens presented on major histocompatibility complex (MHC) is central to immune-mediated cancer outgrowth prevention and response to cancer immunotherapy, such as immune checkpoint therapy (ICT) (e.g., anti-CTLA-4). ICT employs proteins to stop T-cell inhibitors from hindering their ability to kill cancer cells. Two broad types of tumor antigens exist: (1) non-mutant antigens derived from overexpressed proteins or germline proteins expressed in cancer cells and (2) mutant neoantigens that form as a direct consequence of somatic mutations in cancer cells. We have developed novel mouse melanoma tumor lines that express defined mutant neoantigens (mLama4, mAlg8) and non-mutant antigens (Pmel/GP100, p15e, Trp2) presented on MHC-I to cytotoxic CD8$^+$ T-cells. We can track these responses using peptide-MHC tetramers. This will help further define the process by which T-cells recognizing cancer antigens lose function. This project will use preclinical models of cancer formation and progression to characterize the magnitude of the CD8$^+$ T-cell response to tumor neoantigens during cancer immunotherapy. Information from this study may contribute to the development of novel immunotherapies and therapeutic vaccines that can prevent cancer outgrowth and sustain T-cell anti-tumor activity to combat metastasis.

METHODS



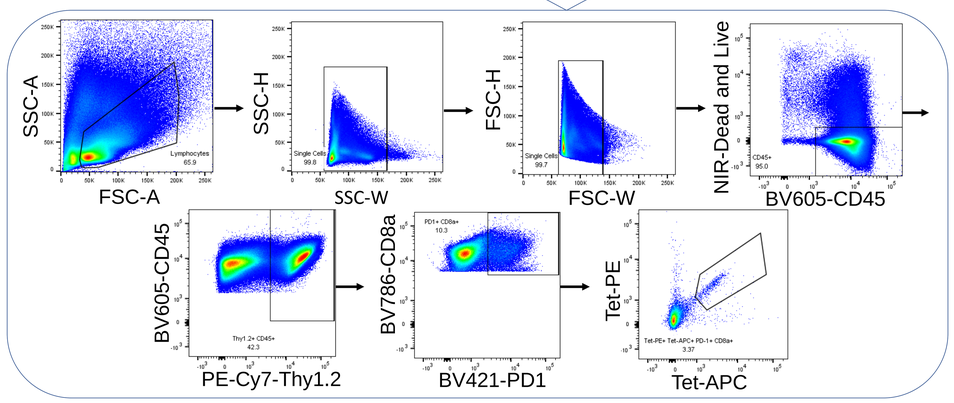


Figure 1. (A) Experimental layout. (B) Gating strategy for detecting neoantigen-specific cells

RESULTS

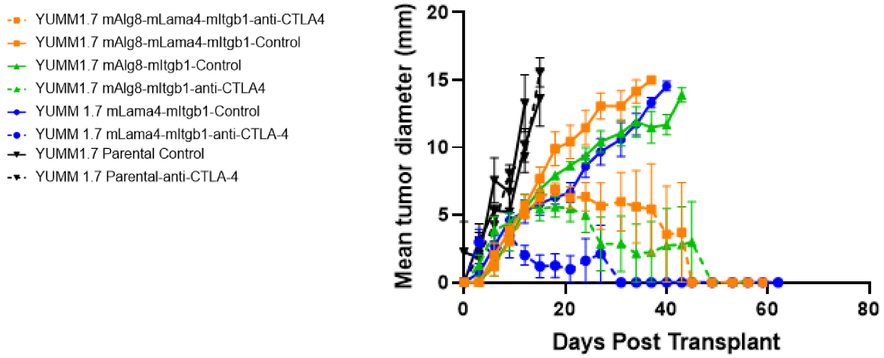
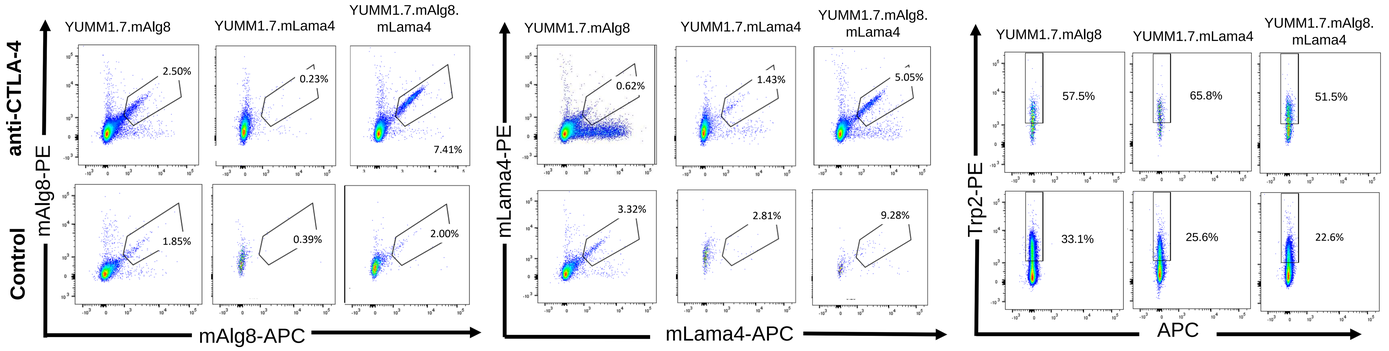


Figure 2. Tumor Growth Curve.
Parental YUMM1.7 grew progressively in both control and anti-CTLA-4 groups. However, enforced expression of mLama4 and/or mAlg8 delayed tumor growth in control mice and facilitated rejection in anti-CTLA-4-treated mice.



All Gated on Live CD45$^+$ Thy1.2$^+$ CD8a$^+$ PD-1$^+$ cells

Figure 3. Neoantigen Specific T-cell Infiltration.
To further identify if the neoantigens are the targets of T cells of these YUMM 1.7 tumor bearing mice, prior to tumor rejection, the tumor from day 16 was harvested and stained with H-2K^b tetramers loaded with their corresponding epitopes. In YUMM1.7.mAlg8 anti-CTLA-4 tumors, 2.50% of the CD8$^+$ T-cells were specific for mAlg8, whereas 7.41% were specific in the presence of mLama4. In YUMM1.7.mLama4 anti-CTLA-4 tumors, 0.62% of the CD8$^+$ T-cells were specific for mLama4, whereas 5.05% were specific in the presence of mAlg8. In YUMM1.7.mAlg8.mLama4 anti-CTLA-4 tumors, 7.41% of the CD8$^+$ T-cells were specific for mAlg8, whereas 5.05% were specific for mLama4. Compared to the anti-CTLA-4 treated groups, a decrease in Trp2 specific CD8+ tumor infiltrating T-cells were detected in the control YUMM1.7 lines expressing mAlg8 and/or mLama4. The Trp2-specific T-cell responses exerted by the expression of each neoantigen seems to have a balancing effect rather than an additive effect in both control and anti-CTLA-4 YUMM1.7.mAlg8.mLama4 tumors.

CONCLUSIONS

The delayed tumor growth and/or rejection depicted in Figure 2 are likely because of the T-cell activation capabilities of the expressed neoantigen(s). Preliminary data in Figure 3 revealed a significant increase in mAlg8-specific CD8$^+$ T-cells and decrease in mLama4-specific CD8$^+$ T-cells in YUMM 1.7 lines expressing both mAlg8 and mLama4 during anti-CTLA-4 treatment. Additionally, a downregulation in CD8+ T-cell response to Trp2 was observed in YUMM1.7.mAlg8.mLama4, possibly due to the neoantigens diverting the response away from the shared antigen Trp2. As compared to the control group, the anti-CTLA-4 group displayed increased intratumoral T-cell infiltration likely due to the anti-CTLA-4 antibody blocking the inhibitory CTLA-4 engagement on T-cells, allowing increased T-cell priming and proliferation. These response differences indicate that the choice of the antigen and the combination of antigens may be a critical factor for efficacious generation of T-cell effector functioning in advanced melanoma patients.

FUTURE DIRECTIONS

Since the results show a difference in T-cell infiltration for each neoantigen, a logical next step would be to investigate whether the expression of one neoantigen influences the other's pMHC-TCR (peptide-major histocompatibility complex- T-cell receptor complex) interaction through epitope spread or immunodominance. Answering this will aid vaccine developers in determining the most effective peptide combination for T-cell mediated destruction of tumor cells. Further investigation of the effects of the neoantigens mLama4 and mAlg8 on pMHC-TCR interaction will result in novel therapies to optimize adaptive immune responses of multiple cancer types. Though neoantigens with high affinity for TCRs and strong adjuvant capabilities are effective targets for immunotherapy, some mutations within cancer cells are not ubiquitous throughout a patient population. Custom vaccines must be produced to familiarize the adaptive immune system with short/long peptide sequences of patient-specific target neoepitopes. Coupled with ICT treatment, therapeutic vaccines may effectively prevent tumor growth and metastasis.

RESPONSIBLE CONDUCT OF RESEARCH

All mouse handling procedures were approved by the IACUC. All references are credited. Patient information was not used in this experiment, eliminating HIPAA related risk.

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REFERENCES

Gubin, M.M. et al. (2014). Checkpoint blockade cancer immunotherapy targets tumor-specific mutant antigens. Nature, 515(7528), 577-581. https://doi.org/10.1038/nature13988

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