Murine models for triple-negative breast cancer with differential responsiveness to immunotherapy

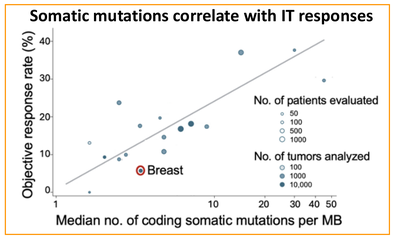
Abstract 2401

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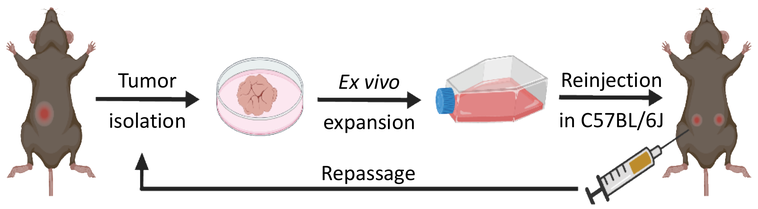
Background

1. Tumor mutation burdens correlate with responses to immunotherapy (IT) including αPD-1 and αCTLA-4 monoclonal antibody treatments [e.g., Pembrolizumab (Keytruda) and Ipilimumab (Yervoy), respectively][1,2]
2. APOBEC3 (A3) hypermutated tumors are candidates for IT[3], but to date, there have been no clinically relevant murine models to study mechanism



3. To address this, we established two mammary cancer cell lines- MM001i, MM008i, from spontaneous tumors arising in conditional p53 heterozygous mice
4. These cell lines were characterized by IT response, infiltrating immune populations (flow cytometry and IHC), transcriptomically (bulk and scRNA-seq), and engineered to express human A3A

Methods



1. MM001i, MM008i cell lines were established in immunocompetent C57BL/6J (WT) mice from spontaneously arising tumors in MMTV-Cre^{+};p53$^{fl/+}$ mice
2. Tumors were treated with αPD-1 ± αCTLA4 *in vivo* to establish baseline immunotherapy responsiveness
3. Tumor infiltrating immune cells were assessed by flow cytometry
4. MM001i and MM008i were transduced with virus expressing human A3A-eGFP or catalytic mutant A3A-E72A-eGFP
5. Clones were evaluated for A3A/A3A-E72A expression by western blot, and for deaminase activity.

References

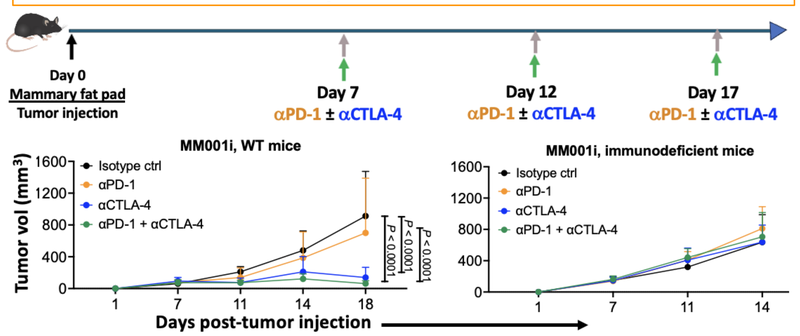
1. Hellmann, M.D., *et al. Cancer Cell*, (**2018**)
2. Yarchoan, M., *et al. New England Journal of Medicine*, (**2017**)
3. Driscoll, C.B., *et al. Nature Communications*, (**2020**)
4. Schmid, P., *et al. New England Journal of Medicine*, (**2020**).

Funding

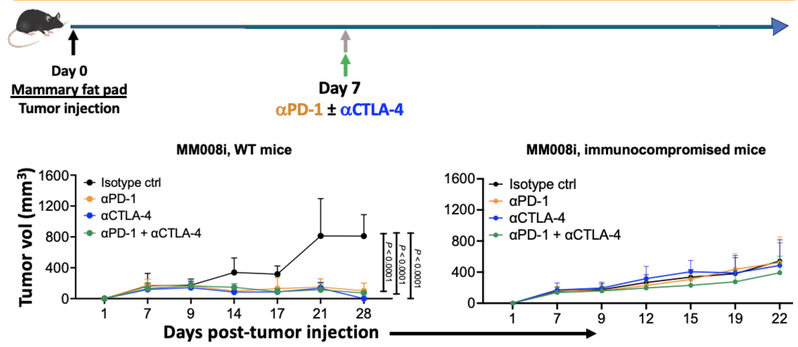
NIH NATIONAL CANCER INSTITUTE · hhmi Howard Hughes Medical Institute · CPRIT Cancer Prevention & Research Institute of Texas

Results

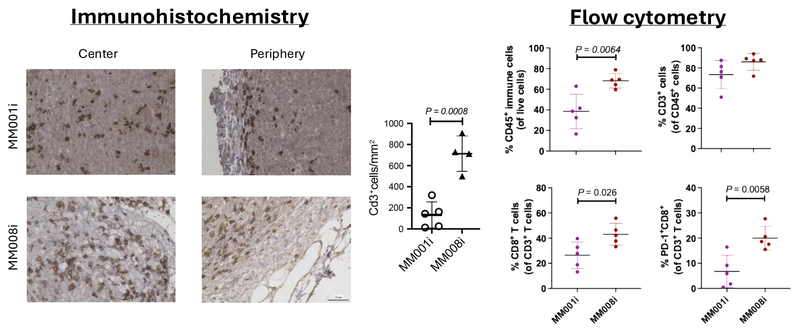
Preferential response of MM001i to αCTLA-4



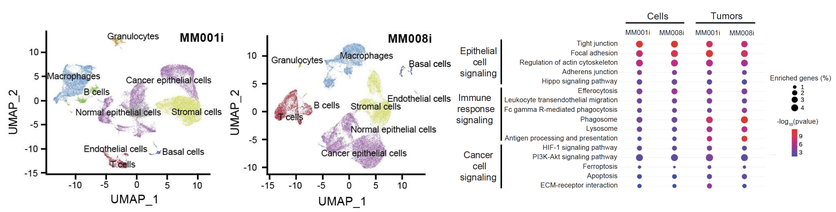
MM008i responds to both αPD-1 and αCTLA-4



MM008i tumors have higher infiltration of PD-1^{+}CD8^{+} T cells

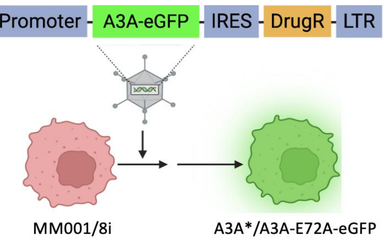


MM008i and MM001i tumors are similar transcriptionally



Results

Schematic of virus transduction for big-bang A3A expression

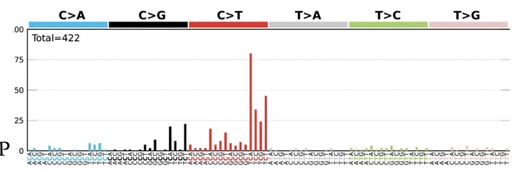


Characterization of A3A(E72A)-eGFP engineered MM001i cells

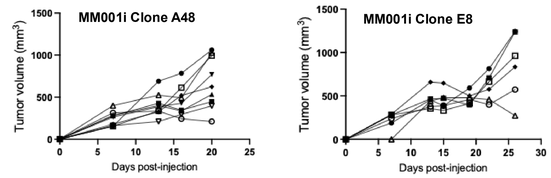
WB and deaminase assay



Clone A48 exonic mutation signature



Tumor growth curves



Conclusions

- Despite originating from the same genetic background, MM008i responds to αPD-1, while MM001i does not. Both cell lines regress with αCTLA-4
- Greater CD3^{+} T cells by IHC, and CD8^{+} T cells and pre-exhausted PD-1^{+}CD8^{+} T lymphocytes by flow cytometry observed in MM008i versus MM001i tumors
- MM001i and MM008i are similar transcriptionally, but MM008i is relatively enriched with immune-activating genes
- MM001i clones engineered with A3A or its catalytic mutant E72A were isolated and have similar expression. Clone A48 has APOBEC3 mutation signature and engrafts into wildtype C57BL/6J mice

NEXT STEPS:

- Uncover the tumor-intrinsic mechanisms behind differences in immune infiltration and immunotherapy response between MM001i and MM008i
- Test impact of A3A mutagenesis on immunotherapy responsiveness *in vivo*
- Determine if A3A can modulate infiltrating immune populations.