Weill Cornell Medicine
Sandra and Edward Meyer Cancer Center

Gut microbiota mimics as a potential source of cross-reactive tumor rejection antigens

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SWIM ACROSS AMERICA ★ MAKING WAVES TO FIGHT CANCER ★

LUDWIG CANCER RESEARCH

Enterome

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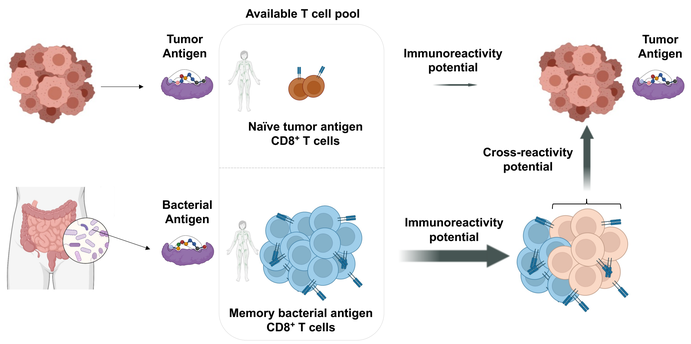
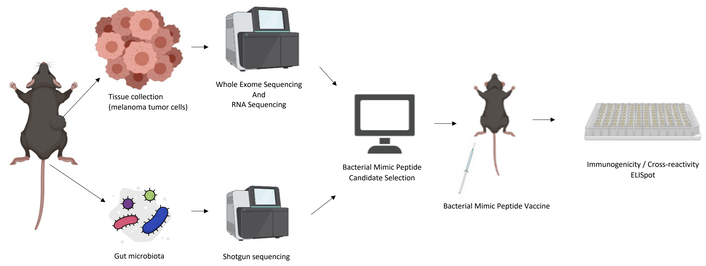
1. Hypothesis



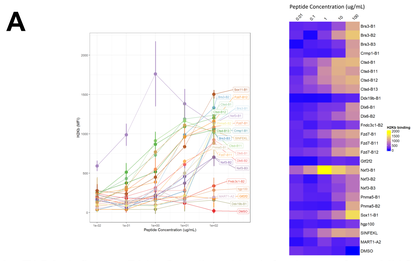
- Interactions between the host immune system and gut microbiota can trigger the formation of pools of bacterial antigen-specific memory CD8+ T cells.
- Some bacterial antigen-specific memory CD8+ T cells may possess cross-reactive potential towards tumor-associated antigens.
- A vaccine strategy aimed at expanding these cross-reactive T cells can trigger enhanced anti-tumor immune responses.

2. Peptide candidate selection pipeline



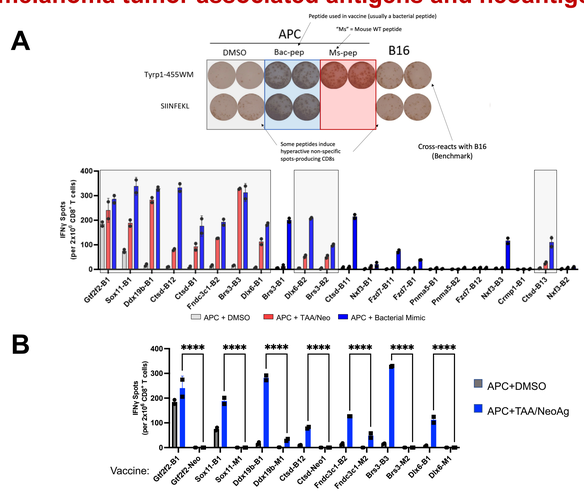
Bacteria harvested from mouse stool samples and murine melanoma cells were sequenced in parallel. The resulting datasets were combined *in silico* and served as the input to a prediction algorithm aimed at selecting bacterial peptides that may trigger an immunogenic response with cross-reactive potential to tumor cells.

3. Predicted MHC I binding correlates with real MHC I binding



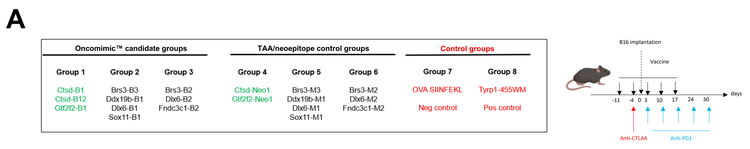
A. TAP2-deficient RMA-S cells with defective surface MHC I expression were incubated with the indicated amounts of bacterial mimic peptides. These exogenous peptides can stabilize pMHC complexes at the cell surface which are then measured by flow cytometry. Peptide affinity is calculated based on the the link between surface levels of MHC I and peptide concentration. **B.** Positive correlations between real and predicted MHC I binding were established.

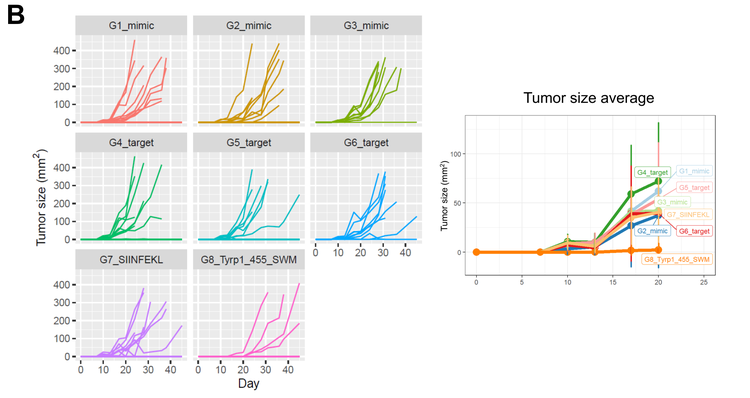
4. Bacterial mimic peptides elicit strong secondary reactivity to melanoma tumor associated antigens and neoantigens



A. Aggregated data of murine melanoma associated ELISpot screens. The best candidates are selected based on immunogenicity (APC + bacterial mimic IFNγ spots) and cross-reactivity (APC+TAA/NeoAg IFNγ spots) and are highlighted by gray boxes. **B.** ELISpot comparing the immunogenicity of bacterial mimic peptides and their respective endogenous peptides. Immunization with endogenous peptides shows little to no immunogenic potential.

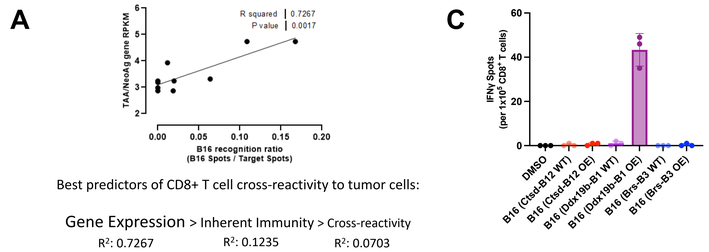
5. Preliminary bacterial mimic peptide prophylactic vaccine trial suggests mild effects on melanoma tumor progression in mice





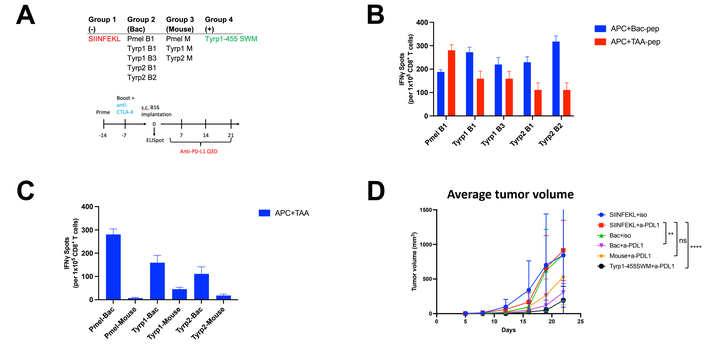
A. Tumor challenge experimental design. Mice were given 2 prophylactic doses and 3 therapeutic doses of vaccines composed of bacterial mimic peptide groups. Anti-CTLA-4 and anti-PD-1 were administered at the indicated timepoints. **B.** Tumor size (mm^2) was measured with calipers at the indicated timepoints post tumor implantation and is represented for individual mice (left) or averaged for every group (right).

6. Target TAA/NeoAg gene expression predicts CD8+ T cell cross-reactivity to melanoma tumor cells



A. Positive correlation between the number of IFNγ spots and the expression levels of full-length antigens by tumor cells. **B.** Target gene expression levels measured from B16 tumors or B16F10 cells. **C.** Overexpression of the mimic target by tumor cells was induced by transiently transfecting B16 cells with pING2 plasmids containing sequences of the full-length antigens. CD8+ T cells isolated from mice immunized with the indicated Oncomimic™ were co-cultured with B16 cells transfected with empty vector (WT), or B16 cells overexpressing the relevant full-length antigen (OE).

7. Prophylactic vaccine with bacterial mimic peptides of known melanoma TAAs is immunogenic and delays tumor progression



A. Tumor challenge experimental design. Mice were given 2 prophylactic doses of vaccines composed of the indicated peptides. Anti-CTLA-4 and anti-PD-L1 were administered at the indicated timepoints. **B.** ELISpot assay for immunogenicity (blue) and cross-reactivity (red) of bacterial mimic peptides. **C.** Immunogenicity of bacterial mimic peptides compared to their respective endogenous peptides. **D.** Tumor volume (mm^3) was measured with calipers at the indicated timepoints post tumor implantation and averaged for every group.

8. Conclusions

- Bacterial Oncomimic™ peptides of murine melanoma antigens with verified MHC-I binding have been identified.
- Immunizing mice with Oncomimic™ candidates elicited a strong secondary reactivity to TAAs/neoantigens. Immunizing with actual TAA/neoantigen did not.
- Vaccine trials with the best Oncomimic™ candidates suggested mild impacts on murine melanoma tumor progression (as tested).
- Predictors of immunoreactivity and cross-reactivity have been identified.
- Immunization with Oncomimics™ targeting known melanoma TAAs lead to significant tumor growth delay.