

Antigen-Specific T Cell Responses to Breast Cancer

Jill.Slansky@UCDenver.edu

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Presenter disclosure information

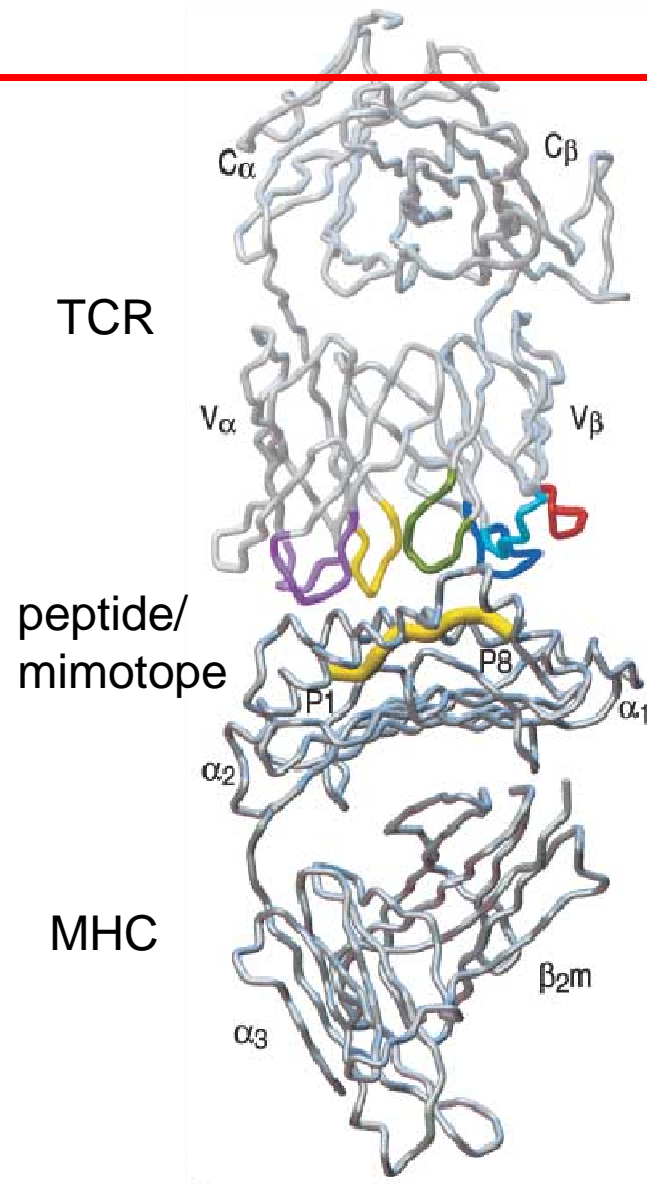
No relationships exist related to this presentation.

Why do we need to know the $\alpha\beta$ -TCR pairs of tumor infiltrating T cells?

- ⊙ The presence of TILs correlates with patient survival.
- ⊙ TILs recognize tumor antigens.
- ⊙ Activated tumor-specific T cells add specificity to cancer vaccines.

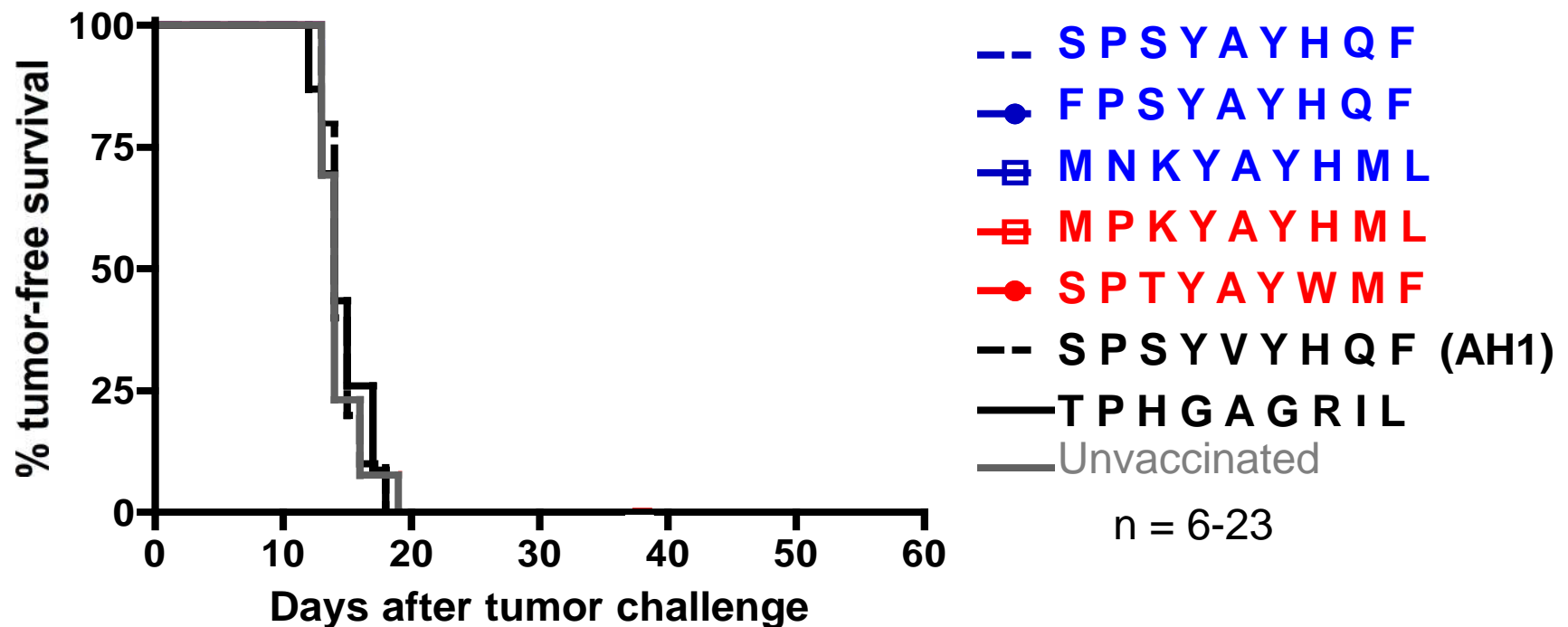
Our ultimate goal is to identify these epitopes and mimotopes for cancer vaccines.

T cell review



The first tumor-specific T cell clone we identified against the colon carcinoma CT26 was “CT”

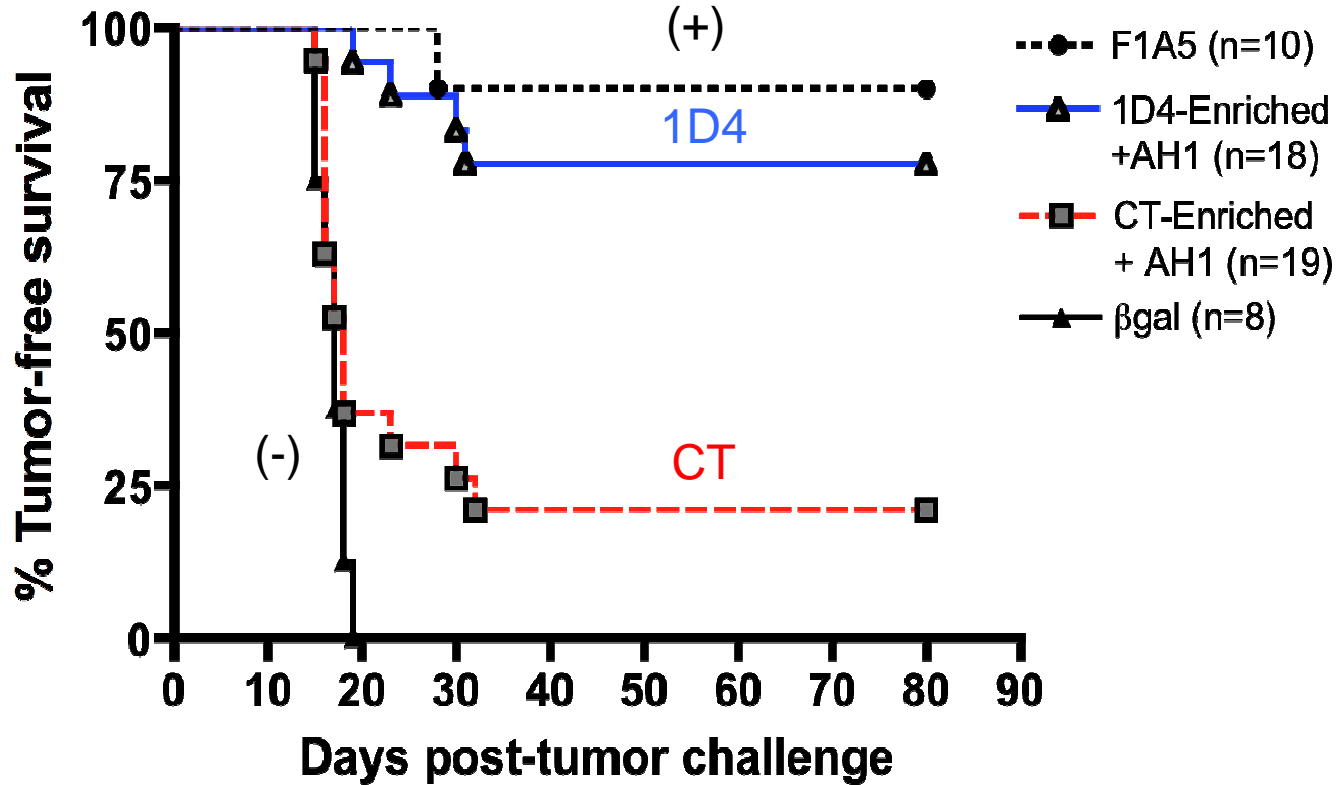
- CT26 is an immunogenic transplantable tumor.
- The dominant MHC1-restricted antigen is gp70₄₂₃₋₄₃₁ (AH1).
- We sub-cloned AH1-specific T cells by limiting dilution. 6 of 6 clones had identical TCR sequences.
- AH1 mimotopes elicit a range of antitumor immunity.



The 1D4 T cell clone is representative of the AH1-specific T cell response

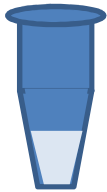
Source of AH1-specific T cells	CT-TCR TRBV13/TRBJ2-7 FCASS SGG AYEQY	1D4-TCR TRBV13/TRBJ2-7 FCASS DGD YEQY
Mimotope vaccination n=118,994	6	10,522
AH1 vaccination n=44,138	0	1,544
TILs, no vaccine n=3,978	0	5
TOTAL n=167,110	0.004%	7.2%

Prime with peptides enriched with representative TCR and tumor-antigen boost results in tumor protection

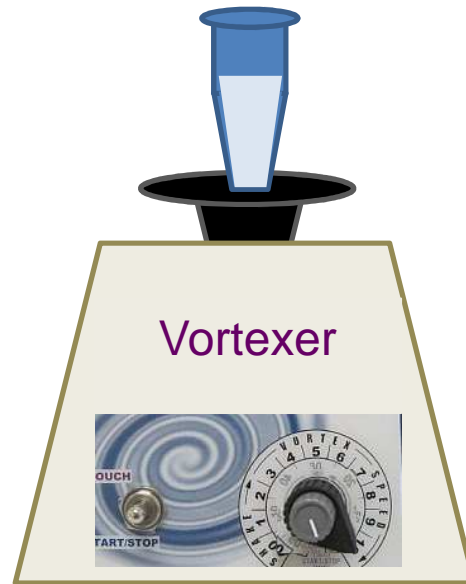


Emulsion RT-PCR of paired $\alpha\beta$ TCR chains

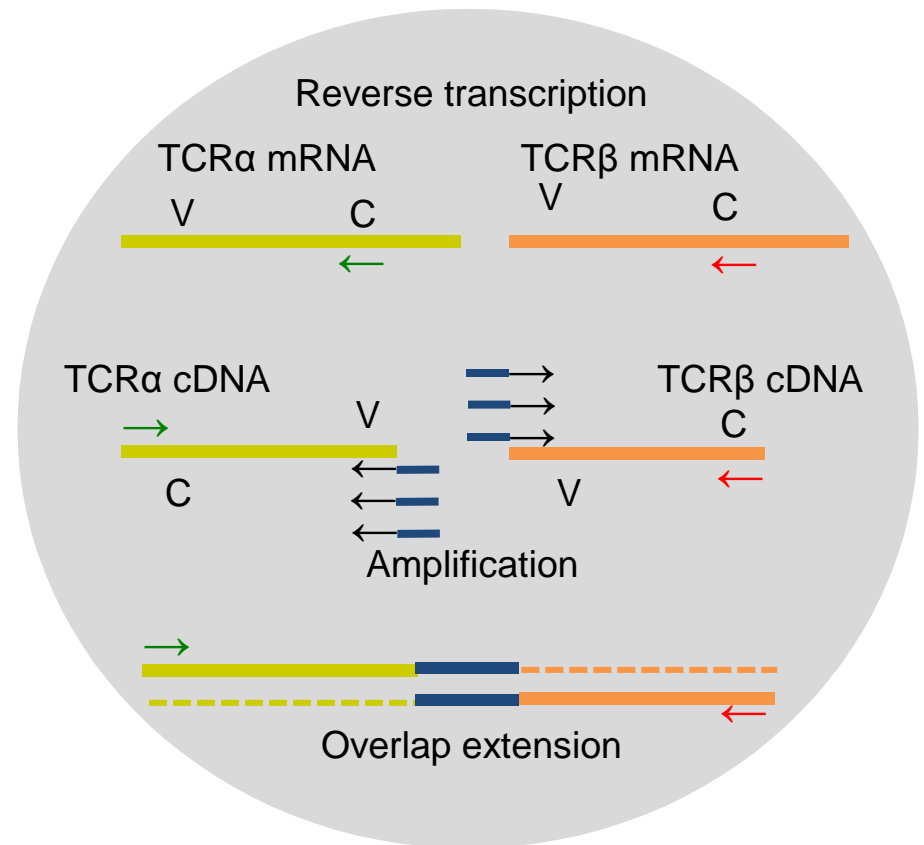
RT-PCR reaction:
 α/β V and C primers
RT-PCR reagents
T cells
50 μ l



Add
300 μ l Emulsion phase
and vortex



Example reaction bubble (200-500 fl)



Preparation of PCR fragment for sequencing

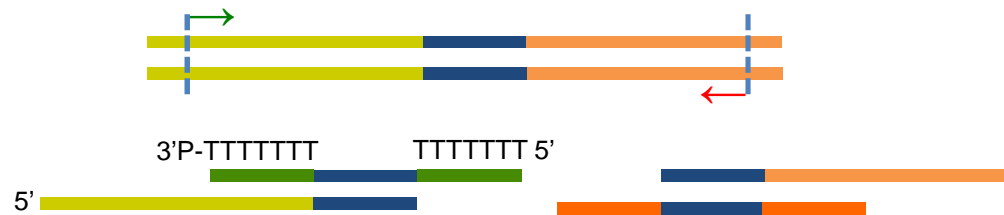
Break emulsion



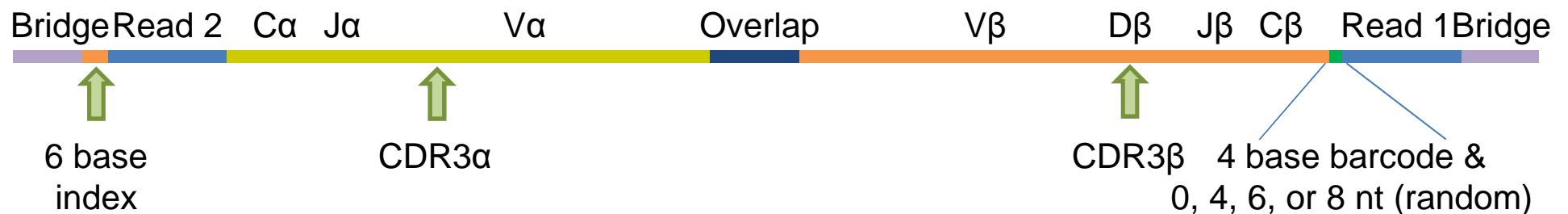
Column purify PCR products



PCR with nested C-region oligos and blocking oligos

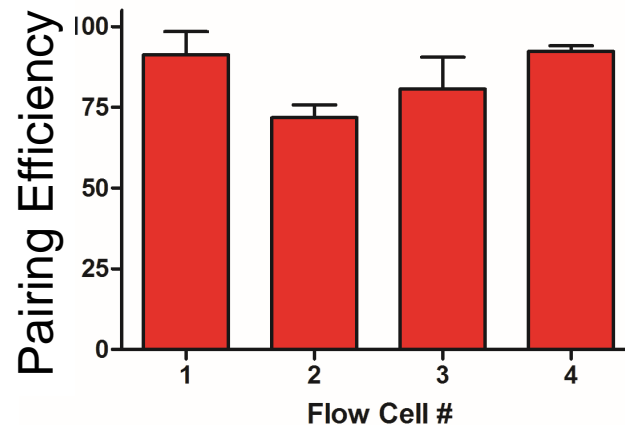
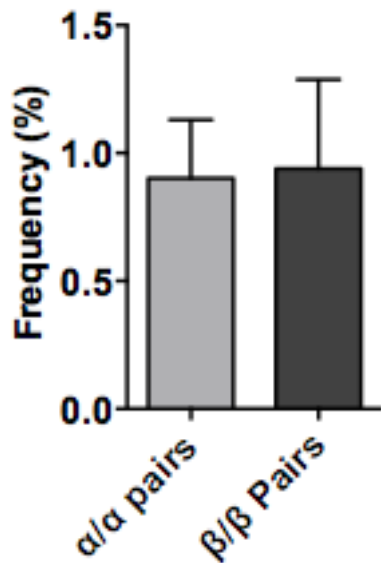


Final PCR to add the bridge and read sites for sequencing (450 bp product)



Confirmation that pairing is correct

- ✓ Confirm that all primers bind
Clear PCR products from productive α chains and β chains
- ✓ Determine number of α/α pairs and β/β pairs
- ✓ Show that pairing is not random
- ✓ Show that titration of known numbers of hybridoma cells into PBMCs emulsion PCR is semi-quantitative.
- Further understand the statistics, significance, and quality of the results obtained.

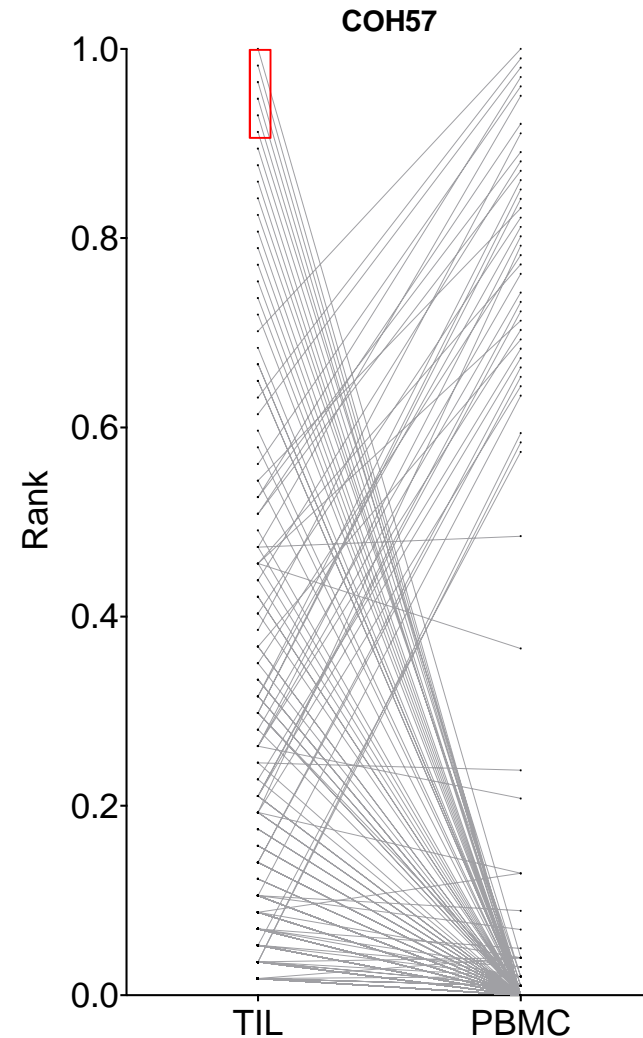
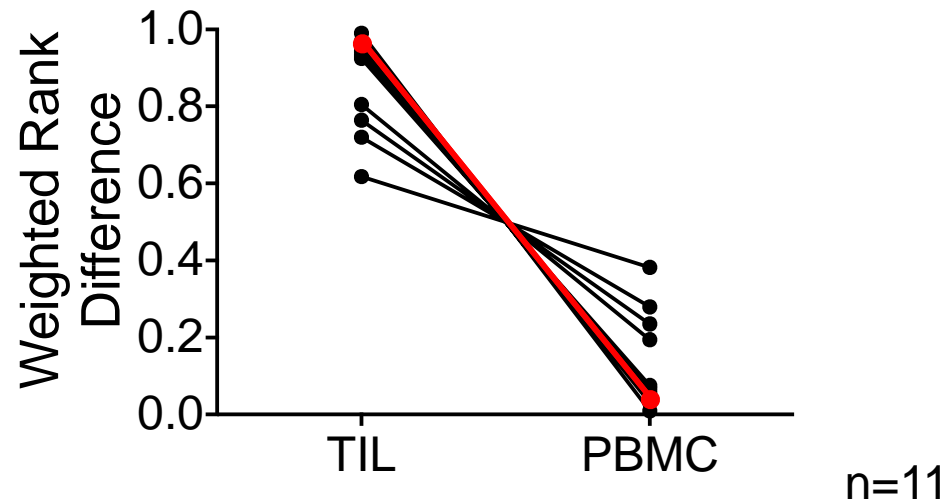


5KC spike	H/H pairs	% M/M pairs	% H/M pairs	% M/H pairs
10	89	10.3	0.3	0.05
5	94	4.7	0.2	1.08
1	98	1.6	0.2	0.013
5% NE	95	1.7	0.7	2.62

Normalized

TCR repertoire from blood and TILs of breast cancer patients are different

Cell Type	Tissue	Cell Counts	Total Reads	Unique pairs
BC57 CD8+ T cells	Tumor	16,500	283,841	1,194
	Blood	30,000	667,982	3,214



Lessons and Take Home Messages

Selected mimotopes stimulate tumor-specific T cells and augment antitumor responses.

Targeting the tumor-specific T cells that are present in the naturally responding repertoire is important in design of peptide vaccines.

Emulsion RT-PCR can be used inexpensively to identify TCR pairs (or other co-expressed messages within a cell).

Comparison of TILs and PBMCs suggests the representative TCRs of the tumor-specific T cell response.

Some “public” TCRs are shared among breast cancer patients, but the cognate antigen is still unclear.

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