Overlap between circulating and intratumoral T cells as a predictor of neoantigen-reactive TIL *ex-vivo* expansion

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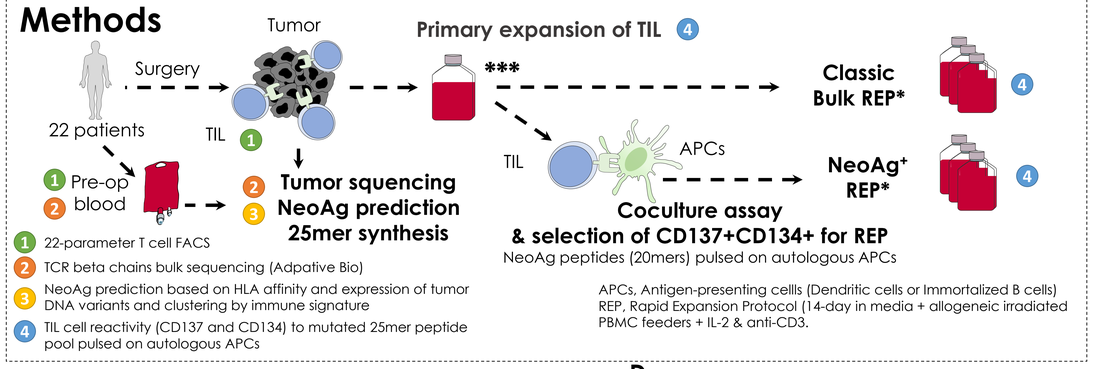
Background & objectives

The amount of neoantigen (neoAg)-reactive tumor-infiltrating T lymphocytes (TIL) infused to patients is associated with clinical response. However, no biomarkers can currently predict whether neoAg-reactive T cells will be expanded from a tumor, and bystander T cells often dominate products administered to patients unlikely to benefit. We have tested a manufacturing method to enrich TIL in neoAg-reactive T cells and investigated **baseline immune variables associated with end product neoAg reactivity**.

#**1**: Test whether the use of pooled long predicted NeoAg peptides can be used to enrich the cell product in reactive TIL.
#**2**: Determine whether immune features of the initial tumor are associated with the capacity to expand NeoAg-reactive TIL.

Methods



Results

Figure 1. Reactivity level of NeoAg+ REP TIL and their association with potential tumor immunogenicity, TCR diversity

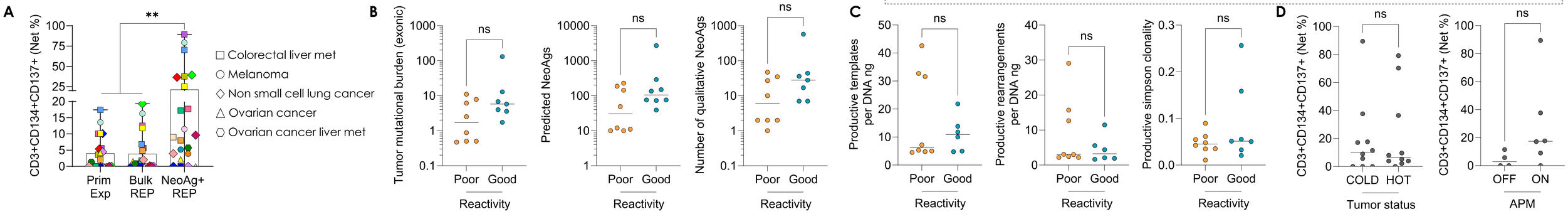


Fig. 1. Legend: A total of 3,985 NeoAg peptides (generally 173 per tumor), out of 25,836 predicted NeoAgs (15%), were used in coculture to enrich for and test TIL reactivity in 22 tumors. **A.** Higher neoAg reactivity obtained after neoAg+ REP compared to classic bulk expansion; 7 highly reactive [>25%], 7 intermediate [5 to 25%], and 8 low [<5%]. **B.** NeoAgs predicted through the detection of non-synonymous mutations by whole exome sequencing, their expression levels by RNA-seq and the *in silico* estimation of peptide affinity to class I MHC. High-quality NeoAg are identified as 25-mers with a predicted positive immunological response chance of >5% using the NMER model described in *Gartner et al. Nat Cancer 2021* **C.** Number of different sequences that can be translated into fully functional TCRs, relative to the DNA quantity (in ng) and, TCR clonality: Simpson index of 1 represent a single TCR clonotype; whereas near zero represents highest diversity of TCR clonotypes (Adaptive Bio). **D.** The detection of immune 'hot' and 'cold' tumors, along with antigen presentation machinery (APM) activity through RNA sequencing

Figure 2. Percent TCR repertoire overlap between pre-operative blood and tumor is associated with end product reactivity to NeoAgs and correlated with blood and tumor T cell phenotypes

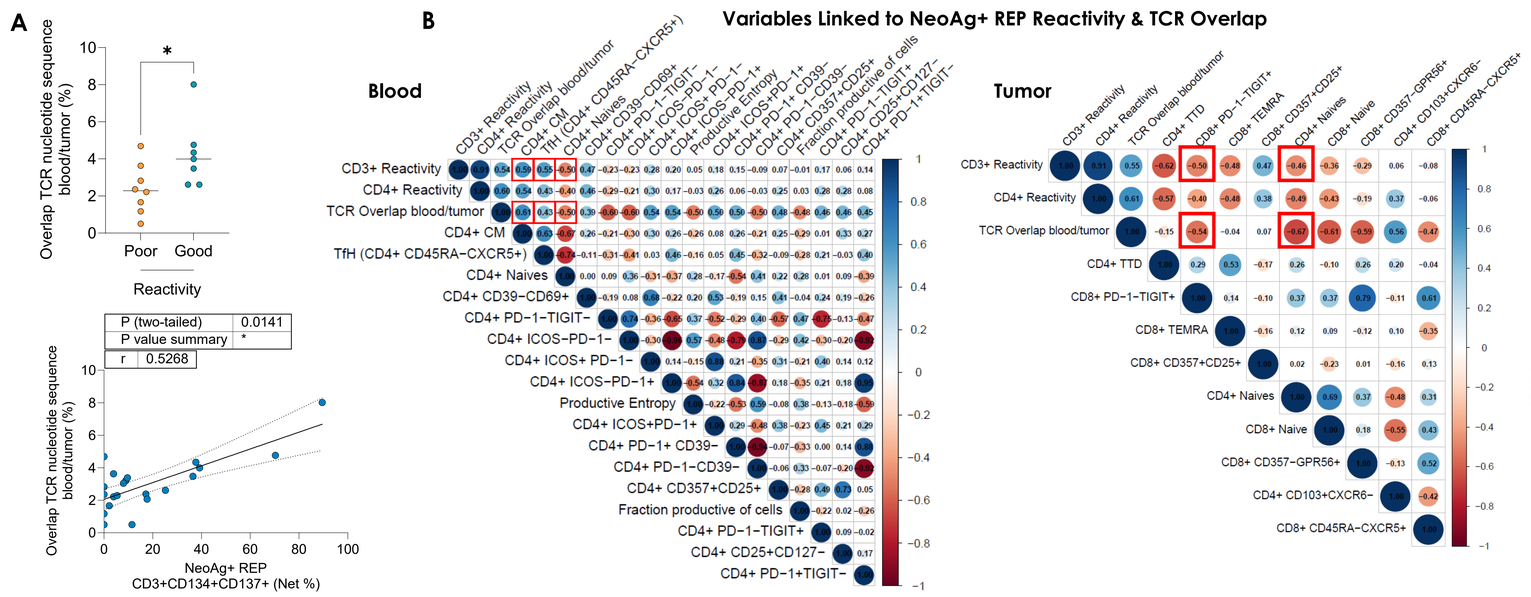


Fig. 2. Legend: A. Correlation between TCR sequence overlap in blood/tumor and NeoAg+ REP reactivity. **B.** Correlation matrix representing the most significantly correlated flow cytometry phenotyping and TCR sequencing from blood (left) or tumors (right) with NeoAg+ REP reactivity and the overlap of TCR sequences between tumor and blood. Circle size represents the r correlation level; red squares indicate correlations common between NeoAg+ REP reactivity and TCR overlap between tumor and blood. *, $P < 0.05$, Mann-Whitney test (A - top).

Figure 3. The capacity to generate neoAg-reactive TIL is associated with distinct baseline blood and tumor T cell phenotypes

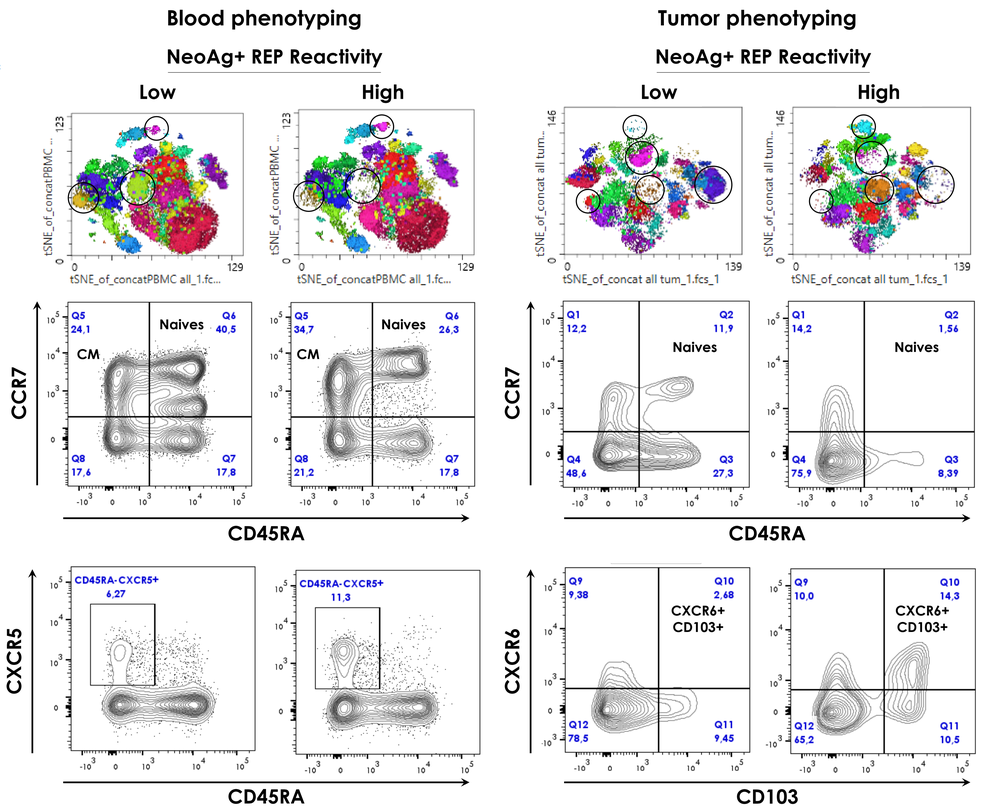


Fig.3 Legend. Top: tSNE analysis of T cells in pre-operative blood (left) and resected tumors (right) utilizing 22-parameter FACS and grouping samples according to TIL end product reactivity (6 highly reactive [>25%] vs 5 low [<5%]). **Bottom**: All events of tSNE analysis merged; illustration of the most discriminant markers identified by tSNE clustering according to low vs high end product reactivity.

Conclusions

1. In a diverse set of cancers, manufacturing based on the selection of TIL reactive to pooled neoAg peptides resulted in significant enrichment compared to classic bulk, unselected TIL manufacturing (with 25% to 89% reactivity observed in ~32% of cases).
2. From the initial tumor, the mutational burden and the number of predicted neoAgs, TCR abundance and clonality, high immune-related gene and antigen-processing machinery gene expression were not significantly associated with TIL end product neoAg reactivity.
3. The degree of baseline TCR overlap between blood and tumor repertoire was the strongest parameter associated with the capacity to expand neoAg-reactive TIL. This immune parameter was associated with more T cells expressing the CXCL13 receptor (CXCR5) in the pre-operative circulating blood, and more tissue resident T cells intratumorly (CD103+ and CXCR6+).
4. Biomarkers combining TCR sequencing and T cell phenotypes in both blood and intra-tumoral compartments may help identify patients from whom tumor-reactive TIL product can be generated. This needs to be validated prospectively in independent datasets.

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