Identification of patient-specific T cell neoantigens through HLA-agnostic genetic screens

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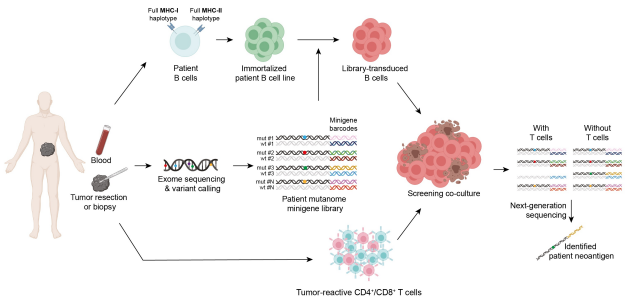
Background

Cancer neoantigens that arise from tumor mutations are drivers of tumor-specific T cell responses, but identification of T cell-recognized neoantigens in individual patients is complicated by their patient-specific nature. Here we develop the first genetic neoantigen discovery platform that allows identification of both CD4+ and CD8+ T cell-recognized neoantigens with high sensitivity and across complete HLA genotypes. This technology should facilitate the development of personalized neoantigen-based cancer immuno-therapies.

Schematic overview of the methodology:



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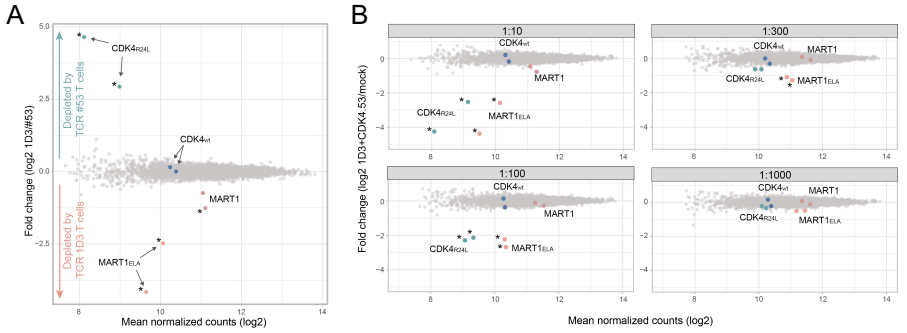
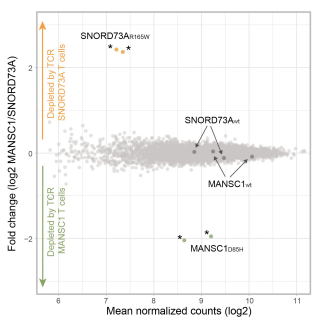
Sensitive HLA class I and class II neoantigen discovery technology: validation experiments

(A) An HLA-A*02:01 B cell line was transduced with a library of 4,764 minigenes that included the $CDK4_{R24L}$ neoantigen, its wild-type counterpart, the $MART1_{26-35}$ epitope (MART1) and the affinity-enhanced $MART1_{26-35*A27L}$ epitope (MART1-ELA). Library-expressing B cells were co-incubated with donor CD8+ T cells engineered to express a $CDK4_{R24L}$-specific TCR, or the $MART1_{26-35}$-specific TCR 1D3. After 72 hours, minigenes from remaining B cells were amplified and quantified by deep sequencing. Dots represent individual minigenes. **(B)** CD8+ T cells expressing either the $CDK4_{R24L}$ or 1D3 TCR were diluted 10-, 100-, 300- or 1,000-fold with mock-transduced T cells to simulate T cell pools with low abundance antigen-reactive T cell populations, and were incubated with library-expressing B cells as in (A).

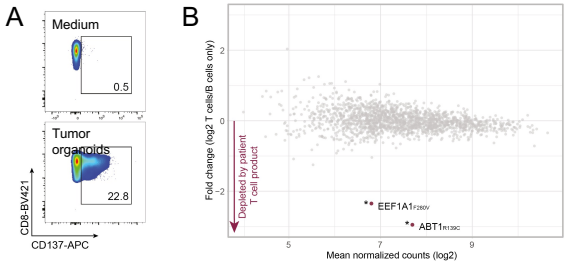


(C) Patient-derived TCRs specific for the MHC class II-restricted $MANSC1_{D85H}$ and $SNORD73A_{R165W}$ neoantigens were expressed in donor CD4+ T cells and used to screen the patient-matched B cell line transduced with the minigene library. In this case, minigenes were subcloned in a vector that couples minigenes to the CD74 signaling motif, to enable processing of minigene products through the HLA class II processing pathway.



Personalized and HLA-agnostic neoantigen screening of patient T cells

(A) PBMCs from a patient suffering from mismatch repair-deficient colorectal cancer were cocultured with matched tumor organoids for two weeks. Tumor-reactivity of the cell product was then assessed by incubating cultured PBMCs with tumor organoids and analysis of CD137 surface expression. **(B)** Non-synonymous tumor mutations from a patient suffering from mismatch repair-deficient colorectal cancer were identified by exome and RNA sequencing, and used to design a personalized mutanome minigene library consisting of 1,834 unique minigenes. Patient B cells were immortalized, transduced with the mutanome library, and incubated with patient PBMCs from (A).



(C) Neoantigen hits identified in (B) were validated by expressing $EEF1A1_{F280V}$, $ABT1_{R139C}$ or the respective wild-type sequences as single minigenes in patient B cells and incubating transduced B cells with organoid-induced patient PBMCs.