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Targeting neoantigens with immunotherapy: Are we limited to pre-existing autologous neoantigen-specific T cells?

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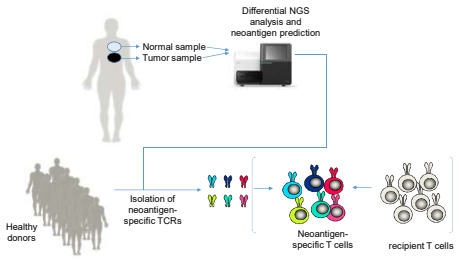
Abstract

Several facts shape our considerations regarding the use of neoantigens as highly specific targets for immunotherapy of cancer. First, adoptive T cell therapy using TILs seems to be most successful if the TILs include T cells specific for antigens resulting from individual mutations. Second, the success of checkpoint inhibitors is often correlated with the mutational load of tumors. However, a mutation must fulfill several criteria to be effective as a neoantigen that can be recognized by T cells. Obviously, the mutation must lead to a novel amino acid sequence (e.g. single amino acid substitution, fusion- or frameshift-sequence), and be located in a gene expressed in tumor cells. Furthermore, a peptide spanning the new sequence needs to be efficiently processed and presented by HLA molecules. Finally, a T cell response must be triggered that can specifically recognize the mutated epitope.

Targeting neoantigens as true patient-individualized epitopes requires robust processes for rational and rapid selection and validation of neoantigens as T cell targets. Currently, the most challenging step is predicting specific T cell responses. Huge efforts have been made to analyze the reactivity of patients' T cells against mutations. However, this approach is limited to the T cell repertoire present in patients at the time of tumor resection or blood draw and might miss potential potent T cell responses that were lacking or no longer present in the patient. In our opinion, only screening the T cell repertoire of several healthy donors can answer the question if a specific mutation can trigger T cell responses. We present proof-of-concept data how we use our high-throughput T cell receptor (TCR) platform technologies and automated processes for fast and efficient screenings of T cells isolated from several partially HLA-matched healthy donors. Promising neoantigens were predicted and T cells responses after stimulation with antigen-presenting cells either transfected with minigene constructs or loaded with peptides were compared. Peptide stimulation triggered specific T cells for most tested mutations, indicating that T cell repertoires of healthy donors can recognize neoantigens when they are forced to be presented. Also, with the clinically relevant approach using endogenously processed antigen encoded by minigenes, specific T cell responses against neoantigens presented on different HLAs were efficiently triggered, although only against some of the tested epitopes. This screening strategy has the aim to develop future TCR-based therapies and can be used for the identification of promising mutations for vaccination or as a source for TCRs for adoptive T cell therapy. Furthermore, generated data can subsequently improve algorithms predicting the immunogenicity of neoantigens.

Experimental setup

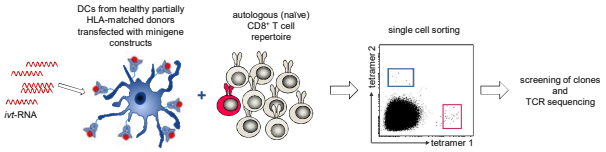


Selection of potential neoantigens

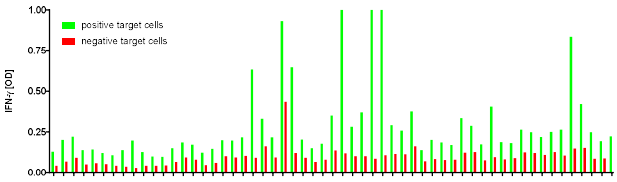
	gene	wt sequence	affinity to MHC	mut. sequence	affinity to MHC	tetramer
HLA-A2	*ZCCHC2*	FTMA**S**LHPA	4	FTMA**L**LHPA	4	✓
	PIGZ	LQAAWQ**R**LQV	471	LQAAWQ**W**LQV	44	✓
	CMTR2	MILPVLSCF**T**	792	MILPVLSCF**I**	51	-
	DTND1	LLARH**R**KI	1123	LLARH**L**KI	59	✓
	LRRK1	QLDALMP**R**L	116	QLDALMP**F**RL	81	-
	SPAG5	KLSLSLSP**S**	3301	KLSLSLSP**L**	90	✓
	EXPH5	RLTLGN**E**FSV	39	RLTLGN**K**FSV	110	-
	NRXN3	W**R**LFWGCIV	8034	W**L**LFWGCIV	111	-
	PLEC	RVPDVQ**D**GV	1833	RVPDVQ**Y**GV	138	✓
	CNKSR3	SP**A**DRSQKI	32854	SL**A**DRSQKI	358	✓
HLA-A24	*METTL2B*	KYWN**D**FYKI	9	KYWN**N**FYKI	6	✓
	SLAMF1	RYLG**D**KYKF	15	RYLG**Y**KYKF	7	✓
	NPSR1	MYGIV**R**TI	33	MYGIV**Q**TI	14	✓
	MRM1	TWGRLVTR**H**F	134	TWGRLVTR**Y**F	86	-
	DNAH3	MWAAKSY**P**SL	316	MWAAKSY**T**SL	191	-
	SPEG	L**S**SVQEEL	24175	L**Y**SVQEEL	224	✓
	GATM	D**H**PLWMSSKW	8197	D**Y**PLWMSSKW	342	-
	ERV3-1	YY**N**ETLGKTL	396	YY**K**ETLGKTL	708	✓

Potential patient-specific neoantigens were selected based on several parameters including predicted MHC binding affinity, difference in predicted binding affinity between wildtype and mutated version, position of the mutation, and biochemical differences between wildtype and mutated residue.

Isolation of neoantigen-reactive T cell clones

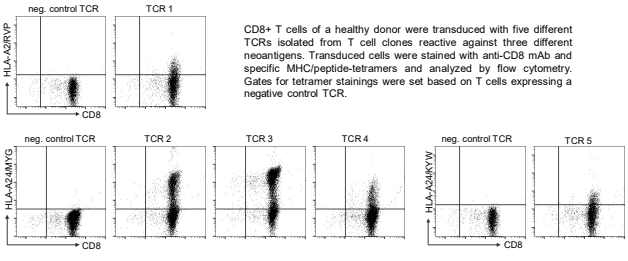


Screening for neoantigen-reactive T cell clones

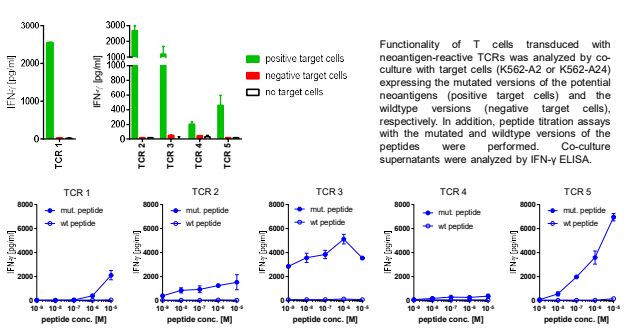


T cell clones were screened by co-culture with target cells (K562-A2 or K562-A24) expressing the mutated versions of the potential neoantigens (positive target cells) and the wildtype versions (negative target cells), respectively. Co-culture supernatants were analyzed by IFN-γ ELISA. Clones with a higher reactivity against the positive target cells compared to the negative target cells are shown. TCRα and TCRβ sequences of the these clones were identified and cloned into a retroviral vector.

Characterization of neoantigen-reactive TCRs

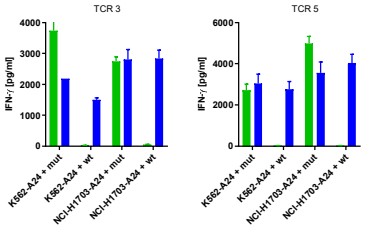


CD8+ T cells of a healthy donor were transduced with five different TCRs isolated from T cell clones reactive against three different neoantigens. Transduced cells were stained with anti-CD8 mAb and specific MHC/peptide-tetramers and analyzed by flow cytometry. Gates for tetramer stainings were set based on T cells expressing a negative control TCR.

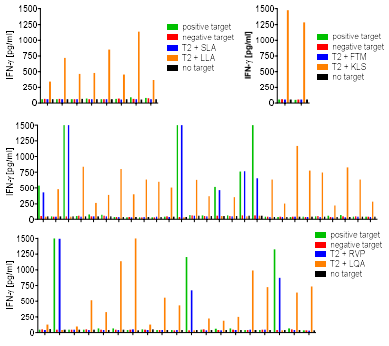


Functionality of T cells transduced with neoantigen-reactive TCRs was analyzed by co-culture with target cells (K562-A2 or K562-A24) expressing the mutated versions of the potential neoantigens (positive target cells) and the wildtype versions (negative target cells), respectively. In addition, peptide titration assays with the mutated and wildtype versions of the peptides were performed. Co-culture supernatants were analyzed by IFN-γ ELISA.

T cells transduced with neoantigen-reactive TCRs were co-cultured with target cells transfected with either the mutated or the wildtype version of the genes (green bars). As target cells K562-A24 or the indication-specific tumor cell line NCI-H1703-A24 were used. All transfected constructs contained an epitope that was recognized by T cells expressing a control TCR (blue bars) to ensure successful transfections. Co-culture supernatants were analyzed by IFN-γ ELISA.



Peptide processing-independent approach



HLA-A2+ DCs were loaded with a mix of six different neoantigen peptides and co-cultured with autologous CD8+ T cells. Neoantigen-reactive T cells were sorted using tetramers and single cell clones were generated. T cell clones were screened with peptide- loaded T2 cells. Clones with a specific reaction pattern were further expanded and their reactivities against target cells transduced with minigene constructs expressing the mutated versions of the potential neoantigens (positive target) and the wildtype versions (negative target), respectively, or against peptide-loaded target were tested. Co-culture supernatants were analyzed by IFN-γ ELISA.

Summary

We showed that T cells reactive against patient-specific mutations can be isolated from healthy, partially HLA-matched donors. This approach can be used as a screening platform for immunogenic neoantigens and as a source for patient-specific, therapeutic TCRs.

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