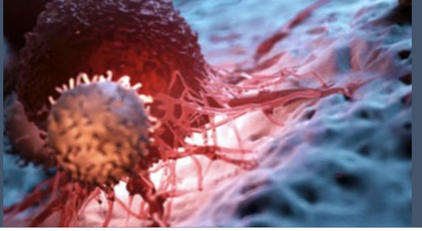

Generating optimal-affinity T cell receptors targeting the shared neoantigen KRAS^{G12V} using the humanized TCR transgenic mouse platform HuTCR

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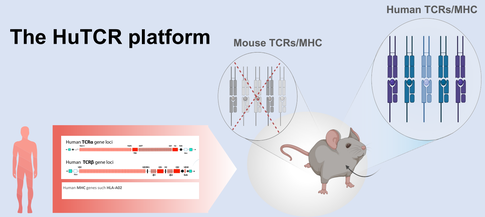
Background

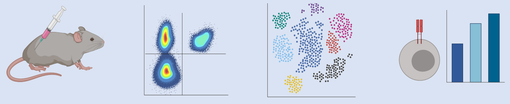
T cell receptor (TCR)-engineered T cell therapy can address key limitations of targeting solid tumors. Neoantigens are excellent therapeutic targets for engineered TCR T cells, as they are highly specific and often homogenously expressed in the tumor. While most neoantigens are patient-specific, the driver oncogene KRAS belongs to rare exceptions of widely shared neoantigens. The KRAS mutations G12C, G12D, and G12V are among the most common mutations in solid tumors[1], including indications with high unmet need, such as pancreatic, colorectal and non-small cell lung cancer. Here we utilize our unique, humanized HuTCR mouse platform to generate TCRs of optimal affinity and high specificity to a KRAS^{G12V} epitope in an HLA-agnostic manner.

The HuTCR platform



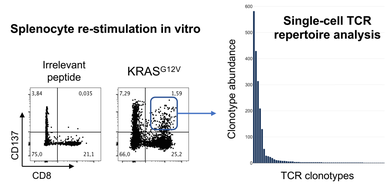
The HuTCR platform[2,3] is based on transgenic, humanized mouse lines that carry the entire human TCR alpha and beta gene loci and single or multiple human HLA molecules. The mice have a broad repertoire of fully human TCRs, while lacking murine TCRs and murine MHC class I molecules.

TCR discovery workflow



- HuTCR mice are immunized with antigenic peptides or DNA vectors encoding full-length antigens or minigenes. Hence, the TCR discovery remains unbiased for HLA/peptide combinations and allows identification of TCRs for immunogenic and naturally processed epitopes.
- After immunization, mice are screened for immune responses by in vitro restimulation of peripheral blood lymphocytes (PBLs).
- Antigen-specific T cells identified by the expression of activation markers are enriched from spleens and lymph nodes of responder mice and the TCR sequences are identified using single-cell sequencing.
- The most frequent TCR clonotypes are synthesized and further characterized.

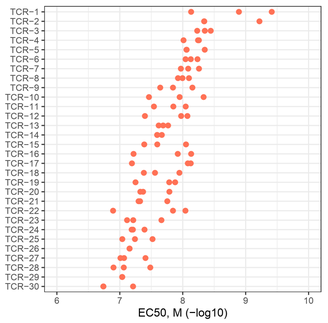
1 TCR generation



Identification of 30 distinct KRAS^{G12V}-reactive, HLA-A*11:01-restricted TCRs

To generate CD8+ T cell responses to KRAS^{G12V} epitopes, HuTCR mice were immunized with peptides or vectors encoding 35-mer minigenes. Splenic T cells from responder mice were restimulated in vitro either with the peptide, or with cells expressing KRAS^{G12V} and the HLA alleles present in the immunized mice. Antigen-specific CD8+ T cells were sorted for single-cell TCR sequencing. TCRs from expanded clonotypes were synthesized, cloned into retroviral vectors, transduced into human PBLs, and tested for specific reactivity to KRAS^{G12V} in vitro. In total, we identified thirty TCRs that recognize the KRAS^{G12V} nonameric or decameric epitopes, [V]VVGAVGVGK, presented by HLA-A*11:01.

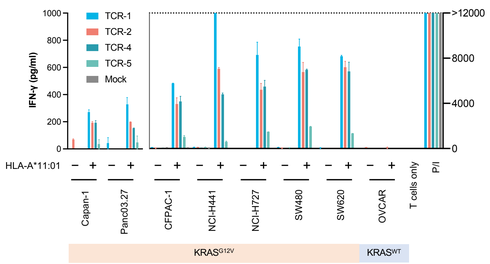
2 Peptide sensitivity



High sensitivity of KRAS^{G12V}-reactive TCRs

The functional avidity of identified TCRs was tested by co-culture of TCR-transduced PBLs with HLA-A*11:01 expressing cells loaded with the corresponding KRAS^{G12V} peptide at concentrations 10^{-6} M to 10^{-12} M. IFN-γ secretion in the culture supernatants was normalized to the maximum IFN-γ secretion levels of each TCR. Each dot represents PBLs of an independent donor.

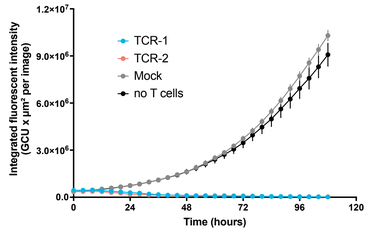
3 Natural antigen recognition



KRAS^{G12V}-reactive TCRs recognize KRAS^{G12V}-expressing cell lines

TCR- or mock-transduced PBLs were co-cultured with cell lines naturally harboring the KRAS^{G12V} mutation, upon or without transduction with HLA-A*11:01. PMA/ionomycin stimulation ("P/I") was used as positive control of T cell activation, whereas OVCAR cells that express the wild-type KRAS allele served as negative control. IFN-γ secretion in the culture supernatants was measured by ELISA.

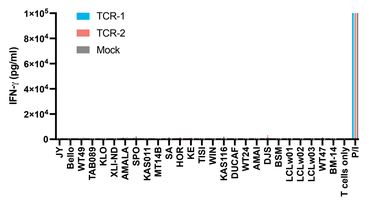
4 Cytotoxicity



Antigen-specific cytotoxicity against KRAS^{G12V}-expressing cell lines *in vitro*

TCR- or mock-transduced PBLs were co-cultured with HLA-A*11:01- and KRAS^{G12V}-expressing SW480 cells labelled with a fluorescent reporter. Cytotoxic activity of PBLs (proportional to reduction of the fluorescent signal) was monitored in real time.

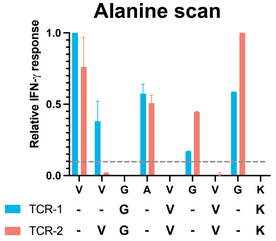
5 Absence of HLA allo-reactivity

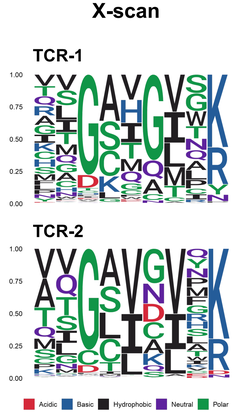


KRAS^{G12V}-reactive TCRs show no alloreactivity to a panel of HLA molecules

TCR- or mock-transduced PBLs were co-cultured with a panel of B-lymphoblastoid cell lines expressing different HLA haplotypes. IFN-γ secretion in the culture supernatants was measured by ELISA.

6 Determination of TCR recognition motifs





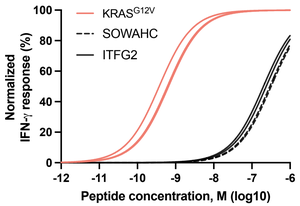
Identification of recognition motifs for TCR-1 and TCR-2

To define which amino acids in nonameric KRAS^{G12V} epitope are essential for TCR-pMHC interaction, TCR-transduced PBLs were co-cultured with HLA-A*11:01-expressing cells loaded with a library of nonameric peptides (at 10^{-7} M) in which amino acids at each position were replaced either with alanine ("alanine scan") or with all 19 alternative amino acids ("X-scan"). Identified motifs for each TCR were mapped to the human proteome. Matched peptides absent in the mouse proteome were experimentally tested for TCR recognition. TCR-1 recognized eight out of 356 tested peptides, whereas TCR-2 recognized two out of 74. The identified peptides were further evaluated for their physiological relevance.

Putative off-targets

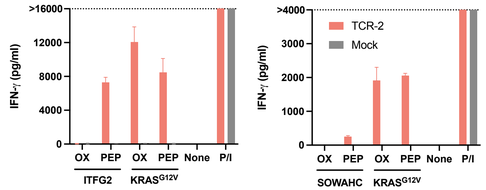
	TCR-1	TCR-2
Ala scan	2/144	2/7
X-scan	6/212	0/67
Total	8	2

7 Absence of off-target reactivity



TCR-2 binds its potential off-target epitopes with a thousand-fold lower avidity

To measure the functional avidity of TCR-2 to the potential off-target peptides ITFG2$_{41-49}$ and SOWAHC$_{350-358}$, TCR-2-transduced PBLs were co-cultured with HLA-A*11:01-expressing cells loaded with the peptides at concentrations 10^{-6} M to 10^{-12} M. The KRAS^{G12V} nonamer was used as positive control. IFN-γ responses are normalized to the maximum IFN-γ secretion levels. Data from two experiments and two donors are shown.



TCR-2 does not recognize putative off-targets when overexpressed or under physiological conditions

TCR-2-transduced PBLs were co-cultured with HLA-A*11:01+ cells that overexpress ("OX") ITFG2, SOWAHC or KRAS^{G12V} (as triple 35-mer minigenes), or pulsed with the corresponding peptides ("PEP"). P/I stimulation was used as positive control of T cell activation, whereas non-modified HLA-A*11:01+ cells served as negative control ("None"). Furthermore, no recognition was observed for cell lines with natural expression of ITFG2 and SOWAHC (data not shown).

Conclusions

- The HuTCR platform generates TCRs that bind to naturally processed, immunogenic epitopes presented by frequent HLA allotypes in an HLA/epitope unbiased way.
- Using the HuTCR platform, we identified a large panel of high-affinity HLA-A*11:01-restricted TCRs specific to the KRAS^{G12V} epitope.
- The identified TCRs mediated recognition of a large panel of KRAS^{G12V}-expressing cancer cell lines, as demonstrated by cytokine responses and cytotoxicity.
- Extensive safety validation workflow ensures high specificity and minimizes risks of off-target reactivity for HuTCR-generated TCRs.

References

1. Thein, Biter and Hong (2020) Therapeutics targeting mutant KRAS. Annu Rev Med 72:15.1-15.16
2. Li, Lampert, et al. (2010) Transgenic mice with a diverse human T cell antigen receptor repertoire. Nat Med 16:1029-1034
3. Obenaus et al. (2015) Identification of human T-cell receptors with optimal affinity to cancer antigens using antigen-negative humanized mice. Nat Biotechnol 33(4):402-407
4. Data analyses and visualizations were performed with GraphPad Prism 9, FlowJo 10.8.0, Loupe VDJ browser 4, and R packages ggplot2 and Immunarch.

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