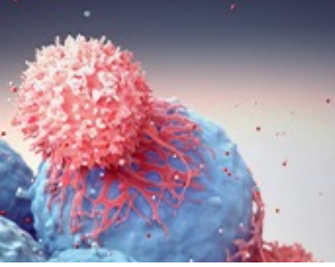

Discovery of novel solid-tumor targets combining a multi-omics based computational workflow and *in vivo* immunogenicity screening in T-knife's MyT™ platform

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Background

A key challenge for cancer immunotherapies is the identification of highly prevalent, tumor-specific T-cell targets that are also immunogenic. Immunogenicity depends on efficient antigen processing and presentation, triggering optimal T-cell activation, which is required for deep and durable anti-tumor immune responses. We previously demonstrated that T-knife's MyT platform[1–3] is a powerful discovery engine to deliver high-affinity, best-in-class T-cell receptors (TCRs) against cancer antigens. Here, we present an innovative strategy for identifying solid-tumor targets, employing a proprietary computational workflow based on multi-omics data combined with selection of highly immunogenic antigens by *in vivo* screening in fully immunocompetent humanized mice.

Methods

A computational target discovery workflow was developed by integrating public and proprietary databases encompassing bulk[4,5] and single-cell gene expression[6], protein expression[7] and antigen presentation[8]. Thresholds and filters were guided by validated tumor targets to prioritize novel targets with limited expression in healthy tissues and highly upregulated gene expression in tumors from indications with high unmet need, especially in late-line treatment. Shortlisted targets were further evaluated using the MyT platform, which consists of humanized mice that express a diverse, fully human TCR repertoire as well as highly prevalent HLA molecules, including HLA-A*02:01. MyT mice were immunized with full-length target antigens, which were then ranked based on target-specific T-cell responses in the peripheral blood over multiple immunization cycles. For selected targets, splenocytes were subsequently analyzed for reactivity to specific HLA-presented epitopes. In the following step, TCRs were isolated from responding T cells and screened using a reporter-based TCR reactivity assay. These pilot TCRs were expressed in human donor T cells and used to confirm recognition of human cancer cell lines.

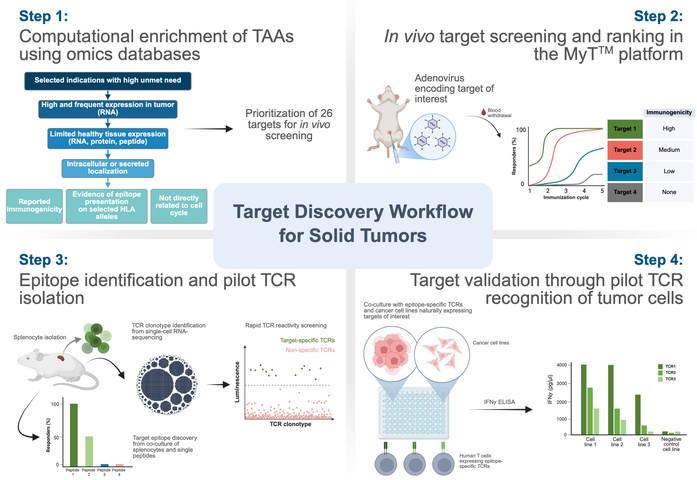

Fig. 1: Integrated discovery workflow for highly prevalent, immunogenic solid-tumor targets. (Step 1) 26 tumor-associated antigens (TAAs) were identified in a computational workflow using omics databases[4–8]. **(Step 2)** Mice of T-knife's MyT platform, expressing a diverse, fully human TCR repertoire restricted to seven prevalent HLAs, were immunized using adenoviruses encoding the target of interest. Based on immune response kinetics, immunogenicity scores (IGScore) were calculated to rank the screened targets. **(Step 3)** Pilot TCRs were isolated by scRNA sequencing from responding T cells and were tested in a rapid TCR reactivity screen (RTS). **(Step 4)** Reactive TCRs were transduced into human T cells and co-cultured with cancer cells naturally expressing the targets of interest to validate the identified solid-tumor targets.

Conclusions

- **We implemented a computational workflow harnessing multi-omics data guided by benchmarks from validated solid-tumor targets to shortlist potential tumor-associated antigens.**
- **We demonstrated that T-knife's MyT platform enables ranking of shortlisted solid-tumor targets by IGScores, reflecting each target's *in vivo* immunogenicity.**
- **Three highly immunogenic epitopes from APOBEC3B and TPX2, not previously reported as functional, were validated by recognition of human cancer cell lines and may have potential for deep and durable anti-tumor immune responses when targeted by immunotherapy.**

Computational workflow prioritizes promising solid-tumor targets for *in vivo* immunogenicity screen

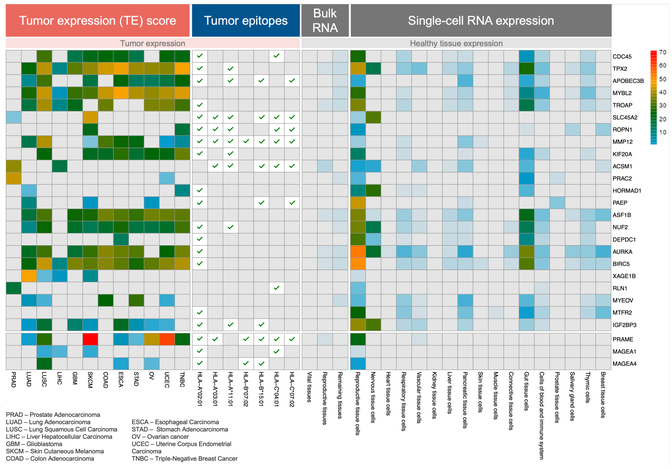

Fig. 2: Expression profiles of shortlisted solid-tumor targets in tumors and healthy tissues. Tumor expression (TE) score was calculated by multiplying target prevalence based on RNA expression[4] (> 5 TPM) by $\log_{10}$ (median RNA expression+1) for each indication. The values were multiplied by 30 for better visualization. Targets with >30% prevalence are shown. Tumor epitopes are ticked if there were one or more epitopes detected by mass spectrometry[8] on the selected HLAs. Bulk RNA expression[5] shows the number of healthy tissues that express target >2 TPM in vital tissues (brain, heart, lungs, liver, kidneys), reproductive tissues (testis, uterus, ovaries, vagina), or the remaining tissues. Single-cell RNA expression[6] shows the number of cell clusters expressing a target >2% per indicated tissue. PRAME, MAGE-A1 and MAGE-A4 are displayed as reference targets.

MyT™ platform enables ranking of prioritized targets based on *in vivo* immunogenicity

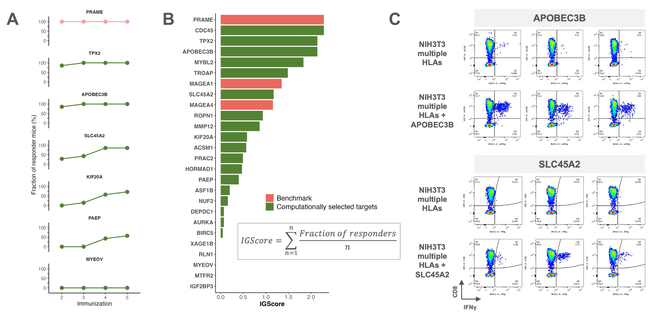

Fig. 3: Immunogenicity of prioritized solid-tumor targets in the MyT™ platform. (A) Response kinetics measured in the peripheral blood of immunized mice (n = 7 per target) are displayed for six representative targets and PRAME as a reference. Repeated immunizations (x-axis) were performed in 4-week intervals. Frequency of responder mice is shown on the y-axis. **(B)** Based on the response kinetics, immunogenicity scores (IGScore) were calculated to rank immunogenicity of screened targets. IGScore was calculated by summing up the fractions of the responder mice in each immunization cycle divided by the number of immunizations (*n*). **(C)** Representative immune responses in mice of the MyT platform. Seven days after immunization, peripheral blood was incubated with NIH3T3 cells expressing the target and all HLAs present in the MyT platform. Cells not expressing the target were used as a negative control. After 6 hours of stimulation, T cells were stained intracellularly for IFNγ.

Literature

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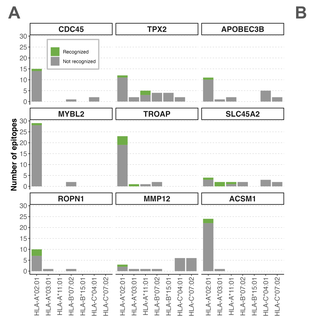
Immune responses encompass one or multiple epitopes across different HLAs including epitopes not previously reported



B

Target	Sequence	HLA
CDC45	SMEHGTVTV	HLA-A*02:01
TPX2	LAGIGQPVKK	HLA-A*02:01
TPX2	EGIGPLPKK	HLA-A*11:01
TPX2	AGIGQPVKK	HLA-A*11:01
APOBEC3B	RLDNGTWVL	HLA-A*02:01
APOBEC3B	YLCYEVERL	HLA-A*02:01
MYBL2	SINNSLVQL	HLA-A*02:01
TROAP	GTSHLPGLLK	HLA-A*03:01
TROAP	TLLEWQDAL	HLA-A*02:01
TROAP	LLRQEVEGL	HLA-A*02:01
TROAP	ATLLEWQDAL	HLA-A*02:01
TROAP	VELQPLLTEI	HLA-A*02:01
SLC45A2	SLYSYFQKV	HLA-A*02:01
SLC45A2	GMYEYGSIEK	HLA-A*03:01
SLC45A2	SSLYSYFQK	HLA-A*11:01
SLC45A2	ALIANPRRK	HLA-A*03:01
ROPN1	EVIGPDGIITV	HLA-A*02:01
ROPN1	VIGPDGIITV	HLA-A*02:01
ROPN1	SALGVTITK	HLA-A*02:01
MMP12	KIQEMQHFL	HLA-A*02:01
ACSM1	TLDPMVIFF	HLA-A*02:01
ACSM1	VIDDKGSIL	HLA-A*02:01

C

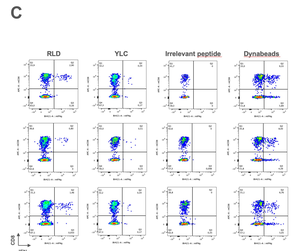


Fig. 4: Epitope discovery using splenocytes from immunized mice restimulated with individual peptides. Selected epitopes previously reported or predicted to bind to HLAs present in the MyT platform were tested as peptides in splenocyte cultures (3–6 mice per target) and T cells were stained for intracellular IFNγ. **(A)** Summary of recognized (green) and non-recognized peptides (grey) are displayed for the prioritized immunogenic targets. **(B)** Reactive epitopes are displayed with their corresponding HLA restriction. **(C)** Representative splenocyte responses (for 3 mice) after 5 hours of stimulation with indicated APOBEC3B peptides.

Rapid TCR reactivity screening identifies pilot TCRs for prioritized solid-tumor targets

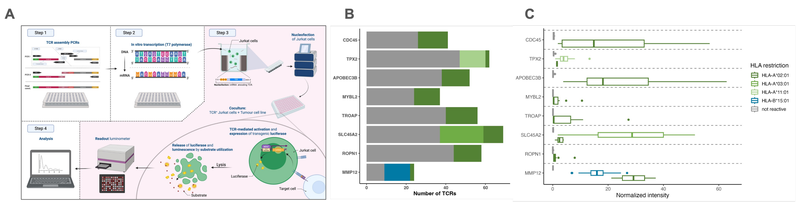


Fig. 5: Rapid screening of MyT platform-derived TCRs for reactivity to immunogenic targets. TCRs of expanded T-cell clonotypes as identified by scRNA sequencing of responsive T cells from immunized mice were subjected to a rapid TCR reactivity screening (RTS) against selected solid-tumor targets. **(A)** Schematic representation of the RTS assay. In short, TCRs from expanded T cell clones were assembled, *in vitro* transcribed and nucleofected into a reporter cell line expressing luciferase upon TCR activation. Reactivity was measured as luminescence after co-culture with T2 cells loaded with peptides or K562 cells expressing the corresponding target and HLA. **(B)** Numbers of expanded TCR clonotypes identified for each target are displayed. Three to six mice were analyzed per target. Reactive TCRs are colored according to their HLA restriction as indicated. **(C)** Normalized TCR reactivity (per target, the median background (non-loaded T2 cells) subtracted and normalized to PMA/ionomycin, shown as % of PMA/ionomycin intensity); colored by HLA restriction; boxplots showing median, q25, q75.

Validation of highly ranked targets via pilot TCR recognition of cancer cells with endogenous target expression

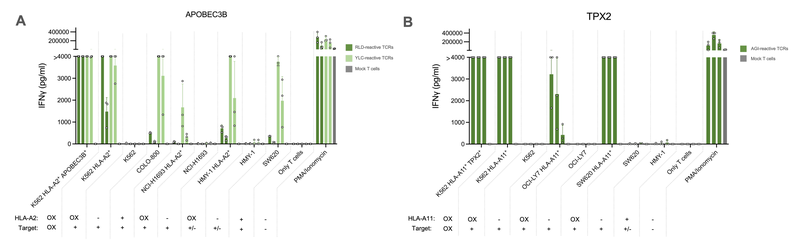


Fig. 6: Co-culture of TCR-T cells with cancer cell lines expressing APOBEC3B or TPX2. (A, B) TCR-transduced T cells were co-cultured overnight with selected cancer cell lines expressing the corresponding targets and HLAs. IFNγ release upon T-cell activation was assessed in the supernatant by ELISA. **(A)** APOBEC3B TCRs with reactivity to the epitopes RLDNGTWVL (RLD) or YLCYEVERL (YLC) and **(B)** TPX2 TCRs with reactivity to AGIGQPVKK (AGI) were tested. RLD, YLC and AGI were not previously reported as immunogenic and functional epitopes. The indicated HLA is either naturally expressed (+), overexpressed (OX), or not expressed (–). APOBEC3B and TPX2 are either overexpressed (OX), expressed >50 TPM (+), 30-50 TPM (+/–), or not expressed (<30 TPM; –). Mock-transduced T cells and T cells without target cells ("only T cells") are negative controls, PMA/Ionomycin was added to T cells as positive control. Data are shown as means of duplicates for 3 donors + SD.