Discovery of novel MAGEC2 epitopes for TCR-T adoptive cell therapy from expanded T cell clones of TIL therapy products

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Overview of Study

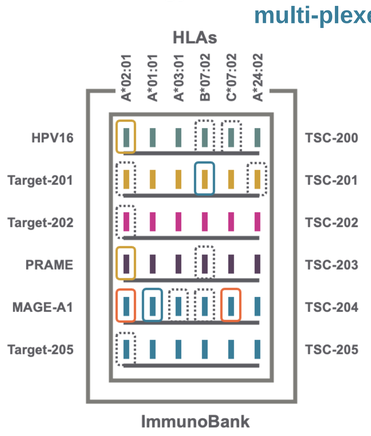
Background: TCR-T adoptive cell therapy is a promising approach to treating solid tumors, but the heterogeneous expression of TCR targets by the tumor and T cell evasion mechanisms are barriers to durable responses. HLA heterogeneity further limits the addressable population. Multiplexing TCR-T products offers a unique strategy to address the heterogenous landscape of targets presented by a diverse array of HLAs, but requires the identification of novel epitopes presented on unaddressed HLAs. TScan's proprietary platform, TargetScan, is an unbiased method to discover the natural targets of T cell clones responding to tumors.

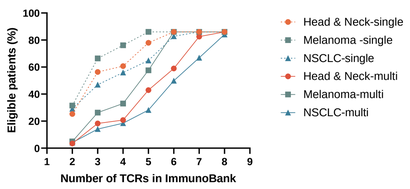
Methods: The target landscape recognized by the most expanded T cell clones derived from clinical melanoma TIL therapy products was evaluated using TScan's TargetScan platform. TCR Targets were identified using a strategy of screening TCRs from the most expanded T cell clones against a peptidome-wide library, and the next most frequent T cell clones against a focused cancer testis antigen (CTA) library. Using this approach, TCRs recognizing targets with a favorable tissue expression profile for targeting with a TCR-T therapy were identified. Individual TCRs were cloned and evaluated for cytotoxicity, cytokine release, and T cell proliferation in response to co-culture with cancer cell lines expressing their cognate antigens.

Results: Peptidome-wide screens of the 10 most expanded TCRs revealed several known targets including the A*02:01 presented MART126-35 epitope as well as previously unknown cancer associated targets with limited tissue specific off-tumor expression including brain tissue. CTA focused screens identified novel targets including a novel clinically relevant B*07:02 presented epitope of the cancer testis antigen MAGEC2. The reactive TCR was identified and exhibited cytotoxicity, cytokine release, and T cell proliferation when co-cultured with B*07:02 expressing cancer cell lines that express MAGEC2 including FTC133, A101D, and SKLMS1. The degree of the response corresponded with the level of MAGEC2 expression in the cell lines.

Conclusion: Using our TargetScan platform, we have shown that expanded T cell clones from clinical TIL products express TCRs that recognize tumor associated targets; the novel B*07:02 restricted epitope of MAGEC2 is a promising target for TCR-T therapy potentially enabling us to target 20% of the US patient population. TargetScan mediated discovery of novel epitopes across a diverse set of HLAs will further enable a multiplexing approach to TCR-T therapy.

TScan's Strategy: build an ImmunoBank of therapeutic TCRs to enable multi-plexed TCR therapy





TScan is building an ImmunoBank of therapeutic TCR-Target pairs to address antigen and HLA heterogeneity. Expanding the frequency of eligible patients requires the discovery of targetable epitopes presented on an array of HLAs. With each addition to the bank, the number of eligible patients increases.

TCRs sequences were sourced from TIL therapy products used to treat melanoma patients

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Patient ID	# of TCRs	Response	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C
LD1	344	PD/**MR**	A*02:01	A*03:01	B*07:02	B*40:01	C*03:04	C*07:02
LD2	37	PD	A*01:01	A*24:02	B*08:01	B*27:05	C*02:02	C*07:01
LD3	39	PD	A*03:01	A*25:01	B*14:01	B*35:01	C*04:01	C*08:02
LD4	61	**SD**	A*03:01	A*24:02	B*07:02	B*15:01	C*03:03	C*07:02
LD5	107	**PR**	A*02		B*07		C*07	
LD6	219	PD	A*01	A*02	B*07	B*08	C*07	C*07
LD7	527	PD	A*02:01		B*15:01	B*40:02	C*02:06	C*03:04
LD8	12	PD/**MR**	A*02:01	A*24:02	B*07:02	B*27:05	C*01:02	C*07:02
LD9	147	PD/**MR**	A*01:01	A*02:01	B*07:02	B*08:01	C*07:01	C*07:02
LD10	81	**SD**	A*02:17	A*32:01	B*18:01	B*40:02	C*02:02	C*07:01
LD11	147	**PR**	A*02:01		B*07:02	B*08:01	C*07:01	C*07:02
LD12	38	**CR**	A*02		B*15	B*35	C*03	C*04

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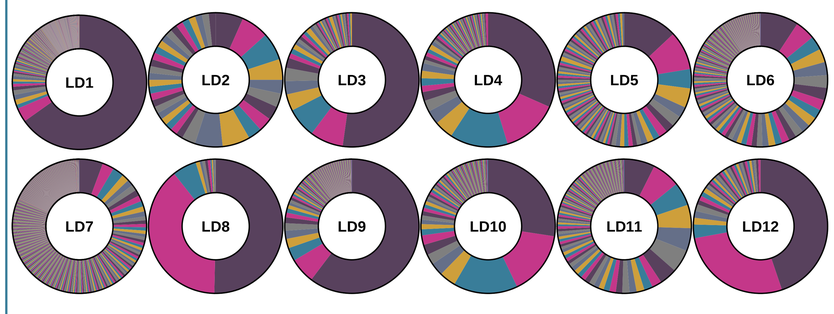

A) Table showing the patient outcome, HLA type, and the number of TCR sequences detected from the CD8 TILs. PD: Progressive Disease, SD: Stable Disease, PR: Partial response, MR: Mixed Response. **B)** TCR diversity in each patient's TIL product. The TCR repertoire in most patient TILs were dominated by a limited set of clones. These predominant TCR sequences were cloned and transduced into donor T cells to be assessed by TScan's TargetScan platform.

TargetScan platform enables the identification of the natural targets of TCRs

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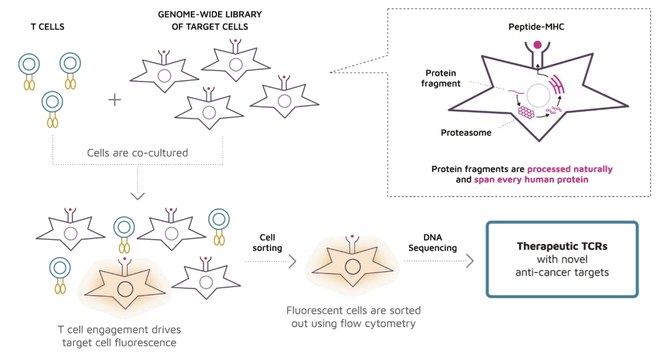

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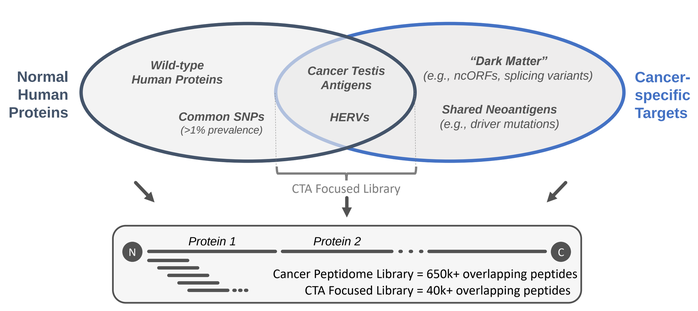
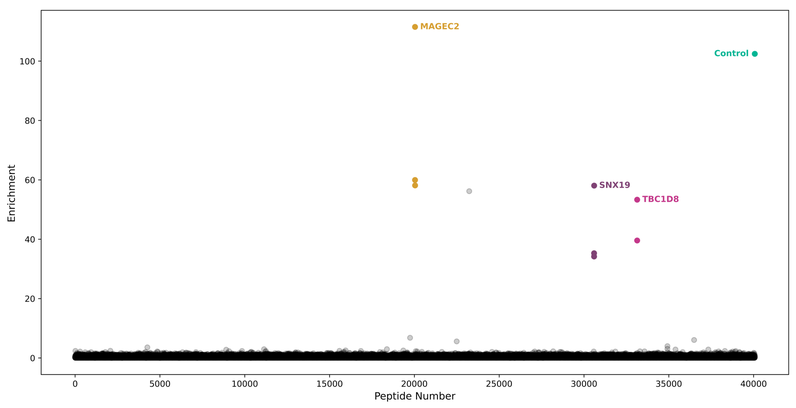


A) TargetScan platform schematic. TCRs of interest are expressed in donor T cells and co-cultured with cells engineered with a granzyme-activated fluorescent reporter and a peptide library. Recognized cells are sorted and analyzed by sequencing to identify the peptides recognized by the TCR. **B)** Schematic illustrating the design of peptide libraries for TargetScan screens. Overlapping peptide fragments spanning the full-length protein sequences in the library enable the identification of the minimal epitope recognized by the TCR from the overlapping regions enriched by the assay. For this study, a cancer peptidome library comprised of more than 650,000 overlapping peptides was used to assess individual TCRs with a >10% frequency in the TIL product, and a cancer testis antigen (CTA) focused library comprised of more than 40,000 overlapping peptides was used to assess pools of TCRs.

TargetScan screens revealed multiple targets of TIL therapy TCRs



Representative TargetScan screen displaying results for 36 of the most abundant TCRs across all patients that were screened using a CTA focused library with cells expressing B*07:02. In addition to the positive control target, three B*07:02 restricted targets were identified.

Target expression profile in cancer and normal tissues

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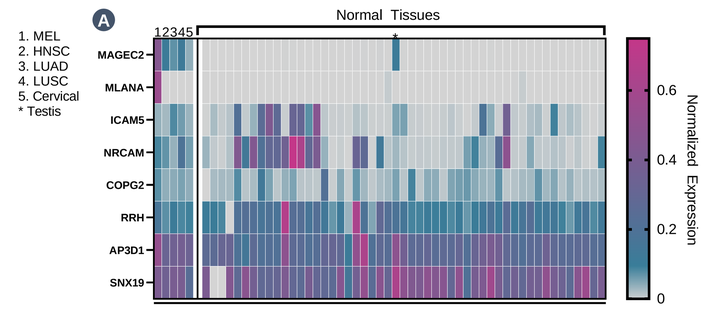

A) Tissue expression of TCR targets identified by TargetScan. Each square depicts the mean of the top quartile of the indicated tissue type. Targets ranged from established melanocyte antigens (MLANA), cancer testis antigens (MAGEC2), targets with limited tissue expression (ICAM5, NRCAM, COPG2), and ubiquitous tissue expression (AP3D1, SNX19). MAGEC2 exhibited a favorable tissue expression for TCR therapy and was evaluated further. **B)** qPCR illustrating MAGEC2 expression in melanoma cancer tissues of various progression showing increasing MAGEC2 expression with disease progression.

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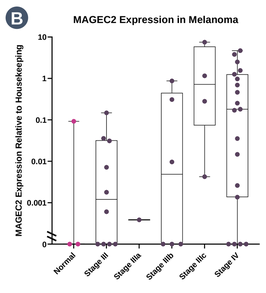

Novel epitopes are immunogenic and recognized by the patient-derived TCR

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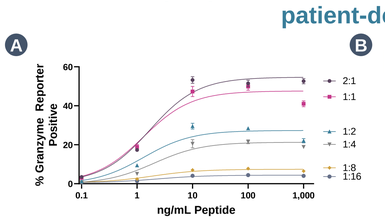

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B*07:02 Model Cancer Cell Lines: RNA Expression (TPM)			
Cell Line	**Origin**	**HLA-B**	**MAGEC2**
FTC133	Thyroid Carcinoma	124.9	312.4
A101D	Melanoma	101.0	232.7
SKLMS1	Leiomyosarcoma	148.8	37.3
A2058	Melanoma	29.5	6.1

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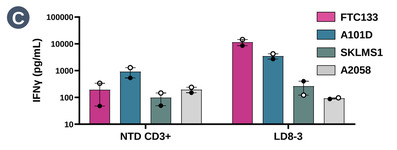

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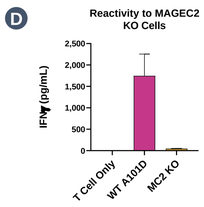

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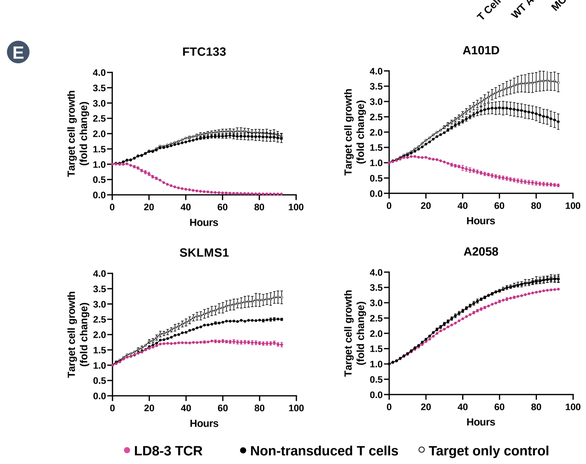

A) Peptide-pulsed B*07:02 monoallelic HEK293T cells engineered with a granzyme-dependent reporter confirmed a dose dependent activity of the MAGEC2 specific TCR LD8-3 to the minimal epitope. **B)** B*07:02 expressing cancer cell lines selected as a model with various expression levels of MAGEC2. **C)** IFN release after co-culture of the LD8-3 TCR with cancer cell lines. **D)** Reactivity of LD8-3 TCR to MAGEC2 KO clones generated with CRISPR/Cas9 illustrating target specificity for LD8-3 mediated reactivity to cancer cells. **E)** Incucyte killing assay measuring LD8-3 mediated killing of MAGEC2 expressing B*07:02 cancer lines. The level of killing corresponded with the level of MAGEC2 expression.

Similar workflows identified a novel A*24:02 presented epitope of MAGEC2

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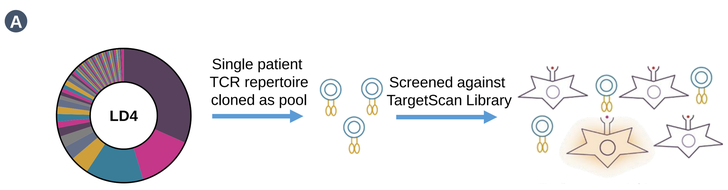

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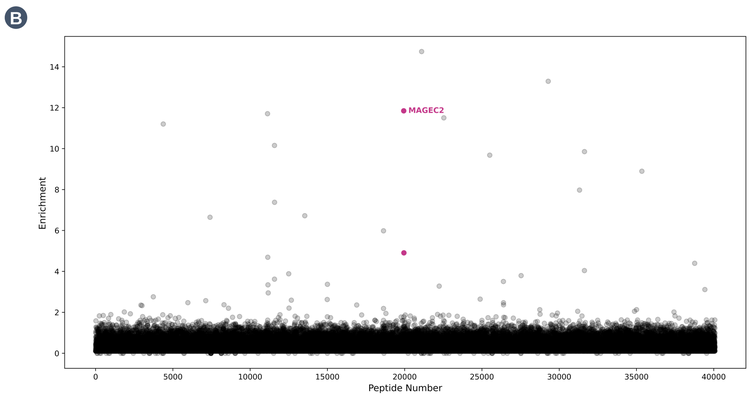

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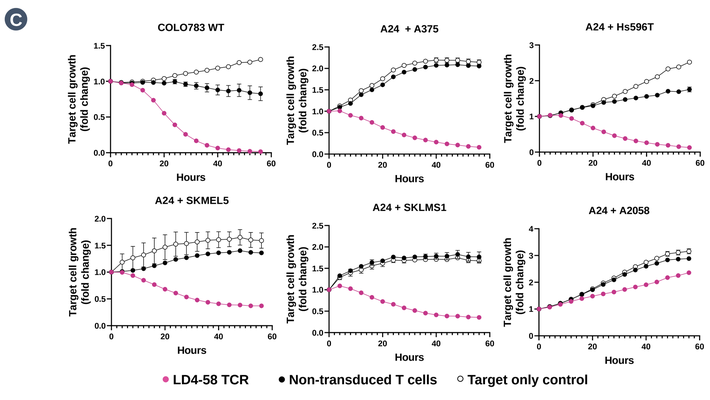

A) Schematic illustrating the screening strategy for the TCR repertoire from LD4. The TCR repertoire of the LD4 TIL product was bulk cloned and transduced into donor T cells for screening against a CTA focused library. **B)** Screen results of LD4 TCRs illustrating a novel MAGEC2 epitope presented on HLA-A*24:02. The TCR was identified as LD4-58, and the minimal epitope determined (data not shown). **C)** Incucyte killing assay measuring LD4-58 mediated killing of an A*24:02 and MAGEC2 expressing cancer cell line, and cell lines with various expression of MAGEC2 engineered to express A*24:02.

 # 364: Non-clinical development of T-Plex component TSC-200-A0201: A natural HPV16 E7-specific TCR-T cell therapy for the treatment of HPV16-positive solid tumors

 # 376: Overcoming tumor heterogeneity – Clinical trial assays to prospectively assign patients customized multiplexed TCR-T cell therapy in Phase 1

 # 390: Discovery of MAGE-A1-specific TCR-T cell therapy candidates to expand multiplex therapy of solid tumors

 # 682: Phase 1 trial of TSC-100 and TSC-101, engineered T-cell therapies targeting minor histocompatibility antigens to eliminate residual disease after hematopoietic cell transplantation

 # 709: Product characteristics and clinical trial design for T-plex, a multiplexed, enhanced T cell receptor-engineered T cell therapy for solid tumors