Neoantigen-specific TCR-T cells targeting shared hotspot mutations for adoptive cell therapy in common epithelial cancers

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Summary

Background

EGFR, *KRAS* and *TP53* have frequent somatic hotspot mutations giving rise to amino acid substitutions in EGFR, KRAS and p53 proteins, respectively. These aberrant proteins can be presented on tumor cells by human leukocyte antigen (HLA) molecules as neoantigens to T cells through their T-cell receptor (TCR). These mutations are critical for the cancer cell and are absent in normal tissue; thus, these shared neoantigens are attractive, tumor-specific targets. A TCR library can be used "off-the-shelf" for any patient with matching somatic hotspot mutation and HLA restriction to enable rapid treatment of a larger patient population. *Sleeping Beauty* transposition is the most advanced non-viral gene transfer technology for TCR-T cells and is appealing for TCR libraries given its low cost, speed, and flexibility.

Methods and Results

In this study, *Sleeping Beauty* transposons were constructed with TCRs targeting EGFR, KRAS and p53 neoantigens restricted by either or both HLA Class-I and HLA Class-II molecules. Donor T cells were co-electroporated with Library TCR transposon and *Sleeping Beauty* transposase and grown *in vitro* to clinical scale quantities (>10^9 TCR-T cells) with high neoantigen-specific TCR expression (>60%). The neoantigen specificity of Library TCRs were confirmed in co-cultures with antigen-presenting cells pulsed with peptides, which was demonstrated by specific IFN-γ secretion and/or up-regulation of 4-1BB. All Library TCRs exhibited high avidity (<1 ng/mL for some TCRs) and negligible recognition of wild-type peptides. Furthermore, TCR-T cells lysed tumor cells with endogenous expression of the somatic mutation and HLA restriction element but did not recognize tumor cells lacking either somatic mutation or HLA restriction element, indicating that the targeted neoantigens are normally displayed on the tumor cell at sufficient levels for specific-killing by TCR-T cells.

Conclusions and future directions

The safety and efficacy of *Sleeping Beauty*-engineered TCR-T cells is currently being investigated in solid tumors (bile duct, colon, lung, pancreas and gynecological cancers) in a Phase I/II clinical trial, conducted in collaboration with MD Anderson Cancer Center. Patients with somatic hotspot mutation and HLA-restriction matched to a TCR in Ziopharm's Clinical TCR Library will be treated with neoantigen-specific TCR-T cells. Overall, Ziopharm's Library TCR-T cells have the potential for safe, durable, objective clinical regressions of cancer.

Library TCR-T cells targeting shared hotspot mutations

HLA allele	Estimated HLA pop. frequency	Mutated gene	Amino acid substitution	Annual incidence of patients with targeted mutation (estimated)	Annual incidence of patients with mutation:HLA
A*11:01	16%	*KRAS*	G12D	64,000	10,200
A*11:01	16%	*KRAS*	G12V	45,000	7,200
C*01:02	13%	*KRAS*	G12V	45,000	5,900
C*08:02	9%	*KRAS*	G12D	64,000	5,700
A*02:01	36%	*TP53*	R175H	4,000	1,400
A*68:01	6%	*TP53*	R248W	2,000	100
DRB1*13:01	14%	*TP53*	R175H	4,000	600
DRB3*02:02	41%	*TP53*	Y220C	1,000	400
DPB1*01:01	6%	*EGFR*	E746-A750del	21,000	1,200
				250,000 (total)	32,700 (total)

Table 1. Selected targets from the Ziopharm TCR-T cell library. Targets of selected TCRs within the Ziopharm TCR library are combinations of HLA restriction and a peptide having a substituted amino acid as the result of a somatic mutations expressed by the tumor. Estimated HLA population frequencies in the United States were generated from http://www.allelefrequencies.net/ from a mean between Caucasian, African-American, Asian-American and Latino-American populations. The mutational incidence was estimated from https://cancer.sanger.ac.uk/cosmic and combined for bile duct, pancreas, ovary, endometrial, lung and colorectal cancers.

Generation of TCR-T cells in peripheral blood T cells by *Sleeping Beauty* transposition

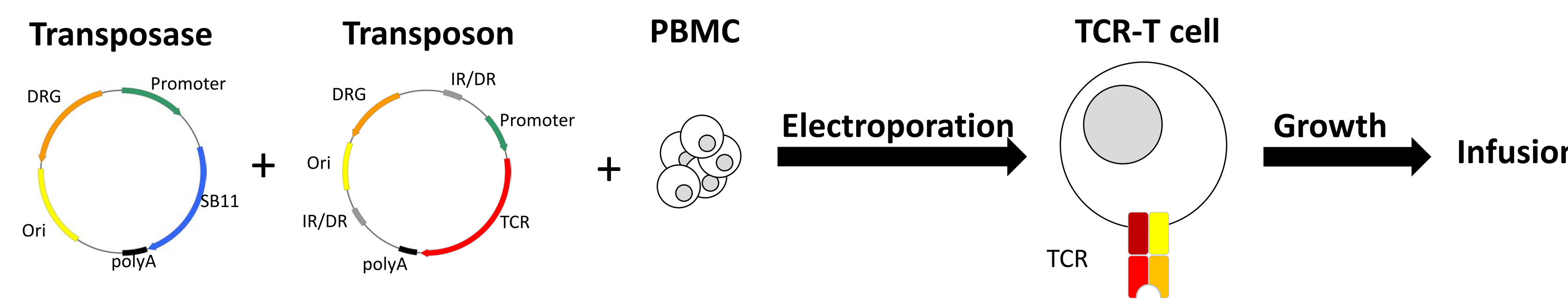

Figure 1. Insertion of neoantigen-specific TCRs in T cells by Sleeping Beauty transposition. SB11 transposase is transiently expressed (non-integrated). Stable expression of TCR is driven by internal promoter within the transposon cassette and integration at flanking IR/DR sequences into TA dinucleotide repeats in the human genome. This non-viral expression system is capable of genetically modifying primary cells, *e.g.,* T cells.

Stable expression of neoantigen-specific TCRs on T cells can be achieved by *Sleeping Beauty* transposition

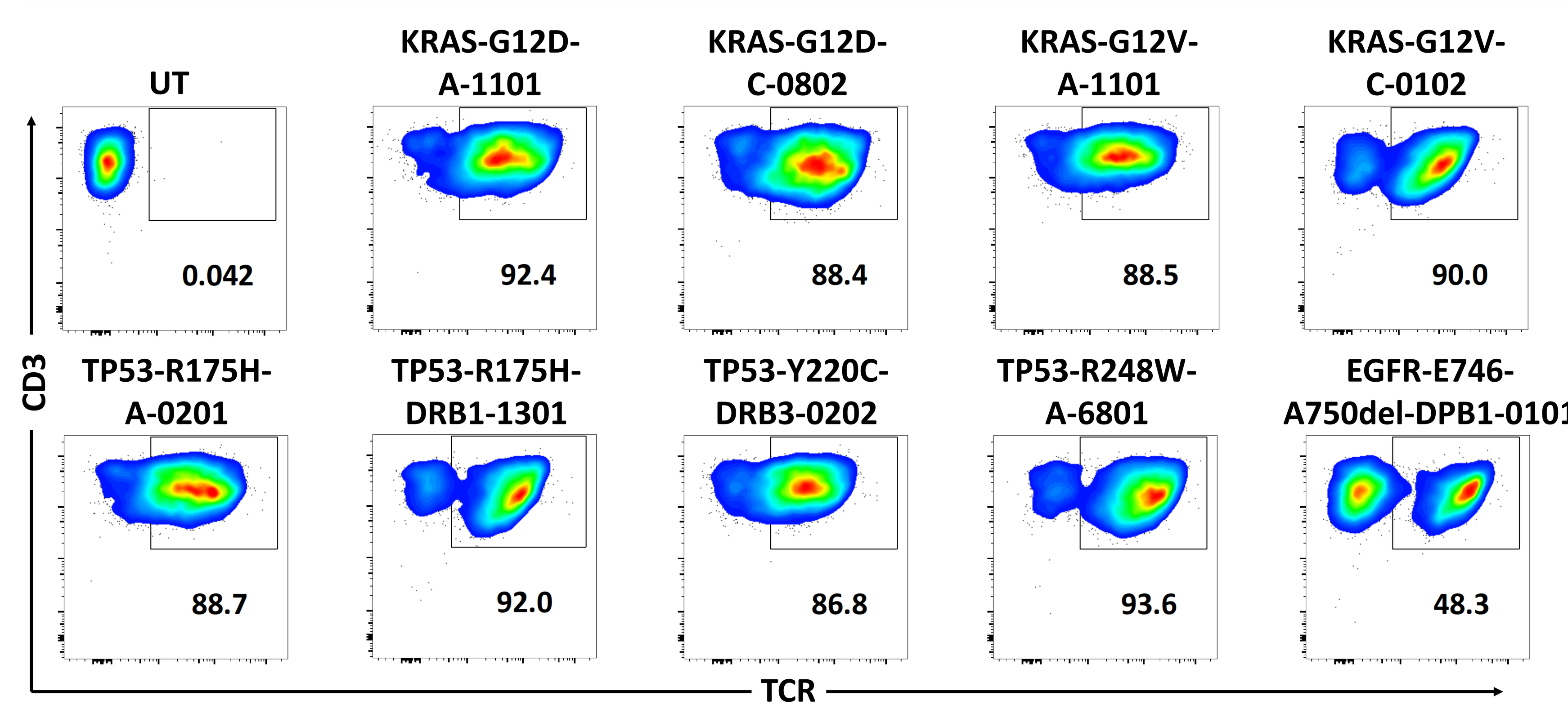

Figure 2. Stable transposition of neoantigen-specific TCRs in human T cells. Representative expression of the introduced TCR (x-axes) in T cells (CD3+, y-axes) from donors (n=4-5) where the specificity of the TCR is displayed above the flow plots. The samples were grown for weeks prior to the staining displayed and indicate stable expression of the neoantigen-specific TCR. Gating was live lymphocytes.

TCR-transposed T cells can grow to clinical scale for targeting neoantigens

Parameter	PD-1	PD-2	PD-3	MFG-1	MFG-2	MFG-3
Viability	89%	84%	89%	95%	96%	91%
% CD3	99%	99%	98%	100%	100%	100%
% TCR	73%	62%	85%	82%	76%	66%
Total cells*	$1.6x10^{10}$	$1.8x10^{9}$	$4.1x10^{9}$	$2.5x10^{10}$	$2.9x10^{10}$	$1.7x10^{10}$

Table 2. Clinical-scale production of neoantigen-specific TCR-T cells. Three independent process development (PD) and three independent manufacturing (MFG) runs were performed in the translational lab or cGMP lab, respectively. After expansion, the cell products were counted and characterized for viability and expression of CD3 and the introduced neoantigen-specific TCR. Each of the MFG runs had dose-level 2 at the time of harvest (>$1x10^{10}$ TCR+ T cells). *The reported total cells are actual values at the time of harvest and did not account for cells discarded in the culture splits; therefore, the cell counts achievable was likely larger in several of the runs.

TCR-T cells were specific to the neoantigen and showed negligible recognition of wild type sequences

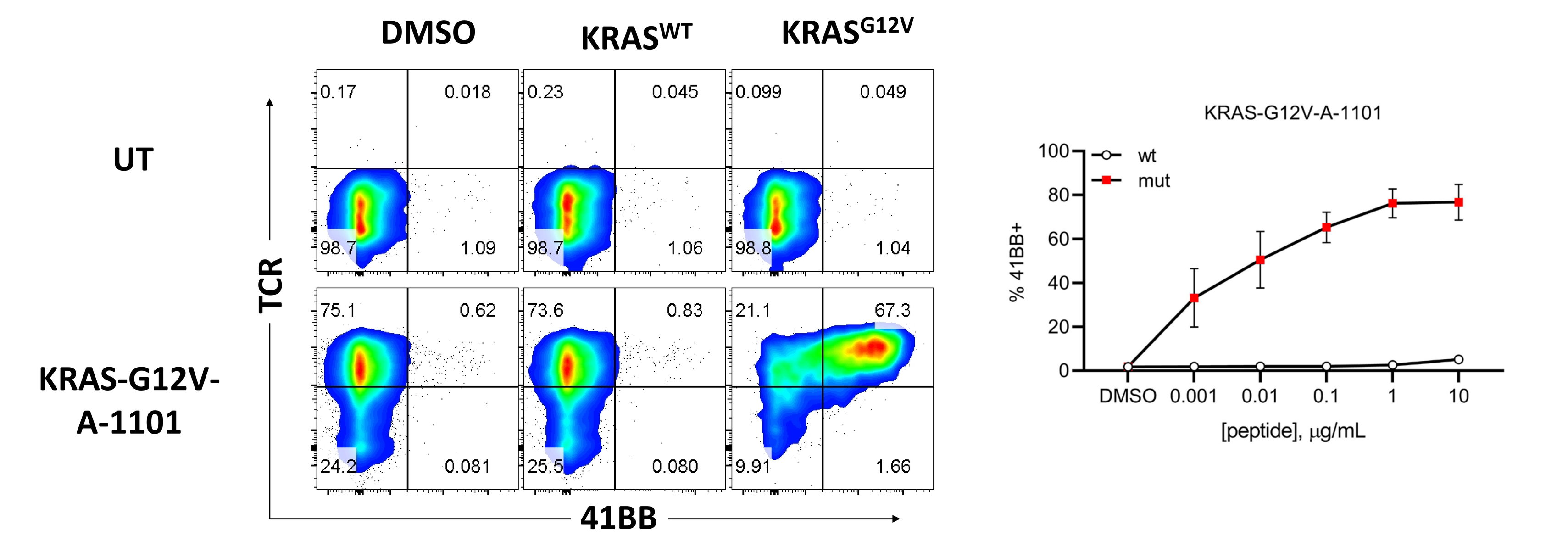

Figure 3. *Sleeping Beauty* transposed TCR-T cells displayed high avidity and specificity for the targeted neoantigen. TCR-T cells were co-cultured with antigen presenting cells pulsed with KRAS peptides either in wild type or mutated variants. Expression of T-cell activation was measured by up-regulation of 41BB on the TCR-T cell surface. Dose response to the mutated, but not the wild type, peptides was observed for both KRAS and p53 neoantigens, demonstrating that TCR-T cells were specific and did not recognize the germline sequences and are, therefore, unlikely to recognize normal tissues. Gates were set on lymphocytes.

Tumor cells with endogenous expression of HLA and mutation were lysed by neoantigen-specific TCR-T cells

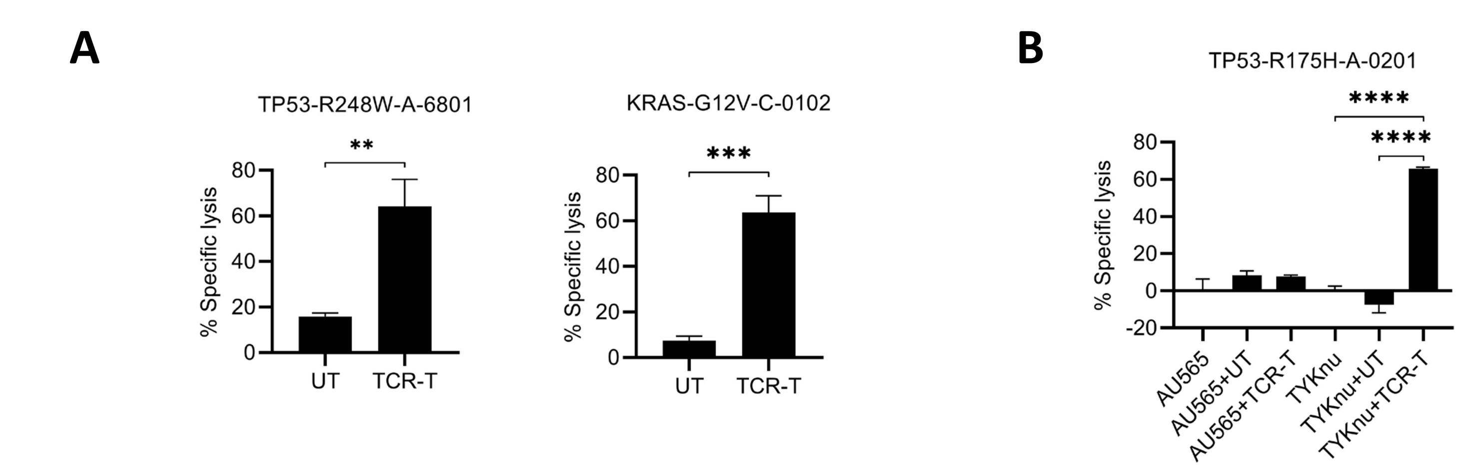

Figure 4. Lysis of tumor cells expressing neoantigen:HLA complexes by *Sleeping Beauty* transposed TCR-T cells. (A) Saos2 tumor cells were transfected with HLA-A*68:01 (left) or HLA-C*01:02 (right) and were pulsed with the neoantigen peptide then co-cultured with TCR-T cells. **(B)** TCR-T cells targeting $p53^{R175H}$ in the context of HLA-A*02:01 were co-cultured with two $p53^{R175H}$ expressing cell lines: AU565 (HLA-A*02:01^{neg}) or TYKnu (HLA-A*02:01^{+}). Tumor killing was evaluated by CellTiter-Glo assay which evaluated viable cells relative to control wells and was used to calculate relative specific lysis. Specific recognition of the tumor cells with matching HLA and mutation, but not tumor cells lacking the HLA restriction, by TCR-T cells and not untransfected T cells showed that specific tumor killing occurred.

Conclusions and future directions

- *Sleeping Beauty* transposition was effective in expressing neoantigen-specific TCRs in donor peripheral blood T cells to generate TCR-T cells.
- TCR-T cells were specific for EGFR, KRAS and p53 neoantigens and could directly kill unmodified tumor cell lines with endogenous expression of HLA and hotspot mutation.
- Translation of these TCR-T cells for clinical use is promising and could be an "off-the-shelf" reagent used for the treatment of cancer for anyone with matching HLA and hotspot mutation.
- A Ziopharm-sponsored Phase I/II clinical trial will evaluate the ability of a library of autologous EGFR, KRAS and p53 neoantigen-specific TCR-T cells to eliminate relapsed/refractory tumors in adult patients with multiple cancer types including lung cancer, gynecological cancers, colorectal cancer, pancreatic cancer and cholangiocarcinoma.