Mutations in critical genes for cell survival and proliferation, e.g., KRAS and TP53, are found as clonal events in multiple tumor types of unrelated people likely due to their importance to the malignant phenotype. T cells recognize products of mutated genes, termed neoantigens, because they are expressed in the tumor but not in the normal tissues; thus, neoantigens are foreign entities from an immunological perspective. T-cell receptors (TCRs) with specificity to the neoantigen in the context of human leukocyte antigen (HLA) on the tumor cell surface can be isolated from the neoantigen-reactive T cell and potentially used for genetically-modified adoptive immunotherapy for any patient with matching mutation and HLA. The purpose of this study was to evaluate the ability of the non-viral Sleeping Beauty transposon/transposase gene transfer system to re-direct the specificity of T cells towards p53 and KRAS neoantigens and to characterize the resulting engineered TCR-T cell populations for specificity and function. Genes encoding the alpha and beta chains of p53 or KRAS neoantigen-specific TCRs were linked by a 2A ribosomal slip site linker and cloned into the clinical SB11 transposase and expanded in vitro. Logarithmic proliferation was observed and resulted in large numbers of highly pure TCR-T cells(>80% by introduced TCR expression). The TCR-T cells were transfected with a plasmid encoding the SB11 transposase and expanded in vitro.}

**Neuroantigens at tumor-specific targets of TCR-T cells**

<table>
<thead>
<tr>
<th>TCR-T cell</th>
<th>TCR-T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cell</td>
<td>T cell</td>
</tr>
<tr>
<td>HLA</td>
<td>HLA</td>
</tr>
<tr>
<td>Neoantigen</td>
<td>T cell</td>
</tr>
<tr>
<td>HLA</td>
<td>T cell</td>
</tr>
</tbody>
</table>

SB11 transposase excises transposon from plasmid DNA backbone

**Sleeping Beauty transposition**

- SB11 transposase cuts genome at TA dinucleotide repeats and ligates transposon into genome
- Stable expression of TCR is driven by internal promoter within the transposon cassette.
- Non-viral expression system capable of genetically modifying primary cells, e.g., T cells.

**Library TCR-T cells targeting shared hotspot mutations**

Expanding Off-the-Shelf TCR Library – Q1 2021 IND Cleared

**Neoantigens at tumor-specific targets of TCR-T cells**

- TCR-T cells specifically recognize neoantigens and kill unmodified epithelial cancer cells

**TCR-T cells**

- TCR-T cells were co-cultured with antigen presenting cells pulsed with KRAS (top) or p53 (bottom) 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.
- Tumor cells expressing the hotspot mutation and either an irrelevant HLA (TCR); HLA-neoAg+) or relevant HLA (TCR); HLA-neoAg+) were co-cultured with no T cells (open shapes), open repertoire untransfected T cells (gray shapes) or TCR-T cells (red shapes). Tumor killing was evaluated by CellTiter-Glo assay which evaluates 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 could occur through this approach.

**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 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 has been cleared by the FDA and will evaluate the ability of a library of autologous KRAS and p53 neoantigen-specific TCR-T cells to eliminate relapsed/refractory tumors in adult patients with a number of cancer types including lung cancer, gynecological cancers, colorectal cancer, pancreatic cancer and cholangiocarcinoma.