

# Abstract# TPS2679: First-in-human phase 1/2 study of autologous T cells engineered using the Sleeping Beauty System transposon/transposase to express T-cell receptors (TCRs) reactive against cancer-specific mutations in patients with advanced solid tumors

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## Background

There have been significant advances in the genetic engineering of T lymphocytes to recognize neoantigens on tumors in vivo, resulting in remarkable cases of tumor regression and remission. Cancer cells frequently harbor KRAS, TP53, and EGFR somatic mutations that can be processed and presented by tumor major histocompatibility complex (MHC) molecules as neoantigens to T cells through their T-cell receptor (TCR). These neoantigens are not present in the normal tissues; thus, they are attractive targets for adoptive T cell therapy. Given the number and complexity of different neoantigen/HLA combinations on solid tumors, a TCR library approach is warranted. We have successfully developed a library of TCR-T cell therapies targeting individual KRAS, TP53 and EGFR mutations. We designed a Phase I/II clinical trial to evaluate the safety and efficacy of TCR-T cell adoptive T cell therapy for advanced solid tumors.

## Development of Neoantigen-Specific TCR-T Cells - *Sleeping Beauty* System

The *Sleeping Beauty* transposase/transposon system can be used as a non-viral gene transfer system in human cells. *Sleeping Beauty* transposase is briefly expressed to integrate the transposon into the genome and is then degraded and eliminated from the T cell. *Sleeping Beauty* transposon is inserted into TA dinucleotide repeats randomly within the human genome (Figure 1A). Co-transfer of *Sleeping Beauty* transposase and transposon into the T cell results in rapid and stable expression of the introduced neoantigen-specific TCR, which allows tumor cell recognition (Figure 1B). The *Sleeping Beauty* system has high flexibility, and low manufacturing time and cost compared to other gene transfer technologies.

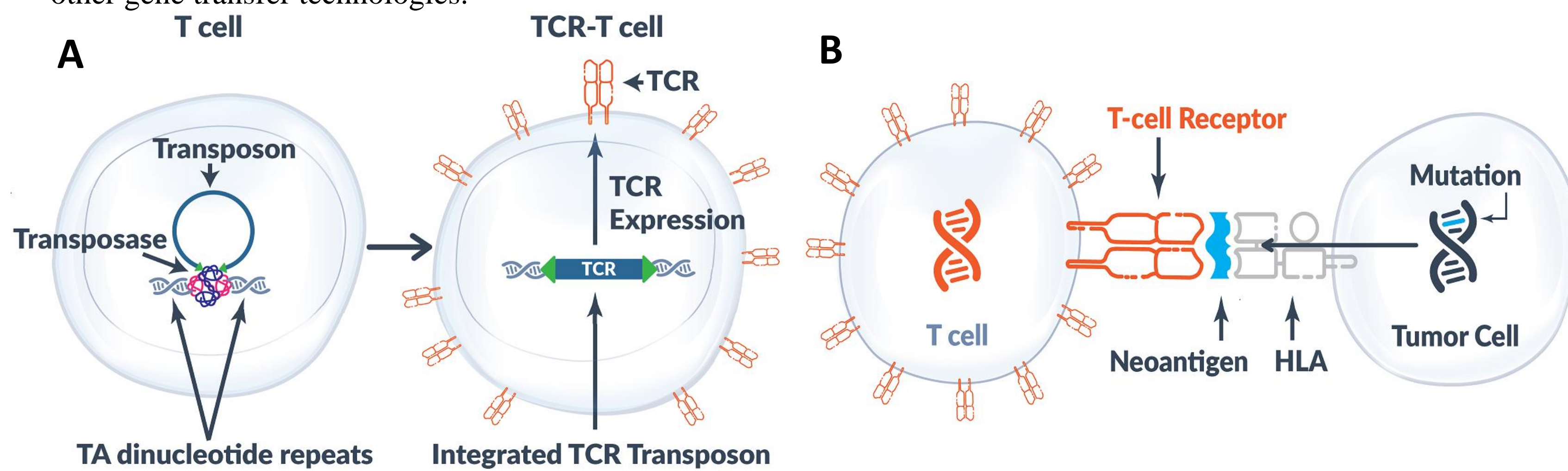
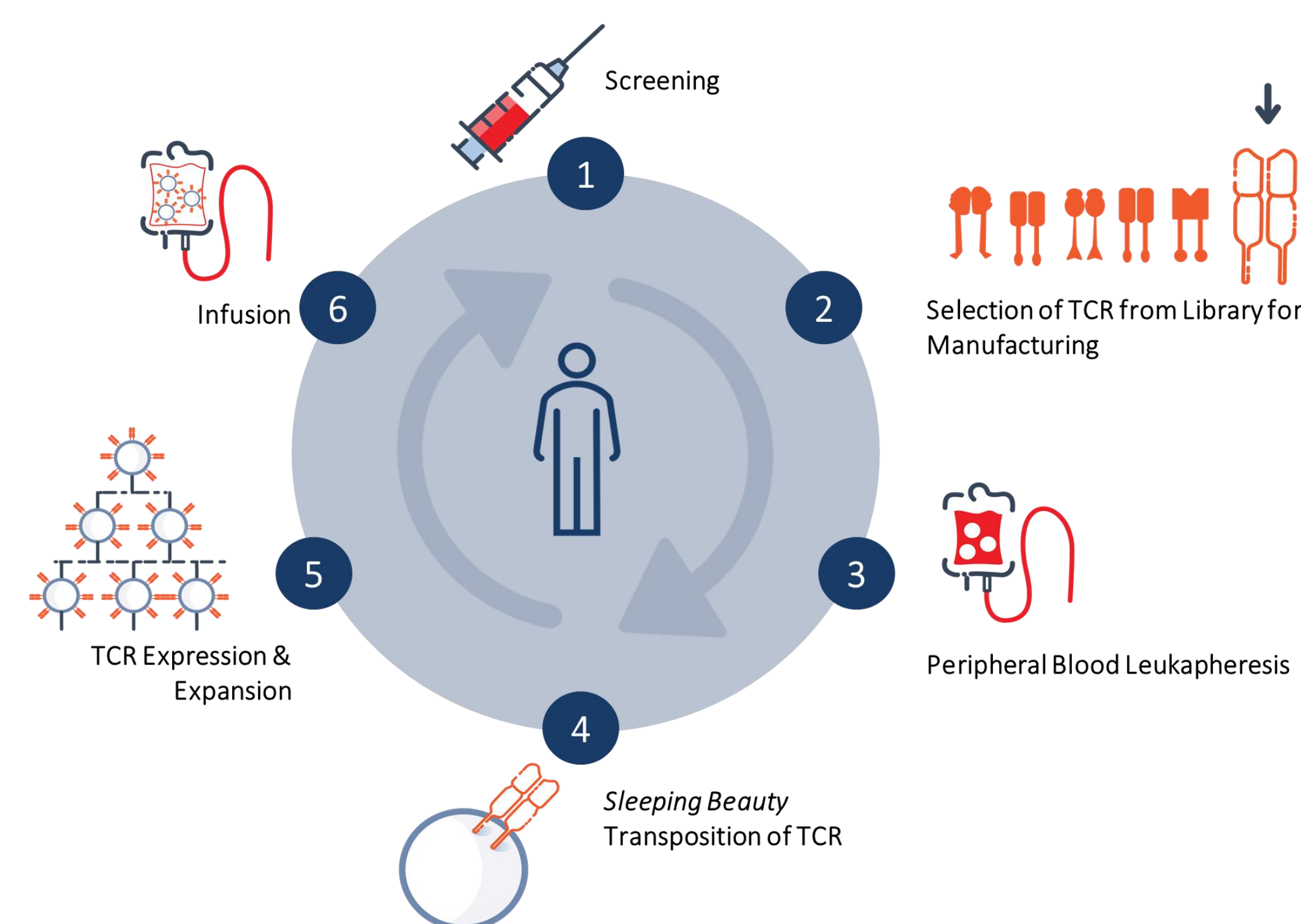


Figure 1. A) Transposon / transposase system for integration into T cell DNA; B) tumor neoantigen recognition by transposed T cell receptor.

## Manufacturing Process for TCR-T cell product – Figure 2



## Study Design

This is a first-in-human Phase I/II trial to determine the safety and efficacy of TCR-T cell adoptive therapy using the *Sleeping Beauty* system for treatment of advanced solid tumors. The trial includes Screening, Pre-Treatment, Treatment and Follow-up Periods. Patients must have a matching somatic mutation(s) and HLA type available in our TCR library to be eligible (Table 1). The following tumor types will be enrolled: non-small cell lung cancer (NSCLC), colorectal (CRC), endometrial, pancreatic, ovarian, and bile duct cancer.

Genes	Mutations	HLA Types
KRAS	G12D	A*11:01 / C*08:02
	G12V	A*11:01 / C*01:02
TP53	R175H	A*02:01 / DRB1*13:01
	R248W	A*68:01
	Y220C	A*02:01 / DRB3*02:02
EGFR	E746_A750del	DPB1*01:01

Table 1. Mutations and HLA matches in library.

During the Pre-Treatment Period, subjects will undergo apheresis for peripheral blood mononuclear cell (PBMC) isolation. The PBMCs will be transposed using the *Sleeping Beauty* system to express the subject's mutation specific TCR. Bridging therapy after apheresis is allowed once the apheresis product has been accepted. During the Treatment Period, patients will undergo lymphodepletion chemotherapy (e.g., cyclophosphamide and fludarabine). The TCR-T cell drug product will be administered by IV infusion. The starting dose level (DL1) is  $5 \times 10^9$  TCR+ cells administered on Day 0. Dose escalation will continue utilizing an accelerated Bayesian Optimal Interval (BOIN) design. Planned escalation dose levels are:  $5 \times 10^9$ ,  $4 \times 10^{10}$  and  $1 \times 10^{11}$  TCR+ Cells. Two Arms are included in the trial: Arm A – TCR-T cell product monotherapy and Arm B – TCR-T cell product and aldesleukin (interleukin-2). Enrollment to Arm B will be decided based on persistence of TCR-T cells at day 28 post-TCR-T cell infusion. In Arm B, subjects will receive aldesleukin infusion starting on Day 0 (within 24 hours of TCR-T cell product infusion) at 720K IU/kg, every eight hours for up to 4 days. The Follow-up Period will begin after the subject completes their Day 28 visit. Clinical and radiologic response will be assessed at 6 and 12 weeks after TCR-T cell drug product infusion and every 12 weeks thereafter until up to 2 years or study discontinuation (e.g., disease progression, initiation of new anti-cancer therapy, consent withdrawn), whichever occurs first. All subjects will continue to be followed in the Long-Term Follow-up Protocol for up to 15 years post-TCR-T cell drug product infusion for safety and efficacy assessment (Figure 3).

## Study Design continued

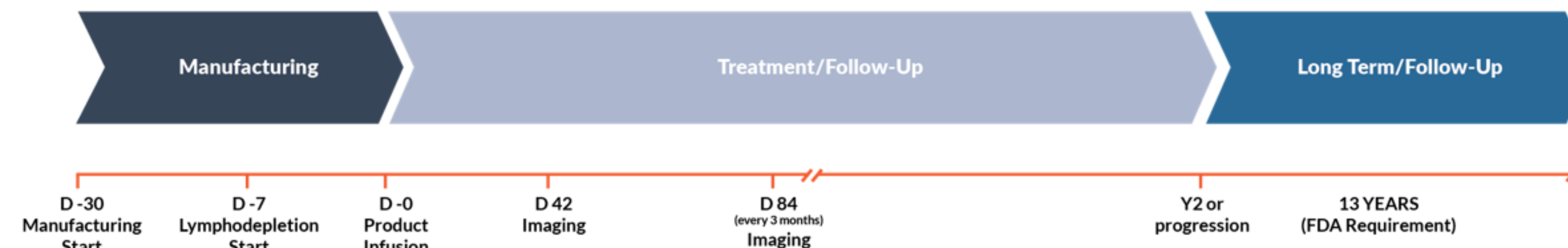


Figure 3. Study Timeline

- ✓ Trial enrolling patients where a TCR matching a neoantigen / HLA pairing is available in our TCR-T library.
- ✓ Phase I is a prospective, open-label, dose-escalation study of TCR-T cells in patients with progressive or recurrent solid tumors who have failed standard therapy utilizing a Bayesian optimal interval design (BOIN) with an accelerated dose escalation.
- ✓ Patients will be enrolled in one of three dose cohorts.

### Phase I Objectives:

- ✓ Define dose limiting toxicity (DLT) and the maximum tolerated dose (MTD) or recommended phase II dose (RP2D).
- ✓ Evaluate the feasibility of TCR-T cell drug product manufacturing.

## Study Population and Key Inclusion / Exclusion Criterion

### Key Inclusion Criteria

1. Patients with tumors that have somatic mutation(s) and HLA type restriction combination matching an available TCR in TCR library.
2. Patients who have previously received at least one line of standard systemic therapy for their advanced/metastatic cancer and have either progressed, recurred, or were intolerant to prior treatment.
  - **Subgroup 1. Gynecologic cancers:** a) **Ovarian cancer:** Subjects who are platinum-resistant, defined as progression on or within 6 months of prior platinum-based regimen; b) **Endometrial cancer:** Subjects who have received at least 2 prior lines of therapy for advanced/recurrent disease (includes adjuvant chemotherapy and/or chemo-radiation; prior hormonal therapy not included).
  - **Subgroup 2. CRC:** At least 2 prior lines of systemic treatment for advanced unresectable or metastatic disease, which must include an irinotecan or oxaliplatin-based therapy and, if eligible, a targeted antibody therapy. Subjects with deficient DNA mismatch repair or microsatellite instability-high CRC must have received treatment with an immune checkpoint inhibitor.
  - **Subgroup 3. Pancreatic cancer:** Subjects who have progressive disease after receiving one prior line of therapy (e.g., FOLFIRINOX, gemcitabine-based therapy).
  - **Subgroup 4. NSCLC:** Subjects with recurrent and/or metastatic disease with disease progression or intolerance to treatment with a PD-1/PD-L1 inhibitor either as a single-agent, or in combination with other immune checkpoint inhibitors (e.g., CTLA-4 inhibitors), and/or platinum-doublet chemotherapy. Subjects with targetable oncogene alterations (e.g., EGFR, ALK, ROS1, RET, MET, NTRK1-3, BRAF) must have had disease progression or intolerance to at least one prior line of targeted therapy.
  - **Subgroup 5. Cholangiocarcinoma:** Subjects must have histologically confirmed cholangiocarcinoma stage II, III, or IV (intra-hepatic, extra-hepatic and perihilar) that is not eligible for curative resection, transplantation, or ablative therapies, and who have progressed after receiving at least one line of standard therapy.
3. Patients must have evaluable or measurable disease per RECIST 1.1 with at least one lesion that can be measured that is not the biopsied lesion.

### Key Exclusion Criteria

1. Known active CNS metastases
2. Concurrent systemic steroid therapy at a dose of  $\geq 10$  mg prednisone daily or equivalent is excluded.
3. Any form of primary immunodeficiency
4. Severe chronic respiratory condition.

## Status of the Study

- The study is active and enrolling in sites in the U.S. since January 18, 2022.
- First patient dosed in April 2022.

## Summary

- Applying the clinical *Sleeping Beauty* transposon/transposase **non-viral** system to generate neoantigen-specific TCR-T cells targeting non-small cell lung, colorectal, endometrial, pancreatic, ovarian, and bile duct cancer
- This proprietary **non-viral gene transfer** platform called *Sleeping Beauty* is utilized to genetically modify the patient's own T cells (both CD4+ and CD8+) using the TCR plasmid.
- The major **advantage** of using **non-viral vectors** is its potential bio-safety (e.g. minimizing insertional mutations/possible secondary malignancies)
- **Non-viral vectors** have also drawn significant attention due to their lower immunotoxicity.
- Phase I portion of the study is expected to complete accrual by the end of 2023
- Phase II portion will include expansion cohorts of non-small cell lung, colorectal, endometrial, pancreatic, ovarian, and bile duct cancer
- **Clinical Trial NCT Number: 05194735**
- **Contact information: Nate Demars, [ndemars@alaunos.com](mailto:ndemars@alaunos.com)**