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Safety and Efficacy of *Sleeping Beauty* TCR-T Cells Targeting Shared KRAS and TP53 Mutations Expressed by Solid Tumors in First-in-Human Phase 1 Study

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Background

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Solid tumors present driver mutations in *KRAS*, *TP53*, and *EGFR* genes on their surface in the context of human leukocyte antigen (HLA) molecules to T-cell receptors (TCRs) expressed by T cells. Non-viral genetic engineering of patient T cells with the *Sleeping Beauty* transposon/transposase system can generate driver mutation-reactive TCR-T cells.

This is a first-in-human phase 1/2 study of autologous *Sleeping Beauty* TCR-T cell therapy for patients with non-small cell lung (NSCLC), colorectal, endometrial, pancreatic, ovarian and bile duct cancers whose tumors contain at least one of the targeted driver mutations in *KRAS*, *TP53*, or *EGFR*. In addition to the target driver mutation, patients must also match on the cognate HLA allele (Table 1). The TCR001-201 phase 1/2 clinical trial is currently open and enrolling on the phase 1 dose escalation portion of the trial (NCT05194735).

Trial Design

Patients

Based on HLA typing and tumor mutation profiles, patients that matched to one of twelve TCRs (Table 1) with one of six relapsed/refractory solid tumor types (NSCLC, colorectal, endometrial, pancreatic, ovarian and bile duct cancer) underwent leukapheresis. Mononuclear cells were enriched from the leukapheresis and TCR-T cells were manufactured using the *Sleeping Beauty* transposon/transposase system in a cGMP facility and formulated fresh for infusion. All patients presented here were treated on Arm A of the study (Figure 1).

TCR-T Cell Treatment

- Lymphodepleting chemo, cyclophosphamide 60 mg/kg day -7 to -6, fludarabine 25 mg/kg day -7 to day -3
- TCR-T cell infusion at assigned dose level (Figure 1) on day 0

Assessments

Tumor responses were evaluated by RECIST1.1 with the first scan collected at week 6, then at month 3 and three-month intervals following (Figure 2). Severity of adverse events (AEs) were assessed according to CTCAE v5.0. TCR-T cellular kinetics and immune monitoring was performed prior to lymphodepletion (baseline), prior to TCR-T cell infusion, and during follow up. Patients are followed on TCR001-201 for up to two years before rolling over to long-term follow up for up to an additional thirteen years (Long-term follow up protocol TCR001-202, NCT05292859).

Figure 1. TCR001-201 Phase 1 dose escalation Bayesian trial design

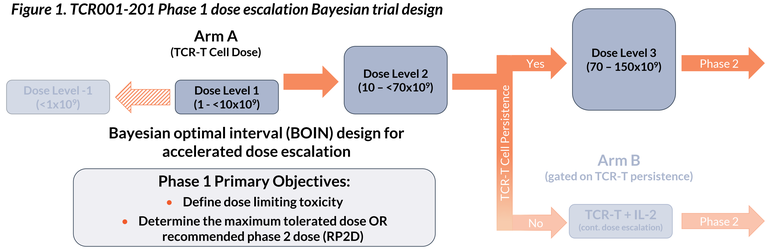
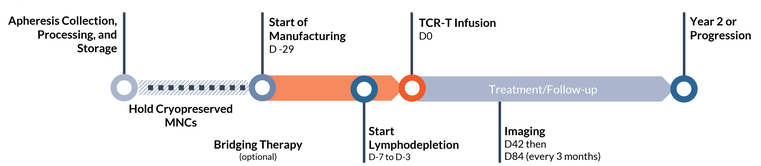


Figure 2. Patient treatment timeline



Treatment Timeline (Figure 2)

- Patients presented here were infused with a fresh TCR-T cell product (as shown above); however, more recent TCR-T manufacturing improvements have shortened manufacturing to 26 days and enabled cryopreservation of the drug product for new patients treated on this trial.

Table 1. TCR Library target tumor mutations and HLA alleles

Gene	Mutation	HLA
EGFR	E746-A750del	DPA1*02:01 + DPB1*01:01
KRAS	G12D	A*11:01
		C*08:02
	G12V	A*11:01
		C*01:02
		DRB1*07:01
TP53	R175H	A*02:01
		DRB1*13:01
	Y220C	A*02:01
		DRB3*02:02
	R248W	A*68:01
	R273C	DPA1*01:03 + DPB1*04:02

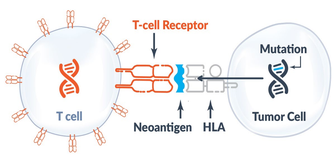
TCR-T library designed to target an expanded patient population with tumors harboring driver mutations.

Table 2. Library TCR target matches in patient population

	No. Patients (%) N=609
Tumor Mutation/HLA Screened[1]	609 (100)
TCR Matched[1]	89 (14.6)

[1]Includes patients from research protocols 2020-0079 (colorectal, pancreatic, and bile duct) and 2020-0762 (NSCLC). Matches based on mutation and HLA.

Figure 3. Neoantigen TCR-T cells recognize tumor-specific driver mutations



Results

Patient Screening and Treatment

Table 2. Number and percentage of patients from screening to treatment

	No. Patients (%)
Screened[1] (N = 14)	14 (100)
Apheresis[2]	9 (64.3)
Intention-to-treat	3 (21.4)
Total Treated with TCR-T cell Product	3 (21.4)

[1]Patient population from TCR001-002 screening protocol as of March 8, 2023. [2]Includes apheresis collections for patients with planned or ongoing manufacturing.

Patient Characteristics and Outcomes

Table 3. Patient demographics, characteristic, and outcomes

Patient ID	Pt-01	Pt-02	Pt-03
Age/Sex	35/F	54/F	59/M
Tumor Type	NSCLC	CRC	PDAC
Primary Sites (Metastatic Sites)	Lung (none)	Sigmoid Colon (diffuse liver)	Pancreas (liver)
No. Prior Regimen[1]	3 (including anti-PD-1, PD-L1, and CTLA-4)	3[2]	2
Bridging Therapy	No	Yes (5FU + Irinotecan)	Yes (Gem/Cis)
Target Mutation/HLA	KRAS p.G12D / HLA-A*11:01	TP53 p.R175H / HLA-A*02:01	KRAS p.G12V / HLA-A*11:01
TCR-T Dose Infused (Dose Level)	9 x 10⁹ (DL1)	64.0 x 10⁹ (DL2)	58.4 x 10⁹ (DL2)
Best Response (wk)	PR (12)	SD (6)	PD (6)

[1]Prior lines of therapy. [2]Second line includes microwave therapy of hepatic lesions. Cis = Cisplatin, CRC = colorectal cancer, Gem = Gemcitabine, NSCLC = non-small cell lung cancer, PDAC = pancreatic ductal adenocarcinoma, 5FU = 5-fluorouracil

Treatment-Related Adverse Events Summary

Table 4. Summary of treatment-related adverse events by patient (by MedDRA preferred term)

Adverse Event (AE) ≥Gr2	Pt-01	Pt-02	Pt-03
Leukopenia	Gr4	Gr4	Gr4
Neutropenia	Gr4	Gr4	Gr4
Lymphopenia	Gr4	Gr4	Gr4
Thrombocytopenia	Gr4	Gr4	Gr4
Aneamia[1]	Gr3	Gr3	Gr3
Cytokine Release Syndrome	Gr2	Gr3	-[2]
Chills/Pyrexia	-	-	Gr2
Hypoxia	Gr2	-	Gr2
ICANS	-	-	-
Neurotoxicity	-	-	-
ALT/AST Increased	-	Gr3	-
Hypoalbuminemia	-	Gr2	-
Hypertension	-	-	Gr3
Sinus Tachycardia	Gr3	-	-
Nausea/Vomiting	Gr2	-	Gr2
Diarrhoea	Gr2	-	-

Treatment-relates AEs with ≥Gr2 severity. [1]includes iron-deficient anemia. [2]Pt-03 experienced Gr1 CRS. Additional adverse events observed, Alopecia (Gr2, N=1), Cancer Fatigue (Gr2, N=1), Influenza like illness (Gr2, N=1), Headache (Gr2, N=1), Hypertriglyceridemia (Gr2, N=1), Hyponatremia (Gr2, N=1). Gr = grade, - = no AE reported

Safety Profile (Table 4)

- No dose limiting toxicities were observed including ICANS or neurotoxicity.
- CRS (Gr 1-3) was observed within one day of TCR-T infusion in all patients and was self-limiting (Pt-01, Pt-03) or resolved with administration of tocilizumab (Pt-02).

Tumor Target Characteristics

Table 5. Target mutation and HLA profile of baseline tumor biopsies

Patient ID	Pt-01	Pt-02[1]	Pt-03
Total No. Somatic Tumor Mutations (Mean VAF)	3 (0.18)	79 (0.22)	268 (0.16)
Target Mutation (VAF)	KRAS p.G12D (0.21)	TP53 p.R175H (0.25)	KRAS p.G12V (0.23)
Target HLA Allele (Germline Zygosity)	A*11:01 (het.)	A*02:01 (het.)	A*11:01 (het.)
HLA Allelic Balance	46%[2]	34%	36%

[1]Archival tumor tissue from prior surgery 3.8 years prior to TCR-T cell infusion. [2]Tumor tissue at progression (6 months post-infusion). het. = heterozygous

Tumor Biomarkers (Table 5)

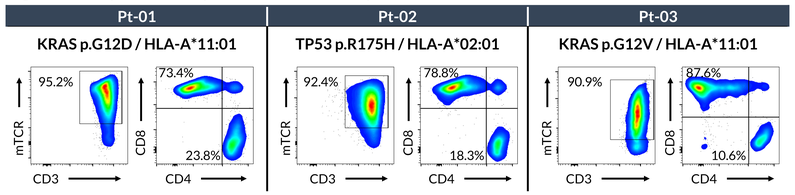
- Tumor biopsies collected approximately one week prior to TCR-T cell treatment (Pt-01 and Pt-03) or from surgical resection (Pt-02, 3.8 years prior) demonstrated the presence of the target mutation and HLA alleles.

TCR-T Infusion Product Characteristics

Table 6. Drug product characteristics and selected release criteria

Patient ID	Pt-01	Pt-02	Pt-03
Total TCR-T Cells	9 x 10⁹	64.0 x 10⁹	58.4 x 10⁹
CD3+ Purity	99.7%	99.7%	99.1%
TCR+ (Transgenic mTCR)	95.2%	92.4%	90.9%
Viability	95.1%	92.5%	92.8%
CD4:CD8 (of TCR+)	0.32	0.23	0.12
Vector Copy Number (VCN)	5	4.2	4.6

Figure 4. Flow cytometric analysis of TCR-T cell infusion products

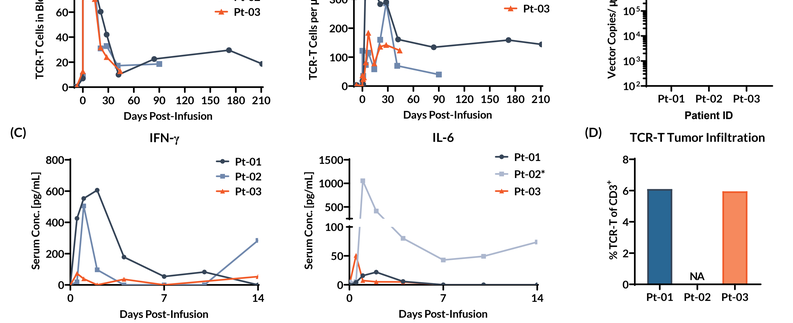


TCR-T Manufacturing (Table 5)

- High doses of TCR-T cells (>10^{10}) were manufactured and administered.
- $CD3^+$ T cell and transgenic TCR purity was very high (≥ 90%).
- Drug product composition contained a mixture of CD4 and CD8 T cells with a greater proportion of CD8 T cells.

TCR-T Cell Kinetics, Tumor Infiltration & Cytokines

Figure 5. Peripheral TCR-T cell kinetics, TCR-T tumor infiltration, and serum cytokine markers



TCR-T Kinetics, Activation, and Tumor Infiltration (Figure 5)

- Continued TCR-T cell persistence up to 7 months post-infusion. (5A)
- TCR-T cells could be detected in expanded TIL from post-treatment biopsies up to 6 months post-infusion. (5C)
- Evidence of immune activation after TCR-T infusion. (5D)

(A) TCR-T cells were measured in blood samples by flow cytometry and quantified as a fraction of the total T cells (left) or absolute counts (right). (B) Peak TCR-transposon vector copy number observed post-infusion measured by ddPCR. (C) Serum cytokines IFN-γ and IL-6 from Day 0 pre-infusion to Day 14 post-infusion quantified by MSD V-plex assay. (D) Percent of mTCR+ cells within the $CD3^+$ T cell gate of tumor infiltrating lymphocytes (TIL) expanded from post-treatment biopsies for Pt-01 (6 months post-infusion) and Pt-03 (7 weeks post-infusion). No biopsy was available from Pt-02. TIL were expanded from tumors using irradiated PBMC feeders, 3000 IU/mL IL-2, and 30 ng/mL OKT3. *Pt-02 received tocilizumab. NA = Not available

Conclusions

- **TCR-T cells demonstrate feasibility of redirected T cell targeting of driver mutations in solid tumors**
  - Patients with NSCLC, CRC, and PDAC were treated with TCR-T cells designed to target driver mutations in *TP53* and *KRAS* including one NSCLC patient with a confirmed partial response at twelve weeks.
- ***Sleeping Beauty* transposon/transposase system is an effective platform for the manufacture of a TCR-T cell library**
  - Infusion products of the first three patients treated on this study were all ≥90% transgenic $mTCR^+$.
  - Doses exceeding 10^{10} TCR-T cells were successfully manufactured and administered.
- **Following infusion TCR-T cells exhibit persistence and tumor infiltration**
  - In peripheral blood, TCR-T cells could be detected in all patients at the last evaluable time point and were detected out to 7 months post-infusion.
  - In two patients with on-treatment biopsies collected at week 7 (Pt-03) and month 6 (Pt-01), TCR-T cells could be detected and isolated from *ex vivo* expanded tumor infiltrating lymphocytes (TIL).
- **TCR001-201 (NCT05194735) is actively enrolling and treating patients in the dose escalation**
  - Future patients will receive cryopreserved TCR-T cells which enables more favorable patient care logistics.