Objective clinical response by \textit{KRAS} mutation-specific TCR-T cell therapy in previously treated advanced non-small cell lung cancer

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Adoptive T-Cell Therapy has Activity in Solid Tumors

- Melanoma: expanded TILs ORR 34-56%\(^1\)
- HPV+ SCC: HPV-TIL ORR 18-28%\(^2\)
- MBC: enriched TILs ORR 67%\(^3\)
- NSCLC: expanded TILs response rate 46%\(^4\)
- CRC: TIL produced durable response\(^5\)
- PDAC: durable response and persistence of TCR-T cells\(^6\)

**KRAS Mutations are Logical Targets for T-Cell Therapy due to High Prevalence in Epithelial Solid Tumors**

1. Judd et al. Mol Cancer Ther. 2021
2. Catalogue of Somatic Mutations in Cancer (COSMIC) database https://cancer.sanger.ac.uk/cosmic
Phase I/II Trial to Determine the Safety and Efficacy of Non-viral TCR-T Cell Therapy for Treatment of Solid Tumors

- ClinicalTrials.gov: NCT05194735
- Solid tumors failed 1+ lines of therapy
- HLA + cancer gene mutation match for TCR library
- Accelerated dose escalation: BOIN design
- 3 dose levels: 1 - <10x10^9 / 10 - <70x10^9 / 70 - 150x10^9
- Objectives: safety / RP2D / manufacturing feasibility

Negrao et al. ASCO 2022 / Morelli et al. ESMO 2022
TCR Library Designed to Target Tumor Neoantigens Derived from Hotspot Mutations

- Common cancer gene mutations: \textit{KRAS, TP53, EGFR}
- Common HLAs: A*02:01 / A*11:01
- Library expansion: identification of novel anti-tumor reactive TCRs
- More TCRs = more eligible patients

<table>
<thead>
<tr>
<th>Genes</th>
<th>Mutations</th>
<th>HLA Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>G12D</td>
<td>A<em>11:01 / C</em>08:02</td>
</tr>
<tr>
<td></td>
<td>G12V</td>
<td>A<em>11:01 / C</em>01:02</td>
</tr>
<tr>
<td>TP53</td>
<td>R175H</td>
<td>A<em>02:01 / DRB1</em>13:01</td>
</tr>
<tr>
<td></td>
<td>R248W</td>
<td>A*68:01</td>
</tr>
<tr>
<td></td>
<td>Y220C</td>
<td>A<em>02:01 / DRB3</em>02:02</td>
</tr>
<tr>
<td>EGFR</td>
<td>E746-A750del</td>
<td>DPB1*01:01</td>
</tr>
</tbody>
</table>
Non-viral *Sleeping Beauty* System Designed to Enable Manufacture of TCR-T Cells without Complex Gene Editing

- Efficient integration without the complexity of gene editing or viral approaches
- Rapid, cost-effective manufacturing
- Accommodates large transgene size
- Expected to be scalable for clinical production
Patient 1: Immune Checkpoint Inhibitor and Chemotherapy Refractory KRAS G12D NSCLC

- 34yo, female, never smoker, lung adenocarcinoma
- Left lower lobectomy and adjuvant cisplatin and vinorelbine x 4 cycles
- Disease recurrence in the lungs 4 months after adjuvant treatment
- KRAS G12D mutation positive / tumor PD-L1 expression = 10%
- Carboplatin, pemetrexed, pembrolizumab x 4 cycles with response followed by pemetrexed and pembrolizumab maintenance x 22 cycles with disease progression
Patient 1: Immune Checkpoint Inhibitor and Chemotherapy Refractory KRAS G12D NSCLC

- Durvalumab + CTLA4 inhibitor + MEK inhibitor on trial – discontinued due to progression
- SHP2 inhibitor single-agent on trial – discontinued due to progression
- Library TCR match:

| KRAS G12D | HLA-A*11:01 |
TCR-T Cells Observed to Specifically Recognize and Kill Targets Expressing KRAS G12D Presented by HLA-A*11:01

Neoantigen-Specific Activation

Effectors Cytokine Secretion

Tumor Killing

Preclinical Data
High TCR Expression and Purity of TCR-T Cells Manufactured with *Sleeping Beauty* Transposition

TCR-T Infusion Product (Patient 1: KRAS G12D/HLA-A*11:01)

<table>
<thead>
<tr>
<th></th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability</td>
<td>95.1%</td>
</tr>
<tr>
<td>Total TCR-T Cells</td>
<td>9x10⁹</td>
</tr>
<tr>
<td>CD3+ Purity</td>
<td>99.7%</td>
</tr>
<tr>
<td>TCR+</td>
<td>95.2%</td>
</tr>
<tr>
<td>CD4:CD8 Ratio</td>
<td>0.32</td>
</tr>
<tr>
<td>VCN</td>
<td>5</td>
</tr>
</tbody>
</table>
Patient 1 Had Manageable Safety Events During Lymphodepletion Chemotherapy

<table>
<thead>
<tr>
<th>Lymphodepletion Drug (LD)</th>
<th>Dose</th>
<th>Days of Administration Prior to TCR-T Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>60 mg/kg</td>
<td>-8, -7</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>25 mg/m²</td>
<td>-8, -7, -5, -4, -3</td>
</tr>
</tbody>
</table>

Note: Day -6 Fludarabine dose withheld

Hospital Admission

Chest Pain - Small R pneumothorax
Related to pre-treatment biopsy

Day

-9 -8 -7 -6 -5 -4 -3

Hypoxia Gr2 / Hypotension Gr3 / Tachycardia Gr3
O2 nasal cannula 3L / hydration w/ albumin
Related to LD chemotherapy

LD = Lymphodepletion
Patient 1 Had Manageable Safety Events After Lymphodepletion Chemotherapy and TCR-T cell infusion

<table>
<thead>
<tr>
<th>TCR-T Cell Target</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS G12D/HLA-A*11:01</td>
<td>9x10^9 TCR+ T Cells (Dose Level 1)</td>
</tr>
</tbody>
</table>

- **TCR-T Cell Target Dose**
  - KRAS G12D/HLA-A*11:01 9x10^9 TCR+ T Cells (Dose Level 1)

**Hospital Discharge Day 11**

**CRS Gr2** – 6hrs post-TCR-T infusion
- Hypoxia / Tachycardia / Fever
- O2 nasal cannula 2L / anti-pyretics / IV fluids / self-limited

**Anemia** – Hb 7.0 – Gr3
- PRBC 1 unit - D5

**Thrombocytopenia** – 22k – Gr4
- Self-limited / no bleeding
Patient 1 Had Transient Elevation in Inflammatory Cytokines Associated with Onset and Resolution of CRS

CRS = Cytokine Release Syndrome
Patient 1: Complete Resolution of Right Lower Lobe Lesion

Baseline 1.3 cm

Week 6 0.0 cm

Week 12 0.0 cm
Patient 1: Reduction of Right Upper Lobe Lesion

- Baseline: 1.3 cm
- Week 6: 1.1 cm
- Week 12: 1.0 cm
Patient 1: Reduction of Right Hilar Lymphadenopathy and of Non-Measurable Right Upper Lobe Lesion

Baseline: 1.5 cm
Week 6: 1.1 cm
Week 12: 1.0 cm
Patient 1 Had a Confirmed Objective Partial Response at Week 12

<table>
<thead>
<tr>
<th>Target Lesions (mm)</th>
<th>Baseline</th>
<th>Week 6</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1: Right lower lobe</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>#2: Right upper lobe</td>
<td>13</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>#3: Right hilar lymph node</td>
<td>15</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td><strong>Sum of Diameters (mm)</strong></td>
<td>41</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td><strong>Percent Change</strong></td>
<td></td>
<td>-46.30%</td>
<td>-51.20%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-Target Lesions</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral lung nodules</td>
<td>Non-CR/Non-PD</td>
<td>Non-CR/Non-PD</td>
<td></td>
</tr>
</tbody>
</table>

**Overall Response**

- Baseline: 
- Week 6: Partial Response
- Week 12: Partial Response
Patient 1 TCR-T Cells Exhibited Rapid Expansion and Ongoing Persistence at Week 12

Circulating TCR-T Cells

<table>
<thead>
<tr>
<th>Time</th>
<th>TCR-T Cells/μL</th>
<th>TCR-T Copies/μg</th>
<th>% TCR-T+ of CD3+</th>
</tr>
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<tr>
<td><strong>Day 4</strong></td>
<td>1,038</td>
<td>5x10^5</td>
<td>98.4%</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
<td>134</td>
<td>2.5x10^4</td>
<td>22.5%</td>
</tr>
</tbody>
</table>

**Gated on CD3+**

- **Baseline**: 0.6%
- **Day 4**: 98.4%
- **Week 4**: 42.0%
- **Week 6**: 10.0%
- **Week 12**: 22.5%
Patient 1 Serum Interferon-γ was Associated with TCR-T Cell Expansion and Persistence

CRS = Cytokine Release Syndrome
Patient 2: Previously Treated Advanced CRC

- 54yo, female, metastatic colorectal cancer
- Progressed on one prior line of therapy (FOLFIRI+Bevacizumab)
- Library TCR match: TP53 R175H, HLA-A*02:01
- TCR-T infusion of $64 \times 10^9$ TCR-T cells - Dose Level 2
Patient 2 Had Manageable Safety Events After TCR-T Cell Infusion

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<td>TP53 R175H/HLA-A*02:01</td>
<td>$64 \times 10^9$ TCR+ T Cells (Dose Level 2)</td>
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CRS Gr3
Fever / Hypotension / Tachycardia / Hypoxia
O2 HFNC 40L 50% and Tocilizumab

CRS Gr2
Hypoxia
O2 Nasal Cannula 4L

HFNC = High flow nasal cannula

CRI-ENCI-AACR SIXTH INTERNATIONAL CANCER IMMUNOTHERAPY CONFERENCE: TRANSLATING SCIENCE INTO SURVIVAL
Patient 2 Achieved Best Overall Response of Stable Disease

<table>
<thead>
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<th>Baseline</th>
<th>Week 6</th>
<th>Week 12</th>
</tr>
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<tbody>
<tr>
<td>#1: Pelvic Mass (mm)</td>
<td>65</td>
<td>48</td>
<td>67</td>
</tr>
<tr>
<td>#2: Retroperitoneal Lymph Node (mm)</td>
<td>27</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>Sum of Diameters (mm)</td>
<td>92</td>
<td>78</td>
<td>95</td>
</tr>
<tr>
<td>Percent Change</td>
<td></td>
<td>-15.20%</td>
<td>21.80%</td>
</tr>
<tr>
<td>New Lesion</td>
<td>No</td>
<td></td>
<td>Liver / Lung</td>
</tr>
<tr>
<td>Overall Response</td>
<td>Stable Disease</td>
<td></td>
<td>Progressive Disease</td>
</tr>
</tbody>
</table>

Note: Patient off study due to disease progression
Treatment of Patients 1 and 2 was Tolerable with a Manageable Safety Profile

- Cytopenias expected with lymphodepletion regimen were observed in both patients
- Manageable CRS observed
  - No mechanical ventilation
  - No ICU admission
  - No vasopressors
- No TCR-T cell related DLTs
- No ICANS

DLT = Dose limiting toxicity; ICANS = Immune effector cell-associated neurotoxicity syndrome
Confirmed Objective Response in Immune Checkpoint Inhibitor Refractory KRAS G12D-mutant NSCLC Treated with TCR-T Cells

- Immune checkpoint inhibitor refractory advanced NSCLC patient treated with TCR-T cells has confirmed partial response
- First confirmed response to TCR-T cell therapy targeting hotspot cancer gene mutation in advanced NSCLC to our knowledge
- KRAS G12D / HLA-A*11:01: viable target for TCR-T cell therapy
Sleeping Beauty System is a Promising Platform for TCR-T Cell Therapy and Trial Enrollment is Ongoing

- First report of successful TCR-T cell therapy using non-viral Sleeping Beauty system for solid tumors
- Proof of concept of manufacturing TCR-T targeting KRAS and TP53
- Ongoing persistence of TCR-T cells at Week 12 at Dose Level 1 in Patient 1
- Phase I dose escalation: enrollment ongoing for patients with advanced solid tumors harboring KRAS, TP53 and EGFR mutations
## Acknowledgements

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- Abhishek Srivastava  
- Ron Weitzman  
- Drew Deniger

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**ClinicalTrials.gov**: NCT05194735

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