Preclinical development of safe and effective T cell receptors specific for mutant KRAS G12D peptide

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Disclosure Information

Loïc Vincent, PhD

I have the following relevant financial relationships to disclose:

- Employee of Affini-T Therapeutics
- Consultant for bit.bio
- Stockholder in Affini-T Therapeutics, Alaya.bio, Takeda
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Targeting oncogenic driver mutations like KRAS strikes at the core of tumor biology

Cancer cells are dependent on oncogenic drivers

KRAS mutations are present in 30% of all solid tumors

Targeting KRAS has been clinically de-risked by approved G12C therapies

Affini-T TCRs have high specificity for KRAS and other oncogenic drivers

Oncogenic driver mutations initiate and maintain cancer growth, and are present in each tumor cell

KRAS represents the most frequently mutated oncogene in difficult-to-treat solid tumors

Recent drug approvals demonstrate single agent activity but need improved duration of response

Affini-T leverages TCRs to attack only cancer cells, utilizing synthetic biology to enhance persistence

Minimizes tumor heterogeneity and escape mechanisms

Provides impact for a high unmet medical need

Robust R&D interest for drugs targeting KRAS

Therapeutic modality with clinical PoC
Identification and characterization of HLA-A*11:01 KRAS G12D TCRs

Christopher A. Klebanoff, MD, Medical Oncologist and Laboratory Head, Memorial Sloan Kettering Cancer Center (MSK), New York, NY

- Immunogenicity and therapeutic targeting of recurrently mutated ‘public’ neoantigens
- SY18 - Dharma Master Jiantai Symposium in Targeted Therapy: Cellular Therapies for Cancer
- April 18, 2023, 12:30-2:00pm (Tangerine Ballroom 2 - WF2)

i) Immuno-peptidomic screen

ii) Public NeoAg biotrust

iii) TCR retrieval

iv) TCR validation

- Recurrent driver mutations
- Prevalent HLA-I alleles

MSK-IMPACT + DARWIN

10x single-cell V(D)J sequencing

DNA barcoded dextramer
AFNT-212: A11 KRAS G12D TCR engineered CD4+ and CD8+ T cells with synthetic receptor for durability

**KRAS G12D TCR**

- KRAS TCR
- Cell Engineering with TRAC/TRBC KO
- Synthetic Biology (e.g., FAS-41BB or ILR)
- CD8αβ Co-Receptor

**Activated CD8+ T Cell**

- CD8αβ Co-Receptor
- FAS-41BB
- FAS-L
- Peptide / MHC Complex
- IL-2
- IFN-γ / TNF-α
- Perforin + Granzyme

**Activated CD4+ T Cell**

- *FAS-41BB represented for illustrative purpose*

**Tumor Cell Death**

- IFN-γ / TNF-α

**KO = Knockout**
Knockout of endogenous TRAC and TRBC could enhance functional avidity of CD4+ and CD8+ TCR-T cells

- Knockout of endogenous TRAC/TRBC (Metagenomi Type V CRISPR/Cas) could
  - eliminate mispairing,
  - improve functional expression of transgenic TCRs and
  - improve signaling by freeing the available CD3 pool.
CD4+ and CD8+ TCR-engineered T cells with knockout of endogenous TRAC and TRBC genes show enhanced activation in presence of mutant KRAS G12D peptide

- Primary CD4+ and CD8+ T cells were transduced with indicated lentiviruses, electroporated with TRAC and TRBC targeting RNPs, expanded for 10 days and treated with mutant KRAS G12D peptide; Activation of TCR-T cells was assessed via CD137 expression
- Primary CD4+ and CD8+ TCR-T cells with knockout of endogenous TRAC/TRBC genes showed enhanced activation upon stimulation with index peptide

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<thead>
<tr>
<th>Construct</th>
<th>WT</th>
<th>dKO</th>
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<tbody>
<tr>
<td>MSK2</td>
<td>31.6</td>
<td>10.1</td>
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<tr>
<td>MSK4</td>
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<td>11.8</td>
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<td>MSK5</td>
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<tr>
<td>TCR091</td>
<td>131.3</td>
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dKO improved EC50 by 3-5 folds over WT
KRAS G12D TCR-T cells with TRAC/TRBC knockout exhibit robust *in vitro* cancer killing in repeat tumor challenge assay

**Tumor Cell killing**  
**HuCCT1 cells (3:1 E:T)**

- WT
- dKO

**Tumor Cell Lysis Efficiency**

- MSK2
- MSK4
- MSK5
- TCR-91
- Mock

- MSK2 dKO
- MSK4 dKO
- MSK5 dKO
- TCR-91 dKO

- Engineered CD4+ and CD8+ TCR-T cells were assessed for function in a tumor cell killing assay across three G12D presenting cell lines

- CD4+ and CD8+ TCR-T cells with knockout of endogenous TRAC/TRBC genes showed robust killing with rechallenge modelling chronic exposure to tumor

* Cell death due to confluency

Rechallenge with addition of more tumor cells
KRAS G12D TCRs to mutant neopeitope are highly selective with low potential for cross-reactivity

<table>
<thead>
<tr>
<th>TCR</th>
<th># Potential Off-Target Peptides</th>
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<tbody>
<tr>
<td>MSK2</td>
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<tr>
<td>MSK4</td>
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- CD4+/CD8+ dKO KRAS G12D TCR-T cells were stimulated with KRAS G12D or mutant peptides in which the cognate amino acid was sequentially changed to all possible 19 amino acids; T cell activity was assessed via secreted IFNγ levels
- No/few potential off-targets were identified for G12D TCRs
KRAS G12D TCR-T cells with TRAC/TRBC knockout demonstrate robust preclinical anti-tumor activity *in vivo*

- NSG mice randomized after SC tumor implantation (5 mice/group)
- Dose: single IV administration of $10 \times 10^6$ KRAS G12D TCR CD4+ and CD8+ T cells (1:1 ratio) on day 9
- CD4+/CD8+ dKO KRAS G12D TCR-T cell therapy induced 100% Complete Responses and 100% Overall Survival
Engineering strategies to overcome the various barriers in solid tumors, restricting efficacy of cell therapies

**Interleukin Receptor (ILR)**

- Signal 3 promotes proliferation
- Increases proliferation, survival in tumor and chemokine receptor expression
- No cytokine independent survival

**FAS-41BB**

- Signal 2 upon FASL binding
- Enhances proliferation upon FASL engagement
- Increases survival in the tumor, provides costimulatory signal, promotes metabolism to support T cell activation and memory development
CD4+ and CD8+ KRAS G12D TCR-T cells equipped with chimeric ILR show enhanced proliferation and tumor cell killing *in vitro*

- CD4+ and CD8+ T cells were engineered with KRAS G12D TCR, CD8αβ, TRAC/TRBC KO and ILR
- Engineered T cells were assessed for activation of pSTAT, proliferation in response to tumor cells and for killing of tumor cells upon persistent exposure from multiple rechallenges
- Inclusion of ILR enhanced potency of engineered KRAS G12D TCR CD4+ and CD8+ T cells

pSTAT = Phosphorylated STAT
cGMP compatible scale-up process for non-viral KI enables efficient genetic engineering of TCR-T cells

Non-Viral Targeted Transgene Integration

- **Key advantages over LVV mediated delivery**
  - Enables larger cargo size
  - Consistent targeting into a desired locus with defined copy number and expression of transgenes
  - Reduced manufacturing complexity and cost
  - Can enable innovative master clinical trial designs

KI = Knockin

KI Efficiency and Functionality in T Cells

- KI yielded up to 44% integration efficiency in primary CD4+ and CD8+ T cells
- KI cells outperformed LVV transduced cells in the rechallenge assay modelling chronic exposure to tumor

Tumor Cell Killing
Panc1 10:1 E:T

Primary T cells

0
10
20
30
40
50
KI efficiency (%)

0
5×10^6
1×10^7
Tumor cell confluence

0
50
100
150
Time (hr)

Rechallenge

Panc01
Mock dKO
LV_TCR91+CD8αβ dKO
LV_TCR91+CD8αβ+ILR dKO
KI_TCR91+CD8αβ
KI_TCR91+CD8αβ+ILR
Summary of findings

• CD4+ and CD8+ T cells engineered with specific mutant KRAS G12D TCRs showed robust anti-tumor activity in vitro and in vivo.

• Knockout of endogenous TRAC and TRBC genes enhanced avidity and cytotoxicity of engineered KRAS G12D-specific TCR-T cells.

• Incorporation of synthetic biology engineering such as a chimeric ILR in engineered KRAS G12D TCR-T cells enhanced proliferation of engineered T cells in response to tumor cells and cytotoxicity against the tumor cells.

• Non-viral KI transgene integration enabled efficient genetic engineering of KRAS G12D TCR-T cells.

• AFNT-212 targeting of KRAS G12D+ tumors in HLA-A*11:01+ patients is poised to enter clinical testing in 2024.
Acknowledgements

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Greenberg Lab

Klebanoff Lab

Metagenomi