Beneficial role of antigen commensalism in mesothelin-targeted T-cell therapy for lung adenocarcinoma

Adam J. Bograd¹, Jonathan Villena-Vargas², Christos Colovos², Stefan Kachala², David R. Jones², Michel Sadelain³, Prasad S. Adusumilli²,³

¹Dept. of Surgery, New York University Medical Center, New York, NY
²Thoracic Service, Memorial Sloan-Kettering Cancer Center, New York, NY
³Center for Cell Engineering, Memorial Sloan-Kettering Cancer Center, New York, NY

No Disclosures
CAR-based Adoptive T-cell therapy

Chimeric Antigen Receptor\(^1\)
(CAR)

- All human components

Adoptive T-cell Therapy\(^2,3\)

1. Sadelain M. *Nat Rev Cancer* 2003
Our laboratory’s adoptive T-cell clinical trials

<table>
<thead>
<tr>
<th>IRB</th>
<th>Target Antigen</th>
<th>Patients Enrolled</th>
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</thead>
<tbody>
<tr>
<td>06-138A</td>
<td>CD19</td>
<td>35</td>
</tr>
<tr>
<td>09-114A</td>
<td>CD19</td>
<td>68</td>
</tr>
<tr>
<td>11-038A</td>
<td>CD19</td>
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<td>11-048A</td>
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<td>CD19</td>
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<td>13-052A</td>
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<td>5</td>
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<tr>
<td>09-036A</td>
<td>PSMA</td>
<td>12</td>
</tr>
</tbody>
</table>

¹Brentjens *Blood* 2011
²Brentjens *Sci Transl Med* 2013
Solid Tumors: Potential Challenges to T-cell Therapy

Impaired T-cell Trafficking to Tumor
- Decreased tumor infiltration\(^1\)
- Lack of “homing mechanism”\(^2\)

Impaired Effector Function
- Increased ratio of regulatory T cells (Tregs)\(^3\)
- Presence of tumor associated macrophages (TAMs)

Selection of Cancer Antigen Target
- Want high tumor expression, low normal tissue expression
- Vital to tumor survival or aggressiveness
- Heterogeneous expression of cancer antigens

\(^1\) Lechner J Immunother 2013
\(^3\) Ganesan J Immunol 2013
Mesothelin as a candidate target antigen in lung adenocarcinoma (ADC)

- **Mesothelin**: Cell-surface tumor antigen overexpressed in multiple solid cancers\(^1\)
- Mesothelin expression is associated with aggressive tumor phenotype and treatment resistance
  - Resistance to chemotherapy (*ovarian ca*)\(^2\)
  - Increased cellular proliferation (*pancreatic ca*)\(^3\)
  - Associated with regional invasion (*mesothelioma*)\(^4\)
  - Decreased overall survival (*triple-negative breast cancer*)\(^5\)

2. Cheng, WF. *Br J Cancer* 2009
Mesothelin (MSLN) is a Candidate Target Antigen in Lung ADC

- Expressed in 69% of lung ADC
- MSLN expression correlated with decreased overall survival
- Associated with kras mut
- Heterogeneous MSLN staining

Normal Lung

Mesothelin TMA

Kachala S. Clin Cancer Res 2014
Mesothelin (MSLN) is a Candidate Target Antigen in Lung ADC

- Expressed in 69% of lung ADC
- Associated with kras mut
- Heterogeneous MSLN staining

In vitro
- ↑ Proliferation
- ↑ Migration/Invasion

N = 1209

Kachala S. Clin Cancer Res 2014
Objective

To determine the efficacy of mesothelin-targeted, adoptive T-cell therapy in lung ADC with heterogeneous antigen expression.
Mesothelin-targeted T cells effectively target lung ADC cells in vitro

- Mesothelin-targeted CAR

Mesothelin CAR M28zG (with CD28 co-stimulation)

- Successfully transduced into T cells of lung ADC patients

- In vitro cytotoxicity

In vitro cytotoxicity of A549GM and H1299GM cells with transduced E:T ratios. EKVX infiltration showed higher cytotoxicity compared to control.
Mesothelin-targeted T cells effectively target lung ADC cells *in vitro*

- Mesothelin-targeted CAR
  - Successfully transduced into T cells of lung ADC patients
- In vitro
  - Effector Cytokine Secretion

![Mesothelin CAR M28zG (with CD28 co-stimulation)](image)

![Cytokine Secretion of Mesothelin CAR T cells](image)
Is mesothelin-targeted T-cell proliferation proportional to mesothelin expression in lung ADC targets?

**Targets**

- A549GM (>97%)
- EKVX (10-20%)
- A549G (4-6%)
- MRC5 (0%)
- MRC5+isotype Negative cont.

**Cell Line (MSLN%)**

**Effector**

- CAR+

**T-cell Isolation**

- Day -7

**Plate Targets**

- 1:10 (1e⁵/well)

**1) Irradiate Targets (40Gy)**

**2) Overlay T cells 1e⁶/well**

**3) Re-Overlay T cells on Targets**

**Day 0**

**Day 7 (q7day)**

**1) T-cell counts (per well)**

**2) Phenotype FACS**

**3) Re-Overlay T cells on Targets**
Mesothelin-targeted T-cell proliferation is proportional to mesothelin expression in lung ADC *in vitro*
Mesothelin-targeted T-cell cytotoxicity in a heterogeneous antigen environment

Can mesothelin-targeted T cells effectively kill low mesothelin-expressing cells in the presence of high mesothelin expressing cells?
Mesothelin-targeted T-cell cytotoxicity in a heterogeneous antigen environment

H1299M

EKVX

M28z

$^{51}$Cr release
Mesothelin-targeted T cells can effectively kill in a heterogeneous mesothelin environment \textit{in vitro}

H1299M\hspace{1cm} MS\textsubscript{LN}\%\hspace{1cm} 98\%

EKVX\hspace{1cm} 20\%
Mesothelin-targeted T cells can effectively kill in a heterogeneous mesothelin environment in vitro.

H1299M

- MSLN%
- 98%

EKVX

- 20%
Orthotopic mouse model of lung ADC

- SCID/bg Mice
- Human Lung ADC
- Intrapulmonary inj.
- Monitoring
  - MRI
  - BLI

Mediastinal lymph node metastasis

Distant metastasis
Systemically delivered mesothelin targeted T cells prolong survival in an orthotopic model of lung ADC.
Mesothelin-targeted T cells, in combination with cisplatin, effectively target lung ADC with heterogeneous mesothelin expression.
Mesothelin-targeted adoptive T-cell therapy

• Translating mesothelin-targeted adoptive T-cell therapy
  – Vector construction and validation ✓
    • First and second generation ✓
  – Anti-tumor efficacy ✓
    • In vitro and in vivo
    • Systemic and intrapleural routes
  – Safety ✓
  – Imaging tumor and T-cells ✓
  – Clinical trial protocol ✓
  – Manufacturing and quality control ✓
    • Cell bank and vector ✓
  – Regulatory approvals ✓
    • NIH RAC approval, IND, (IRB revisions, FDA)
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Mesothelin expression in lung ADC is heterogeneous