In vitro Selection and Engineering of a Human Leukocyte Antigen-Independent T-Cell Receptor (HiT) Recognizing Human Mesothelin

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HLA-Independent TCRs (HiTs) bind directly to cell surface target proteins

<table>
<thead>
<tr>
<th>TCR T-cell</th>
<th>CAR T-cell</th>
<th>TRuC T-cell</th>
<th>HiT T-cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g. afamitresgene autoleucel</td>
<td>e.g. tisagenlecleucel</td>
<td>e.g. gavocabtagene autoleucel</td>
<td>Adaptimmune</td>
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<tr>
<td>Adaptimmune</td>
<td>Novartis</td>
<td>TCR² Therapeutics</td>
<td>e.g.</td>
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</tbody>
</table>
Isolation of a HiT recognizing mesothelin using phage display

HiT binds directly to mesothelin

Surface plasmon resonance (SPR)

\[ K_D = 0.8 \, \mu M \]
\[ T_{1/2} = 6.6 \, s \]

HiT T-cell activated by mesothelin

- \( K_D \) dissociation constant; HiT, HLA-independent TCR; HLA, human leukocyte antigen; MSLN, mesothelin; T_{1/2}, half-life
HiT T-cell activation level is mesothelin expression dependent

**Mesothelin Expression**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IFNγ (pg/mL)</th>
<th>Granzyme B (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capan-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNG-M</td>
<td></td>
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<tr>
<td>HCT-116</td>
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<tr>
<td>HeLa</td>
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<tr>
<td>Capan-1</td>
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<tr>
<td>HepG2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLD-1</td>
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<td></td>
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<tr>
<td>DLD-1.A2B2m</td>
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<tr>
<td>A375</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cama-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-BR-3</td>
<td></td>
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<tr>
<td>K562</td>
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</tbody>
</table>

**Legend:**
- ntd: non-transduced
- red: mesothelin HiT T-cells

**Acronyms:**
- HIT: HLA-independent TCR
- HLA: human leukocyte antigen
- IFNγ: interferon gamma
HiT T-cells kill primary tumor-derived cells *in vitro*

Capan-2 pancreatic cell line

Lung PDX tumor

**Dead target cells**

- **Control T-cells**
- **mesothelin HiT T-cells**
- **Targets Alone**

**Time (h)**

0 | 24 | 48 | 72

**5**

HiT, HLA-independent TCR; HLA, human leukocyte antigen; PDX, patient-derived xenograft
HiTs are active in both CD4 and CD8 T-cells and are not inhibited by soluble protein.

No CD8 or CD4 dependency

Anti-CD8 antibody blocks pHLA TCR activity

Reporter Gene Activity

Exogenous mesothelin (μM)

HiT not inhibited by soluble mesothelin

*Value not tested

ctrl, control; HiT, HLA-independent TCR; HLA, human leukocyte antigen; IFNγ, interferon gamma; MSLN, mesothelin; pHLA, peptide–human leukocyte antigen; All T-cells manufactured with Adaptimmune proprietary technology
HiT induces strong, dose-dependent and persistent tumor regression in vivo

- untreated
- Non-transduced T-cells
- 0.3 x10^6 T cells
- 1 x10^6 T cells
- 3 x10^6 T cells

Mean ± SEM, n = 6–8 per group

HiT, HLA-independent TCR; HLA, human leukocyte antigen; MSLN, mesothelin;
All T-cells manufactured with Adaptimmune proprietary technology
HiTs are an exciting new therapeutic modality for targeting extracellular proteins on tumors.
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  - Jaelle Foot
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  - Sara Boiani
  - Terri Cornforth
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  - Andrew Gerry
  - Chris Herring

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  - Adaptimmune is partnered with Astellas to co-develop a mesothelin-targeted HiT

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