ACTR (Antibody Coupled T-cell Receptor): A universal approach to T-cell therapy

European Medicines Agency
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The Evolution of Cancer Immunotherapy

The drug is...

...a molecule normally made by immune cells (e.g., cytokines, monoclonal antibodies)

...an engineered molecule targeting immune cells (e.g., checkpoint inhibitors)

...an immune cell that attacks disease (cellular immunotherapy)

Source: New York Times
Chimeric Antigen Receptor (CAR)-expressing Autologous T cells (CAR-T)

- Each CAR requires an optimized antibody fragment for a specific single antigen
- CAR constructs substantially overlap across groups
- Primary foci to date: B cell antigens CD19 and BCMA
  - Acute lymphoblastic leukemia (ALL)
  - Chronic lymphocytic leukemia (CLL)
  - Non Hodgkin lymphoma (NHL)
  - Multiple myeloma (MM)
- CAR construct designs against additional tumor antigens ongoing
<table>
<thead>
<tr>
<th>Institution</th>
<th>CAR design</th>
<th>Patient population</th>
<th>Outcome</th>
<th>Toxicities</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSKCC</td>
<td>CD28, CD3ζ</td>
<td>• n = 32 adults</td>
<td>91% CR</td>
<td>• B-cell aplasia</td>
<td>NCT01044069</td>
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<tr>
<td></td>
<td></td>
<td>• R/R B-ALL</td>
<td></td>
<td>• CRS</td>
<td>(REF. 13)</td>
</tr>
<tr>
<td>UPenn/CHOP</td>
<td>4-1BB, CD3ζ</td>
<td>• n = 30 children and young adults</td>
<td>90% CR</td>
<td>• B-cell aplasia</td>
<td>NCT01626495</td>
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<tr>
<td></td>
<td></td>
<td>• B-ALL</td>
<td></td>
<td>• CRS</td>
<td>(REF. 15)</td>
</tr>
<tr>
<td>NCI</td>
<td>CD28, CD3ζ</td>
<td>• n = 20 children and young adults</td>
<td>70% CR</td>
<td>• B-cell aplasia</td>
<td>NCT01593696</td>
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<td></td>
<td></td>
<td>• B-ALL</td>
<td></td>
<td>• CRS</td>
<td>(REF. 17)</td>
</tr>
<tr>
<td>Fred Hutchinson</td>
<td>4-1BB, CD3ζ</td>
<td>• n = 20 adults</td>
<td>83% CR</td>
<td>CRS</td>
<td>NCT01865617</td>
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<tr>
<td></td>
<td></td>
<td>• B-ALL</td>
<td></td>
<td></td>
<td>(REF. 18)</td>
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</tbody>
</table>

Preconditioning chemotherapy was used in all the trials shown in this table. B-ALL, B-cell acute lymphoblastic leukaemia; chemo, chemotherapy; CHOP, Children’s Hospital of Philadelphia; CR, complete response; CRS, cytokine-release syndrome; Fred Hutchinson, Fred Hutchinson Cancer Research Center; MSKCC, Memorial Sloan Kettering Cancer Center; NCI, National Cancer Institute; R/R, relapsed and/or refractory; UPenn, The University of Pennsylvania.
CAR-T cell potential risks

ACTR T cells + Antibodies: Combining Two Proven Anti-tumor Adaptive Immune Responses

- Distinct, potent and specific mechanisms of immune response to invasion have evolved
- Adaptive immune responses sculpt highly specific recognition of the invader
- Redirecting T cell responses combined with Ab-mediated cellular cytotoxicity maintains specificity while enhancing immune response

**cell autonomous**

- One component
- (control must be built into the cell)

**cell extrinsic**

- Two component
- (control can be outside of cell)
Antibody-Coupled T cell Receptor (ACTR): A Next-Generation Platform

- Specific for one type of cancer
- Always ‘on’ after infusion into the patient

ACTR

- Universal – ACTR T cell can attack many different cancers
- Activity is controlled by antibody dosing

**CAR**

- targets tumor
- triggers T-cell

**ACTR**

- Antibody: tumor targeting
- CD16: Fc receptor
- 4-1BB: co-stimulation
- CD3ζ: TCR signaling
Rituximab binds ACTR T cells with affinity comparable to endogenous CD16

Example of expression of ACTR-V158 on T cells and binding of rituximab to ACTR-expressing cells

<table>
<thead>
<tr>
<th></th>
<th>ACTR-V158 (Jurkat cells)</th>
<th>ACTR-F158 (Jurkat cells)</th>
<th>ACTR-V158 (T-cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 $K_D$ (nM)</td>
<td>631</td>
<td>1700</td>
<td>598</td>
</tr>
<tr>
<td>Experiment 2 $K_D$ (nM)</td>
<td>606</td>
<td>1750</td>
<td>708</td>
</tr>
<tr>
<td>Experiment 3 $K_D$ (nM)</td>
<td>596</td>
<td>1610</td>
<td>701</td>
</tr>
<tr>
<td>Average $K_D$ (nM)</td>
<td>611</td>
<td>1690</td>
<td>669</td>
</tr>
<tr>
<td>Standard deviation of $K_D$ (nM)</td>
<td>18</td>
<td>70</td>
<td>62</td>
</tr>
</tbody>
</table>

• Most wildtype IgG1 antibodies bind CD16 with a monomeric affinity in the range of 200-600 nM
• Published affinity of rituximab for CD16-V158 is 660nM and 2000nM for CD16-F158
ACTR T cell specificity: Fc-CD16 affinity vs. avidity

Monovalent binding interaction is not productive

Multivalent interactions drive signaling

Sub $\mu$M affinity

Sub fM avidity
ACTR T cells Kill Tumor Cells *In Vitro*

ACTR T cells kill cancer cell lines in the presence of the right targeting antibody

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Marker</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daudi</td>
<td>CD20</td>
<td>Rituximab</td>
</tr>
<tr>
<td>SK-BR-3</td>
<td>Her2</td>
<td>Trastuzumab</td>
</tr>
<tr>
<td>NB1691</td>
<td>GD2</td>
<td>Anti-GD2</td>
</tr>
</tbody>
</table>

ACTR T cells kill primary cells from chronic lymphocytic leukemia (CLL) patients when combined with Rituxan

Specificity: Effect of Non-Targeting Antibodies

**Non-specific IgG does not trigger ACTR T cell killing**

Neuroblastoma cells incubated with ACTR T cells in the presence of either anti-GD2 antibody or nonspecific IgG.

**Targeting mAbs do not trigger ACTR T cell killing if the target is not present**

Daudi (CD20+) cells incubated with mock T cells + rituximab or ACTR T cells in the presence of rituximab, trastuzumab, or anti-GD2 mAb.
• Daudi cells expressing luciferase were injected in NSG mice on day 0.

• Mice receiving rituximab were injected once weekly for 4 weeks starting on day 4.

• Mice receiving ACTR were injected with ACTR-expressing T cells (2.5 x 10^6) on day 5.
Tuning ACTR Activity

ACTR cell killing can be controlled by adjusting the antibody dose

- Potential to maximize therapeutic index
- Potential to access targets with low levels of off-tumor expression

### Lymphoma

- **Rituximab (μg/mL)**
  - % Cell Killing vs. antibody dose

### Breast cancer

- **Trastuzumab (μg/mL)**
  - % Cell Killing vs. antibody dose

### Neuroblastoma

- **Anti-GD2 (μg/mL)**
  - % Cell Killing vs. antibody dose

**Potential safety issues**

**Potential loss of efficacy**
Ongoing Phase 1 Trial: ACTR T cells + Rituximab (ATTCK20)

**Design**

- Safety and feasibility in patients with B-cell CLL or B-cell NHL refractory/relapsed to chemotherapy including rituximab
- ACTR expressed by mRNA electroporation
- Patients receive rituximab one day prior to ACTR T cell infusion; repeat ACTR dosing in recent study amendment
- Dose-escalation (traditional 3+3) with option for intra-patient escalation

**Status**

- Enrolling to high, multi-dose cohort
ACTR Construct Can Be Delivered as an mRNA

- ACTR can be transiently expressed as an mRNA by electroporation
- CARs delivered in this way have shown clinical activity

T cells electroporated with ACTR mRNA efficiently express the receptor (assessed 24 h after electroporation by flow cytometry)

T cells electroporated with mRNA express ACTR for about 6 days ex vivo

T cells electroporated with ACTR mRNA efficiently kill cancer cells (CD20+) in the presence of rituximab
ACTR Pharmacokinetics

- Peripheral blood from ATTCK20 mRNA patient isolated post-infusion and characterized by flow cytometry
- ACTR T cells expand in vivo to ~15% of the total T-cell population by day 3, drop to background by day 7
Ongoing Phase 1 Trial (United States): ACTR T cells + Rituximab (ATTCK-20-2)

• Stable ACTR transgene product expression following viral vector transduction (vs. mRNA transfection)

• **Automated, closed T cell manufacturing**
  system in a centralized facility

• Patient population with relapsed or refractory CD20+ non Hodgkin B-cell lymphoma subtypes:
  • diffuse large B-cell lymphoma
  • primary mediastinal B-cell lymphoma
  • mantle cell lymphoma
  • transformed follicular lymphoma
  • Grade 3b follicular lymphoma

• **Multiple rituximab infusions; single ACTR T cell infusion**
  • Flu/Cy lymphodepleting chemotherapy
Expanding Unum’s Target Space

- A wide range of tumor-targeting antibodies in clinical development

- Combination approach with ACTR provides significant opportunities for to rapid pipeline expansion and accelerated development

- A critical need exists for regulatory mechanisms facilitating early development of novel combinations such as ACTR T cells + Ab

naked antibodies

antibody-drug conjugates

湎

CA125 CA200 EGFL7 CD80 CXCR4 α5β1 CTLA4 CD55 CD40 CD56 CD200 CD20 CD44 CD70 CD30 CD138 CD33 CD38 CD74 CD79b CD22 CD200 CD200 CD22 MUC1 CEACAM5 EpCAM mesothelin HER2 LIV melanin NaPi2b antibody-drug conjugates

naked antibodies

湎

CA125 CA200 EGFL7 CD80 CXCR4 α5β1 CTLA4 CD55 CD40 CD56 CD200 CD200 CD22 MUC1 CEACAM5 EpCAM mesothelin HER2 LIV melanin NaPi2b
## Current Unum Pipeline

<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Pre-Clinical</th>
<th>Phase I</th>
<th>Phase II</th>
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<tbody>
<tr>
<td>mRNA ACTR + rituximab (ATTCK20)</td>
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<tr>
<td>ACTR087 + rituximab (ATTCK-20-2)</td>
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<tr>
<td>SGI collaboration A</td>
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<tr>
<td>SGI collaboration B</td>
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<tr>
<td>Preclinical targets</td>
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ACTR T cell platform: Summary

• Novel immunotherapeutic targets and platforms are transforming oncology

• The ACTR platform combines the potential immunologic benefits of engineered tumor-directed T cells with monoclonal antibodies

• The ACTR platform is distinct from other adoptive T cell therapies (e.g. CAR-T cells), with potential differences in therapeutic index
Thank you!