A Novel CD19-Anti-CD20 Bridging Protein To Prevent and Reverse Relapses from CAR-CD19 T Cell Therapy

Characterization, *in vitro* and *in vivo* activity

Paul D Rennert, President & CSO, Aleta Biotherapeutics
Approved CAR-CD19 Therapies Have Changed the Clinical Treatment Paradigm For B-cell Tumors…

CAR-CD19 T cell therapies have been approved for subsets of r/r NHL and ALL

Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma


Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia

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CAR-CD19 therapy: responses & relapses

- CAR-CD19 therapy produces high complete response rates in NHL and ALL
- The high CR rate is impacted by relapses
  - Some have relapsed with CD19-negative or dim tumors
  - Some have had a suboptimal response and have failed to clear the tumor burden
- Patients can relapse with CAR-CD19 T cells still present

<table>
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<tr>
<th>CR rate</th>
<th>relapse rate</th>
<th>% CD19-relapses</th>
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<tr>
<td>72.4%</td>
<td>35%</td>
<td>41%</td>
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r/r pediatric and adult ALL and NHL (multiple centers, CD28 and 4-1BB based-constructs*)

* calculated from Majzner & Mackall, Nat Med. 2019
Most relapses occur in the first 6 months or less

**Adult NHL**
Axicabtagene Ciloleucel, Ph1

**Pediatric ALL**
Tisagenlecleucel, Ph2

Neelapu, Locke et al 2017. NEJM
Maude et al 2018. NEJM
Can we rescue the CAR-CD19 activity?

- We created a CD19 – anti-CD20 bridging protein
  - The CD19 extracellular domain will bind anti-CD19 CARs and will bind CD20 on the target tumor cell
  - Re-stimulate any CAR19 present in the patient by providing additional antigen
- CD20 is expressed in virtually all NHL cases and 30-40% of ALL cases
- CD20 expression is relatively insensitive to selection pressure: CD20 escapes are uncommon

This protein bridges CAR-CD19 T cells to tumor cells, forming a cytotoxic synapse and triggering tumor cell killing.
Retargeting and therefore reactivating CAR-CD19 T cells

CD19 anti-CD20

anti-CD19 CAR-T Cell

B Cell Tumor

CD20
Characterizing bridging proteins

### Cell binding (anti-CD19-PE)

- **293-CD20 cells**

### Cytotoxicity (+ CAR-CD19 T cells)

<table>
<thead>
<tr>
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<th>binding EC$_{50}$</th>
<th>cytotoxicity IC$_{50}$</th>
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<tbody>
<tr>
<td>prototype</td>
<td>6.7 nM</td>
<td>143 pM / 8 ng/ml</td>
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<tr>
<td>candidate - 1</td>
<td>3.6 nM</td>
<td>66 pM / 3.8 ng/ml</td>
</tr>
<tr>
<td>candidate - 2</td>
<td>1.3 nM</td>
<td>22 pM / 1.3 ng/ml</td>
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- Small protein
- High affinity binding
- Potent mediator of CAR-CD19 cytotoxicity
Delivering the bridging protein

- Clone into a CAR-CD19 lentiviral construct as a dual-targeting therapy
- Engineer as an extended half-life biologic for injection
A candidate bridging protein cloned downstream of a CAR-CD19

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<th>binding $EC_{50}$</th>
<th>cytotoxicity $IC_{50}$</th>
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“CD20-bridging CAR19 T cell”

![Diagram of CAR-CD19 and Bridging protein]
CD20-bridging CAR19 T cell activity in vitro

- Bridging protein secretion measured at 10 ng/ml in vitro
- High affinity binding to CAR-CD19 and to CD20 (1-2 nM)

JeKo (CD19+/CD20+)

OC1-Ly3 (CD19-/CD20+)

CD20-bridging CAR19 T cells
Two means of delivery

• Cloned into a CAR-CD19 lentiviral construct

• Engineered as a biologic with extended half-life

  - Added to a novel series of CD20 binders
  - Albumin binding provides FcRN-mediated half-life extension of ~ 3 weeks
  - Importantly, binding to albumin does not functionally multimerize the bound protein, cf. Fc-fusions
**In vitro** characterization of half-life extended bridging proteins

- Highly purified monomeric proteins from standard mammalian expression culture
- Do not induce tonic signaling when bound to CAR-CD19
- Stable at 37°C in serum x 3 days: no aggregation, no clipping
- Potent binding, potently cytotoxic in the presence of CD20-positive cells and CAR-CD19 T cells

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<th>Prototype bridging protein - anti-albumin</th>
<th>Candidate bridging protein</th>
<th>Candidate bridging protein - anti-albumin</th>
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<tr>
<td><strong>IC$_{50}$ (ng/mL)</strong></td>
<td>1</td>
<td>0.04</td>
<td>0.04</td>
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<tr>
<td><strong>IC$_{50}$</strong></td>
<td>14.4 pM</td>
<td>0.84 pM</td>
<td>0.69 pM</td>
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In vitro model of antigen heterogeneity

- This simple experiment demonstrates that the CAR-CD19 can kill directly (targeting CD19) and indirectly (via the CD19-anti-CD20 bridging protein)
In vitro model of antigen escape using dual-antigen-positive cells

- JeKo cell line is derived from a mantle cell lymphoma
- These cells brightly express CD19 and CD20
- The vast majority of individual JeKo cells are CD19+/CD20+
- Regardless, these cells are highly proliferative and prone to antigen escape
Establishing antigen escape

- JeKo cells targeted by CAR-CD19 T cells

**Day 1**

- 1:1
- 0.3:1
- 0.1:1

**B cells**

**CAR-CD19 T cells**

**Day 6**

**CD19+/CD20+**

**Day 13**

**CD19-/CD20+**
Reversing antigen escape

- JeKo cells targeted by CAR-CD19 T cells, then by CAR-CD19 T cells with or without bridging protein

Rest x 48 hours

Add excess CAR-CD19 cells (10:1)

Add excess bridging protein (1 µg/ml) *(candidate BP – anti-albumin)*

Add CAR-CD19 T cells and bridging protein *(candidate BP – anti-albumin)*

ESCAPE

mixed populations

CD19- cells untouched

no cell death

all target cells eliminated
Delivering the bridging protein *in vivo*

- CD20-bridging CAR-CD19 T cells

- Extended half-life biologic for injection
Nalm6-luciferase model (CD19+, CD20-)

- Nalm6-luciferase, pre-implanted iv x 3 days
- Challenged with CAR-CD19 or CAR-CD19 with bridging protein delivery

![Graph showing flux total over days for untreated mice, untransduced T cells, CAR-CD19 T cells, and CAR-CD19 T cells + bridging protein (100ug/q3w).]

Untreated mice
Untransduced T cells
CAR-CD19 T cells
CAR-CD19 T cells + bridging protein (100ug/q3w)
CD20-bridging CAR19 T cells
Evolution of escape in the JeKo model (CD19+, CD20+)

Day 18
Untreated, UTD

Day 28
Untreated mice, untransduced T cells
CAR-CD19 T cells

Day 32
CAR-CD19 T cells + bridging protein
CD20-bridging CAR19 T cells
Next steps

• Allow relapse to occur in the JeKo model, then add bridging protein to reverse
• Evaluate PDX models if available (ie. CD19-/CD20+ or mixed phenotype)

• IND in 16 -18 months, Phase 1 dose escalation (single and multiple dose)
• We will enroll patients who are relapsing at < 6 months post-CAR-CD19 infusion
• The Phase 1 PD marker is simple – do the CAR-CD19 T cells expand above baseline?
• CAR T expansion is the single best predictor of therapeutic efficacy; the PD marker is clinically relevant
# Acknowledgements

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<th>Scientific Advisory Board</th>
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<td>Christine Brown, City of Hope</td>
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<tr>
<td>Alyssa Bir</td>
<td>Funding</td>
<td></td>
</tr>
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61st ASH Annual Meeting, December 7-10, 2019, Orlando, FL