CTL019 - tisagenlecleucel (Kymriah)

Overview

Abhijit Chakraborty, PhD
# Meeting Agenda

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00 – 10:20a</td>
<td>CART Overview and Landscape</td>
<td>Dr. Abhijit Chakraborty, PhD Novartis</td>
</tr>
<tr>
<td>10:20 – 10:40a</td>
<td>Clinical Perspective on CTL019 in pediatric and young adult ALL</td>
<td>Dr. Lamis Eldjerou, MD Novartis</td>
</tr>
<tr>
<td>10:40 – 10:50a</td>
<td>qPCR methods for characterizing cellular kinetics</td>
<td>Dr. Reinhold Pollner, PhD Navigate Biopharma</td>
</tr>
<tr>
<td>10:50a – 11:00a</td>
<td>Flow cytometry approaches for characterizing functional persistence</td>
<td>Dr. Naveen Dakappagari, PhD Navigate Biopharma</td>
</tr>
<tr>
<td>11:00a – 11:25a</td>
<td>Impact of Clinical Pharmacology on CART Development</td>
<td>Dr. Karen T Mueller, PharmD, MS Novartis</td>
</tr>
<tr>
<td>11:25a -12:00p</td>
<td>Q&amp;A</td>
<td>All</td>
</tr>
</tbody>
</table>
FDA approval of first CAR-T cell therapy signifies watershed moment in cancer treatment
History of CAR T cell Therapy and Tisagenlecleucel

First CAR T cell proposed
1998

Preclinical studies of second generation CAR T
2006

First in human studies of first generation CAR T cells
2010

Tisagenlecleucel, a second generation CAR directed against CD19, enters the clinic
2012

First presentation of data in ALL
2013

Tisagenlecleucel receives breakthrough therapy designation by the FDA for R/R pediatric ALL (first CAR T cell therapy to receive breakthrough designation)
2014

First results from multicenter trials presented
2015

Tisagenlecleucel receives breakthrough therapy designation by the FDA for R/R DLBCL
2016

First tisagenlecleucel cells are processed at the Morris Plains facility
2017

Submission in pediatric ALL
February 2017

Approval in pediatric ALL
August 30

The University of Pennsylvania and Novartis announced global collaboration to research, develop, and commercialize CAR T cells
2008

Novartis sets up a GMP-approved manufacturing site specific for cellular therapies in Morris Plains, NJ
2015

Tisagenlecleucel receives breakthrough therapy designation by the FDA for R/R pediatric ALL (first CAR T cell therapy to receive breakthrough designation)
2016

First results from multicenter trials presented
2015

First in human studies of first generation CAR T cells
2010

Preclinical studies of second generation CAR T
2006

First CAR T cell proposed
1998

CAR, chimeric antigen receptor; DLBCL, diffuse large B-cell lymphoma; FDA, United States Food and Drug Administration.

Novartis Oncology

Business Use Only
Relapsed/Refractory B-ALL in Pediatric and Young Adult Patients

• More than 3,000 children and young adults under the age of 20 are diagnosed with ALL in the United States annually\(^1\)
  – 80%-85% of childhood ALL cases are B-cell ALL\(^2\)
  – The majority of these will express CD19, a marker found on the surface of B cells at all phases of differentiation on their surface\(^3\)

• Despite current treatment options, ~15% pediatric and young adult patients with ALL experience relapsed/refractory (r/r) disease\(^4\)
  – Relapsed ALL represents the fourth most prevalent cancer and the leading cause of cancer death in children\(^5\)

• Children whose disease is refractory to chemotherapy, or who have relapsed after an allogeneic stem cell transplant have poor outcome\(^3\)-\(^8\)
  – Children who fail induction therapy have a survival of only 30%\(^3\)
  – Survival after second and 3\(^{rd}\) relapse are 27% and 15% respectivley\(^6\)

HSCT, hematopoietic stem cell transplant.
CD19: a Rational Target for Therapy

• CD19 is expressed on B cells and B-cell precursors and is not expressed on bone marrow stem cells or other tissues

FL, follicular lymphoma; GC, germinal center; MCL, mantel cell lymphoma; MZL, marginal zone lymphoma; WM, Waldstrom's macroglobulinemia.

Tisagenlecleucel: a CD19-directed Genetically Modified Autologous T Cell Immunotherapy

- The tisagenlecleucel CAR consists of T cell activation domains coupled to an anti-CD19 single-chain variable fragment

CTL019 is a living drug designed to target CD19+ B cells

Mechanism of action data is based on in vitro/in vivo data
Overview of Tisagenlecleucel Therapy in the Clinic

1. **Leukapheresis:** Patient’s white blood cells are collected, cryopreserved, and shipped to the manufacturing facility\(^1\)\(^-\)\(^3\)

2. **Selected T cells:** T cells are genetically transduced ex vivo with a lentiviral vector encoding the anti-CD19 CAR\(^1\),\(^3\)

3. **Modified T cell expansion:** Tisagenlecleucel cells undergo ex vivo expansion on magnetic antibody-coated beads\(^1\)\(^-\)\(^3\)

4. **Lymphodepleting chemotherapy:** the patient may receive a preparative lymphodepleting regimen before T cell infusion\(^1\)\(^-\)\(^3\)

5. **Modified T cell infusion:** Tisagenlecleucel cells are shipped back to the clinic and infused into the patient\(^1\)\(^-\)\(^3\)

---


CTL019 Expansion and Persistence in Representative Patient Profile

- Similar cellular kinetic profiles observed in all responder patients

Thudium Mueller, K et al  Blood 2017
Cellular Kinetics Across Indications

Thudium Mueller, K et al  Blood 2017

Novartis Oncology
Higher expansion seen in responding patients across multiple indications

Thudium Mueller, K et al. Blood 2017
Control of Cell Product Through Final Product Release Testing Requirements

Appearance and description
• Color

Safety
• Bacterial endotoxins
• Sterility
• Mycoplasma
• Determination of VSV-G DNA by quantitative PCR (qPCR, surrogate for replication competent lentivirus)

Purity
• Percentage of viable T cells
• Determination of transduction efficiency by CAR qPCR
• Cell viability

Impurities
• Determination of residual beads by microscopy
• Percentage of viable CD19+ B cells

Identity
• Identity by CAR qPCR

Quantity
• Total cell count
• Number of viable cells (calculated)
• Dose (calculated)

Potency
• Determination of CAR expression by flow cytometry
• Release of IFNγ in response to CD-19 expressing target cells
Consistent T-cell Product From Variable Patient Material

Leukapheresis Material

Tisagenlecleucel Final Product

Dedicated Manufacturing Facility

- Novartis acquired a 170,000 square-foot cell-manufacturing facility in Morris Plains, NJ in 2012
- Used to manufacture > 250 batches of tisagenlecleucel during Novartis-sponsored clinical studies as the sole supply site for US clinical trials
  - Also used to manufacture tisagenlecleucel for global trials in ALL and DLBCL
- Designed to support commercial production
Summary

- Novartis has accrued significant experience in manufacturing patient-specific CAR T cells in global, multicenter trials
- Novartis has developed a consistent manufacturing process for tisagenlecleucel
- Consistent product quality has been demonstrated by extensive product testing, including assessment of product T cells, vector copies per cell, CAR functionality
- Positive clinical outcomes observed across the allowable dose range and range of product quality attributes
- The cellular kinetics highlight the importance for characterizing clinical activity of CAR-T cells

Novartis. Data on file (Presentations for the July 12, 2017, Meeting of the Oncologic Drugs Advisory Committee).
CTL019 (tisagenlecleucel) Clinical Efficacy and Safety

In pediatric and young adult patients with relapsed/refractory B-cell acute lymphoblastic leukemia

Lamis Eldjerou, MD
Medical Director US Oncology
Novartis Pharmaceuticals Corp.
Clinical Trials of Tisagenlecleucel in Pediatric and Young Adult ALL


Database lock for interim data analysis (final analysis for ELIANA [US manufacturing]).

CR, complete response; CRi, CR with incomplete recovery; FPFV, first patient first visit; ORR, overall response rate.


Supportive: Pedi-CART
1 site; N = 59; CR at 1 month, 93%
15 MAR 2012 – 30 NOV 2015
Collaboration with University of Pennsylvania

Supportive: ENSIGN
9 sites; N = 29; ORR (CR + CRi) at 6 months, 69%
14 AUG 2014 – 1 FEB 2016

Pivotal: ELIANA
25 sites; N = 68; ORR (CR + CRi) at 3 months, 83%
8 APR 2015 – 23 NOV 2016

Follow-up AUG 2021

Follow-up APR 2022

Follow-up AUG 2018

Database lock for interim data analysis (final analysis for ELIANA [US manufacturing]).

63 patients were evaluable for efficacy. FPFV to data cutoff for interim analysis (final analysis for ELIANA [US manufacturing]).
ELIANA: Trial Design

Inclusion Criteria:
- 2nd or greater bone marrow relapse or primary refractory B-cell ALL
- ≥5% bone marrow lymphoblasts
- Age 3 years at the time of screening to age 21 years at the time of initial diagnosis

Lymphodepleting chemotherapy

- Fludarabine (30 mg/m^2 IV daily for 4 doses) plus cyclophosphamide (500 mg/m^2 IV daily for 2 doses)

Büchner J et al. Haematologica. 2017;120(S2) [abstract S476].
Dose used in clinical trials
ELIANA (Study B2202)

• Single IV infusion

• Protocol-specified dose range
  – 0.2 to $5.0 \times 10^6$ transduced viable T cells/kg
    (for pts $\leq 50$ kg, weight adjusted)
  – 0.1 to $2.5 \times 10^8$ transduced viable T cells (for pts $>50$ kg)

• Wide dose range considered acceptable because there is no relationship between expansion in vivo and dose

Büchner J et al. *Haematologica*. 2017;120(S2) [abstract S476].
ELIANA: Efficacy and Cellular Kinetic Endpoints

**Primary endpoint**
- Overall remission rate (ORR=CR+CRi) within 3 months after CTL019 administration (by IRC)

**Key secondary endpoints**¹
- Remission rate (CR/CRi) with MRD-negative bone marrow within 3 months

**Other efficacy endpoints include**
- Duration of remission (DOR)
- Overall survival (OS)

**Clinical Pharmacology Endpoints**
- Expansion and Persistence
- Dose Response, Exposure-Response, Dose-Exposure
- Immunogenicity

¹ Key secondary endpoints also include ORR and MRD in patients receiving CTL019 manufactured in the US.
## ELIANA: Baseline Characteristics

<table>
<thead>
<tr>
<th>Select baseline characteristics</th>
<th>Full analysis set</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N=68</strong></td>
<td></td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>12 (3-23)</td>
</tr>
<tr>
<td>Sex, %</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>75</td>
</tr>
<tr>
<td>Asian</td>
<td>9</td>
</tr>
<tr>
<td>Black</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
</tr>
<tr>
<td>Previous lines of therapy, median (range)</td>
<td>3 (1-8)</td>
</tr>
<tr>
<td>Prior HSCT, %</td>
<td>59</td>
</tr>
<tr>
<td>Primary refractory, %</td>
<td>9</td>
</tr>
<tr>
<td>Morphologic blast count in bone marrow, %, median (range)</td>
<td>73 (5-99)</td>
</tr>
</tbody>
</table>

## ELIANA: Overall Remission Rate

<table>
<thead>
<tr>
<th>Primary end point</th>
<th>Efficacy Analysis Set (N = 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR (CR+CRi) within 3 months(^a,(^b)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Best overall response (BOR), n (%)</td>
<td></td>
</tr>
<tr>
<td>CR(^c)</td>
<td>40 (63%)</td>
</tr>
<tr>
<td>CRi(^d)</td>
<td>12 (19%)</td>
</tr>
<tr>
<td>No response</td>
<td>5 (8%)</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>Day 28 response</td>
<td>53 (84%)</td>
</tr>
<tr>
<td>CR or CRi with MRD negative bone marrow(^e,(^f)</td>
<td>52 (83%)*</td>
</tr>
</tbody>
</table>

\(^a\) ORR was calculated based on the first 50 patients who received tisagenlecleucel and have completed ≥ 3 months’ follow-up or discontinued earlier. Requires remission status to be maintained for ≥ 28 days without clinical evidence of relapse.

\(^b\) One-sided exact \(P\) value threshold .0057 (adjusted for interim). The null hypothesis of ORR ≤ 20% was rejected.

\(^c\) CR was defined as < 5% of blasts in the bone marrow, no evidence of extramedullary disease, and full recovery of peripheral blood counts (platelets > 100,000/\(\mu\)L and absolute neutrophil counts [ANC] greater than 1000/microliter) without blood transfusion.

\(^d\) CRi was defined as < 5% of blasts in the bone marrow, no evidence of extramedullary disease, and without full recovery of peripheral blood counts with or without blood transfusion.

\(^e\) MRD negative was defined as MRD by flow cytometry less than 0.01%.

\(^f\) One-sided exact \(P\) value threshold .0057 (adjusted for interim). The null hypothesis of MRD negative remission rate ≤ 15% was rejected.

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**ELIANA: Overall Remission Rate**

- ORR was consistent across subgroups evaluated

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
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<tbody>
<tr>
<td>Overall</td>
<td>63</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>26</td>
</tr>
<tr>
<td>≥10-&lt;18</td>
<td>25</td>
</tr>
<tr>
<td>≥18</td>
<td>12</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
</tr>
<tr>
<td>Male</td>
<td>35</td>
</tr>
<tr>
<td>Race</td>
<td></td>
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<tr>
<td>White</td>
<td>46</td>
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<tr>
<td>Asian</td>
<td>6</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Hispanic or Latino</td>
<td>14</td>
</tr>
<tr>
<td>Other</td>
<td>49</td>
</tr>
<tr>
<td>Response status at study entry</td>
<td></td>
</tr>
<tr>
<td>Primary refractory</td>
<td>6</td>
</tr>
<tr>
<td>Relapsed disease</td>
<td>57</td>
</tr>
<tr>
<td>Prior SCT therapy</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>28</td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
</tr>
<tr>
<td>Enrollment bone marrow tumor burden</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>41</td>
</tr>
<tr>
<td>Low</td>
<td>22</td>
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<tr>
<td>Complex karyotypes (≥5 unrelated abnormalities)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>45</td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
</tr>
<tr>
<td>Any high-risk mutations</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43</td>
</tr>
<tr>
<td>Yes</td>
<td>20</td>
</tr>
<tr>
<td>Down syndrome</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>57</td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
</tr>
</tbody>
</table>

**ORR (95% CI)**
ELIANA: Duration of Response

- DOR: 75% relapse-free 6 months after onset of remission

Büchner J et al. Haematologica. 2017;120(S2) [abstract S476].

Bulmathis Oncology
Duration of remission was consistent across trials.

Even with longer-term follow-up in the single-center study, the median duration of remission has not been reached.

Büchner J et al. *Haematologica*. 2017;120(S2) [abstract S476].

**Patients at risk**

<table>
<thead>
<tr>
<th></th>
<th>ELIANA</th>
<th>Pedi-CART</th>
<th>ENSIGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>52</td>
<td>52</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
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<tr>
<td>18</td>
<td></td>
<td>8</td>
<td>5</td>
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<tr>
<td>21</td>
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<td>2</td>
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<tr>
<td>24</td>
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<td>30</td>
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<td>36</td>
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</tr>
<tr>
<td>39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td></td>
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</tr>
</tbody>
</table>
CTL019 (tisagenlecleucel)
Clinical safety
Lamis Eldjerou, MD
Medical Director US Oncology
Novartis Pharmaceuticals Corp.
WARNING: CYTOKINE RELEASE SYNDROME AND NEUROLOGICAL TOXICITIES

• Cytokine Release Syndrome (CRS), including fatal or life-threatening reactions, occurred in patients receiving KYMRIAH™ (tisagenlecleucel). Do not administer KYMRIAH to patients with active infection or inflammatory disorders. Treat severe or life-threatening CRS with tocilizumab [see Dosage and Administration (2.2, 2.3), Warnings and Precautions (5.1)].

• Neurological toxicities, which may be severe or life-threatening, can occur following treatment with KYMRIAH, including concurrently with CRS. Monitor for neurological events after treatment with KYMRIAH. Provide supportive care as needed [see Warnings and Precautions (5.2)].

• KYMRIAH is available only through a restricted program under a Risk Evaluation and Mitigation Strategy (REMS) called the KYMRIAH REMS [see Warnings and Precautions (5.3)].
ELIANA: Overall Safety

<table>
<thead>
<tr>
<th></th>
<th>After CTL019 Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Time (N = 68)</td>
</tr>
<tr>
<td>Grade 3/4 AEs, %</td>
<td>85</td>
</tr>
<tr>
<td>Suspected to be study drug related, %</td>
<td>72</td>
</tr>
</tbody>
</table>

- The majority of AEs occurred in the first 8 weeks after CTL019 infusion
## ELIANA: AEs of Special Interest

<table>
<thead>
<tr>
<th>AESI within 8 weeks after infusion, %</th>
<th>All Grades, %</th>
<th>Grade 3, %</th>
<th>Grade 4, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine release syndrome</td>
<td>78</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>Cytopenias not resolved by day 28</td>
<td>37</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Infections</td>
<td>43</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Neurological events</td>
<td>44</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Tumor lysis syndrome</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

- 2 deaths within 30 days of CTL019 infusion (1 ALL, 1 cerebral hemorrhage)
- No deaths due to refractory CRS
- No cases of cerebral edema reported

Büchner J et al. *Haematologica*. 2017;120(S2) [abstract S476].

Novartis Oncology
**ELIANA: Cytokine Release Syndrome**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 68)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to onset, days</td>
<td>3.0 (1-22)</td>
</tr>
<tr>
<td>Duration of CRS, days</td>
<td>8.0 (1-36)</td>
</tr>
<tr>
<td>ICU admission, %</td>
<td>46</td>
</tr>
<tr>
<td>Anticytokine therapy, %</td>
<td>38</td>
</tr>
<tr>
<td>Hypotension that required intervention, %</td>
<td>51</td>
</tr>
<tr>
<td>High-dose vasopressors, %</td>
<td>25</td>
</tr>
<tr>
<td>Intubation, %</td>
<td>15</td>
</tr>
<tr>
<td>Dialysis, %</td>
<td>10</td>
</tr>
</tbody>
</table>

CRS was graded using the Penn scale and managed by a protocol-specific algorithm.¹

---

Neurological Events by CRS Grade

<table>
<thead>
<tr>
<th>CRS</th>
<th>Any-Grade Neurological Events, %</th>
<th>Grade 3 Neurological Events, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CRS (n = 15)</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>Grade 1/2 (n = 21)</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>Grade 3 (n = 14)</td>
<td>50</td>
<td>14</td>
</tr>
<tr>
<td>Grade 4 (n = 18)</td>
<td>67</td>
<td>33</td>
</tr>
</tbody>
</table>

• Numerically higher neurotoxicity incidence and grades were observed with higher-grade CRS

• The majority of neurological events occurred during CRS or shortly following CRS resolution

• The most common any-grade neurological events were encephalopathy (12%), confusional state (10%), and delirium (10%)

Büchner J et al. *Haematologica*. 2017;120(S2) [abstract S476].
Conclusion:

• The efficacy of tisagenlecleucel was demonstrated in 3 clinical trials in more than 150 pediatric and young adult patients with R/R B-ALL.

• Tisagenlecleucel has demonstrated prolonged remissions, median duration of remission was not reached.

• Well-characterized safety profile for tisagenlecleucel including a restricted REMS program for the management of CRS and neurotoxicity.

• CRS was manageable at sites with appropriately trained staff.

• No cases of cerebral edema.

• Majority of safety events occurred within the first 8 weeks, and no new safety findings relative to the single-center data.

• Tisagenlecleucel offers a new option for pediatric and young adult patients with r/r B-cell ALL.

Büchner J et al. Haematologica. 2017;120(S2) [abstract S476].
Applications of QPCR Assays in CAR-T Cell Therapies and Clinical Trials

November 15, 2017
Reinhold Pollner
Bioanalytical Endpoints

Flow Cytometry

Transgene qPCR
Specialties

- Develop high-complexity custom assays for oncology biomarkers
- Global Clinical Trial Testing

Various Technology Platforms

Anatomic & Hematopathology

Multiplex IHC & RNAscope

High Complexity Flow Cytometry

Cytogenetics

Custom Assay Development

Molecular Assays (NGS, NanoString)

Project Management

Companion Diagnostics Development (CoDx)

Solid & Heme Tumors

Clinical Trial Testing

Data Management

NAVIGATE BIOPHARMA SERVICES, INC.

A Novartis Subsidiary

Novartis Oncology

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CAR-T CELL THERAPY

Clinical Trial Testing
QPCR CAR-T Transgene Assays (murine and human)
QPCR VSVg Assay

Long-Term Safety Follow Up (15 Years)
QPCR CAR-T Transgene Assays (murine and human)
QPCR VSVg Assay

Identity Testing
RT-QPCR CAR-T Lentivirus Assays (murine and human)

QC Release/GMP testing
QPCR CAR-T Transgene Assays (murine and human)
QPCR VSVg Assay
Unified CAR-T QPCR Assay Workflow

Samples: 0.2-2 mL Frozen Whole Blood or Bone Marrow in EDTA tubes

DNA Isolation: Qiagen DNA Blood Mini/Midi Kit / MinElute Qubit

QPCR: 20-200 ng input Set Up: QIAgility 96 or 384 well ViiA7

Qubit, QIAgility, ViiA7
**LOD, LLOQ, ULOQ, DYNAMIC RANGE AND LINEARITY**

**Limit of detection (LOD):** 2.8 copies/rxn (95% detection rate)

**Lower limit of quantitation (LLOQ):** 10 copies/rxn or 50 copies/µg (100% detection rate, imprecision <35% CV, recovery 80-120%)

**Linearity (transgene and reference gene assays):** > 0.99 RSquare

**Dynamic range:** 10 – 1,000,000 copies/rxn
TRANSGENE CART-T MONITORING OVER A ONE-YEAR PERIOD

Log Average Copy Numbers/µg vs. Time Points

Time Points

Log Average Copy Numbers/µg

BLOOD
BONE MARROW

SPECB1

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CONCLUSIONS

• QPCR assays play an important role in CAR-T Cell Therapies and in support of clinical trials

• Primary applications of qPCR assays are transgene monitoring and safety testing

• QPCR has unique advantages related to
  • Accurate quantitation
  • Automation
  • Fast turnaround time
  • Ease of use
  • Different low- and high-throughput platforms (96- and 384-well plates)
  • Sensitive target detection
CAR-T CELLULAR KINECTS BY FLOW CYTOMETRY

Naveen Dakappagari, PhD
AAPS Annual Meeting, San Diego, Nov 2017
CELLULAR KINETICS: CRITICAL ANALYTICAL CONSIDERATIONS

1. Validation Hurdles (CAR-T Detection & Specimens)

2. Stability of CAR-T Cells (Multicenter Trials)

3. Real World Verification of True Sensitivity [LLOQ]
ASSAY VALIDATION: DESIGN PRINCIPLES & LIMITATIONS

Key challenges: generation of anti-idiotypic antibody & availability of CAR-T transfected patient cells

Advanced method validation guidelines: O’hara et al., J imm. Met., 2011

Orthogonal test: QPCR (spearman correlation = rho > 0.9, p = 0.0001)
CAR-T Stability: Evolution of Cell Preservation Methods

Premise: Activated (CAR) T cells are highly labile. Preservation methods explored during clinical development

**Single Clinical Site**

- **Peripheral Blood**
- **Separation Medium**
- **Plasma**
- **PBMC**
- **CFG**
- **Granulocytes**
- **RBCs**

**Frozen PBMC**

**Benefits:** Pure cell populations for easier enumeration

**Risks:**
- Variable recovery of T cells
- Does not allow accurate estimation of circulating CAR-Ts

**Blood Frozen in a Proprietary Fixative**

**Benefits:** Simple 1-step preservation

**Risks:**
- High formaldehyde hinders optimal detection of CAR-T epitope

**Heparinized Whole Blood**

**Benefits:**
- Best reflection of circulating CAR-T burden
- Widely utilized in diagnostic flow cytometry

**Risks:** Limited sample stability (5-day)

**Multiple Centers**
FIXATION REDUCED CAR-T EPITOPE DETECTION*

*Representative patient data from early development
CAR-T Cell Identification by Multicolor Flow Cytometry

Lyse & Stain with antibody cocktails

Viable WBCs → Mononuclear cells → Resolution of T-cells from Monocytes → Hone in on CAR-T

CAR-T Positive

CD8+

32.3

CD4+

46.1

CAR-T Negative

CD8+

40.4

CD4+

54.9

Flow Cell

Viable Cells

99.6

MNCs

44.8

Dump (CD14_16_20)

CD3-

23.6

CD3+ T-cells

68.0

CART19

1.04

CART19-

98.9

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NEED FOR REAL WORLD VERIFICATION OF TRUE SENSITIVITY [LLOQ]

Analytical Validation (spike in)

Expected CAR-T: 21% 10% 7.5% 5% 2.5% 1% 0.5% 0%

Observed CAR-T: [Images showing flow cytometry plots]

LLOQ: 0.5% >100 Events

Clinical Verification (Patient Specimens)

LLOQ: Revised to 1.0%, >100 events based on patient data

Patient #1: Low WBC

0.7% = 11 events!

Patient #2: Skewed distribution

0.01% = >100 events!
Summary: Flow Cytometry, A Powerful Tool for CAR-T Trials

Cellular Kinetics

- AICD/Anergy
- Treg control - Yes/No?
- Memory – Yes/No?

Immune System Balance

- Tolerance-promoting milieu
  - TGFβ
  - IL-2, IL-2, Ig, IL-2/anti-IL-2 complex
  - retinoic acid
  - IL-10
  - anti-inflammatory agents (AAT, anti-IL-6, anti-TNFα)

- Rejection-promoting milieu
  - ischemia-reperfusion
  - infection
  - proinflammatory cytokines (IL-6, TNFα, IL-1β, IL-12)
Clinical pharmacology and Biopharmaceutics
Karen T. Mueller, PharmD., MS
Clinical Pharmacology of CTL019 in r/r pediatric and young adult ALL

• Objectives
  – Investigate and characterize the relationship of patient characteristics and clinical outcomes (efficacy/safety) with
    – CTL019 expansion, persistence
    – CTL019 dose
    – Tocilizumab impact on CTL019 cellular kinetics
    – Impact of immunogenicity on cellular kinetics and clinical response

• Two pediatric and young adult r/r ALL studies pooled
  – ENSIGN (n=29) and ELIANA (n=50)
  – Dose: Single infusion
    – >50 kg : 0.1 x 10^8 to 2.5 x 10^8 transduced viable T cells
    – <= 50 kg : 0.2 x 10^6 to 5.0 x 10^6 transduced viable T cells/kg
Responding Patients Have Rapid Expansion in Peripheral Blood and Bone Marrow with Persistence

**Peripheral Blood**

- **CTL019 transgene (copies/µg DNA)**
- **Elapsed time from infusion, mo**

**Bone Marrow**

- **CTL019 transgene (copies/µg DNA)**
- **Elapsed time from infusion, mo**

Data: ELIANA

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# Summary of CTL019 Cellular Kinetic Parameters in Responding and Non-Responding Patients

- Responding patients showed earlier and higher expansion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Statistics</th>
<th>Pooled data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CR/CRI N=62</td>
</tr>
<tr>
<td>AUC(_{0-28d}) (copies/µg DNA×days)</td>
<td>n</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Geo-mean (CV%)</td>
<td>318000 (177.8)</td>
</tr>
<tr>
<td></td>
<td>Fold change CR/CRI vs NR</td>
<td>2.0</td>
</tr>
<tr>
<td>C(_{max}) (copies/µg)</td>
<td>n</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Geo-mean</td>
<td>34700 (155.4)</td>
</tr>
<tr>
<td></td>
<td>Fold change CR/CRI vs NR</td>
<td>1.7</td>
</tr>
<tr>
<td>T(_{max}) (days)</td>
<td>n</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>9.91 (0.00764, 27.0)</td>
</tr>
<tr>
<td>T(_{last}) (days)</td>
<td>n</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Median (min, max)</td>
<td>102 (17.8, 380)</td>
</tr>
</tbody>
</table>

Data pooled from ELIANA AND ENSIGN.

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Selected Intrinsic and Extrinsic Characteristics do not Influence CTL019 Expansion

- Other factors (eg, ethnicity/race) showed no effect on expansion

Data pooled from ELIANA AND ENSIGN. Lower and upper whiskers extend to the most extreme points with 1.5*IQR of Q1 and Q3 respectively.
CTL019 Cells Continue to Expand and Persist After Administration of Tocilizumab

Data pooled from ELIANA AND ENSIGN.
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**CTL019 Expansion and Safety Events**

- Patients with higher CRS grades\(^1\) tend to have greater expansion (C\(_{\text{max}}\))

![Box plot diagram showing C\(_{\text{max}}\) values for different CRS grades.](image)

- Patients with neurological toxicity or cytopenias did not have differences in expansion

Lower and upper whiskers extend to the most extreme points with 1.5*IQR of Q1 and Q3 respectively.

Note: One patient with No CRS had a Cmax ~320,000 copies/ug, data not presented

Pooled data from ENSIGN and ELIANA

Relationship between maximal expansion and peak cytokines

A. Cmax of Transgene, copies/μg

B. Cmax of Transgene, copies/μg

Spearman $r = 0.4304$
$P = 0.0010$

Spearman $r = 0.63$
$P = 0.001$

Thudium Mueller, K et al  Blood 2017
CSF Penetration and Neurological Events in Study B2101J

3a. CTL019 Transgene, copies/μg

3b. CTL019 Transgene in CSF, at Day 28, copies/μg

Grade of Neurologic Events

n = 25
n = 11
n = 11

Thudium Mueller, K et al  Blood 2017
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Higher expansion and more severe CRS seen in patients with higher tumor burden at baseline

Thudium Mueller, K et al  Blood 2017

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**CTL019 Expansion and Dose**

- Across a wide range of doses, expansion and dose are independent.

\[ C_{\text{max}} = 54117.33 - 318.3 \times \text{CTL dose} \quad (r^2 = 0.001) \]

- **Product transduction efficiency**, cell viability and total T cells do not impact expansion and persistence.
# CTL019 Dose-Response Analysis

- High clinical response rates across all dose quartiles for patients ≤ 50 kg
- No apparent difference in response rates across dose quartiles for patients > 50 kg

<table>
<thead>
<tr>
<th>Response</th>
<th>Quartile 1 (Dose ≤2.0×10^6 N=20)</th>
<th>Quartile 2 (Dose: &gt;2.0×10^6 to ≤3.2×10^6 N=20)</th>
<th>Quartile 3 (Dose: &gt;3.2×10^6 to ≤4.5×10^6) N=20</th>
<th>Quartile 4 (Doses &gt;4.5×10^6 N=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 28 response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>5 (20.0)</td>
<td>10 (41.7)</td>
<td>5 (20.8)</td>
<td>5 (20.8)</td>
</tr>
<tr>
<td>CRi</td>
<td>12 (48.0)</td>
<td>8 (33.3)</td>
<td>17 (70.8)</td>
<td>12 (50.0)</td>
</tr>
<tr>
<td>No response</td>
<td>4 (16.0)</td>
<td>3 (12.5)</td>
<td>0</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (16.0)</td>
<td>3 (12.5)</td>
<td>2 (8.3)</td>
<td>5 (20.8)</td>
</tr>
<tr>
<td>Day 28 response rate (CR+CRi)</td>
<td>17 (68.0)</td>
<td>18 (75.0)</td>
<td>22 (91.7)</td>
<td>17 (70.8)</td>
</tr>
</tbody>
</table>
CTL019 Dose and Safety

- No apparent relationship between dose and grade 3 or 4 CRS
- No apparent relationship between dose and grade 3 or 4 neurological events or cytopenias

Data: ELIANA

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Tisagenlecleucel by B-Cell Recovery

Semi-logarithmic view

CTL019 transgene levels (copies/μg of genomic DNA)

Time (days)

Arithmetic means

B-cell recovery times

≤3 months

>3 and ≤6 months

>6 months

Sustained B-cell aplasia
Key dose-related findings with CTL019 from clinical trials

Based on dose range used in clinical trials

Efficacy

• Responses observed across entire dose range studied
• Response rates were similar across all dose quartiles
• Probability of response at lowest doses tested carries a favorable benefit-risk

Safety

• No impact of dose on CRS (all grades, Grade 3-4)
• No impact of dose on neurotoxicity or cytopenias
Humoral Immunogenicity: Impact of Pre-existing and Treatment Induced Anti-mCAR19 Antibodies on DOR

- Pre-existing and treatment induced immunogenicity do not impact the DOR or EFS

DOR, duration of response.

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CTL019 Summary of Clinical Pharmacology Results (ELIANA and ENSIGN)

- Across a wide dose range, CTL019 exhibits high efficacy rates in children and young adults with ALL
- No relationship between CTL019 dose and safety (CRS, neurologic, cytopenias)
- High expansion and long term persistence (>1 year) of transgene observed in responder patients
- Higher grade CRS is associated with greater expansion
- No impact of selected intrinsic or extrinsic factors on CTL019 cellular kinetics
- CTL019 cells continue to expand and persist after administration of tocilizumab
- No impact of pre-existing or treatment induced anti-mCAR19 antibodies on cellular kinetics, response rates or EFS
- No relationship between cellular kinetics and select manufacturing attributes

Conclusion

- Cellular kinetics support the CTL019 dose range, safety and efficacy profile in pediatric and young adult patients with ALL
Acknowledgements

• All of the patients, families, physicians and medical staff that contributed to the CTL019 program

• Clinical teams, scientists and manufacturing staff supporting the ELIANA and ENSIGN trials