Severe Neurotoxicity in the Phase 2 Trial of JCAR015 in Adult B-ALL (ROCKET Study): Analysis of Patient, Protocol and Product Attributes

Mark J. Gilbert, MD
Chief Medical Officer, Juno Therapeutics, Inc.

This presentation will focus on the investigation of severe neurotoxicity
Comprehensive clinical study data available at 2017 SITC Poster P217
Presenter Disclosure Information

Mark J. Gilbert, MD

The following relationships exist related to this presentation:

Juno Therapeutics, Inc, Employee
Juno Therapeutics Inc, Ownership
Objectives

• ROCKET Phase 2 Trial Background
• Review the Neurotoxicity Events from ROCKET Phase 2 Trial
• Review the Pathology & PK Findings from ROCKET Phase 2 Trial
• Exploratory Analyses between Fatal Neurotoxicity & CMC/Clinical Data
• Lessons Learned for Cell Therapy Field Moving Forward
JCAR015: CD19-CAR T Cell Product Candidate in Phase 2

Construct & Process Based on Phase 1 MSKCC CAR T Cell Product 19-28z

**CAR CONSTRUCT**

- CD19 Expressing Cell
- CD19 Epitope
- SJ25C1-like binding domain
- CD28 domain
- CD3ζ domain

**PHASE 1 vs PHASE 2 PROCESS**

**MSK 19-28z Ph1 Process**
- SELECTION & ACTIVATION
- Immuno-magnetic beads
- RETROVIRAL TRANSDUCTION
- Institution-sourced vector
- EXPANSION
- ~9 Day Culture
- CRYOPRESERVATION
- In fixed volume

MSK process using raw materials sourced for Ph 1

**JCAR015 Ph2 Process**
- SELECTION & ACTIVATION
- Immuno-magnetic beads
- RETROVIRAL TRANSDUCTION
- Commercially-sourced vector
- EXPANSION
- ~9 Day Culture
- CRYOPRESERVATION
- In fixed volume

Juno process using commercially sourced raw materials

**Patient Apheresis Material**

**CAR T Cell Drug Product**

SJ25C1-like binding domain
CD3ζ domain
T cell
CD28 domain
CD19 Epitope
Highlights of ROCKET Phase 2 Trial Design

Dosing & Lymphodepletion Based on Phase 1 Single Institution Trials at MSKCC & FHCRC

<table>
<thead>
<tr>
<th>Stage</th>
<th>Product Manufacture</th>
<th>Apheresis</th>
<th>Restage</th>
<th>Morphologic (≥5% blasts on BM)</th>
<th>Follow-up Efficacy &amp; Safety Evaluations</th>
</tr>
</thead>
</table>

**Low Dose**
- (1 × 10⁶ CAR+ cells/kg)

**High Dose**
- (3 × 10⁶ CAR+ cells/kg)

14-42d

Lymphodepletion regimen:
- 1-3 g/m² CY
- or CY/FLU 30-60 mg/kg × 1
- & 25 mg/m² × 3 days

1° Endpoint = Confirmed complete remission ± hematological recovery at Day 28 post last JCAR015
(Study met 1° endpoint with 47% rate; see poster P217 for more details)

**Key Inclusion Criteria**
- ≥ 18 years
- Relapsed or refractory morphological (> 5% blasts in BM) CD19 positive disease
- 1st salvage or greater [incl. post Allo HSCT]
- ECOG 0-2
- Prior blinatumomab permitted

**Key Exclusion Criteria**
- Isolated extramedullary disease, Burkitt’s
- Active CNS involvement with disease (CNS3) or CNS pathology
- Active GvHD
- Active infection
- Prior gene therapy

### Neurotoxicity Shift Observed During Phase 2 ROCKET Trial

**Outcomes for 32 Patients Treated With Morphological Disease (Total Treated = 381)**

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Patients, N</th>
<th>CR/CRi rate</th>
<th>Severe CRS Grade 3/4</th>
<th>Severe NTX Grade 3-5</th>
<th>Treatment Related Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY</td>
<td>14</td>
<td>6/14 [43%]</td>
<td>1/14 [7%]</td>
<td>3/14 [21%]</td>
<td>Grade 5 events: 0</td>
</tr>
<tr>
<td>Flu/CY</td>
<td>8</td>
<td>5/5 [100%]; 3 NE</td>
<td>3/8 [38%]</td>
<td>7/8 [88%]</td>
<td>Grade 5 events: 3 [all NTX events]</td>
</tr>
<tr>
<td>CY post-hold</td>
<td>10</td>
<td>4/8 [50%]; 2 NE</td>
<td>3/10 [30%]</td>
<td>8/10 [80%]</td>
<td>Grade 5 events: 2 [both NTX events]</td>
</tr>
<tr>
<td>CY or Flu/CY</td>
<td>31</td>
<td>23/30 [77%]; 1 NE</td>
<td>13/31 [42%]</td>
<td>11/31 [35%]</td>
<td>Grade 5 events: 3 [no NTX events: 2 sepsis, 1 CRS]</td>
</tr>
</tbody>
</table>

**ROCKET 15001 Phase 2**

**MSKCC 09-114 Phase 1**

- **Data reported across various CAR T trials include at least 15 cases of grade 5 neurotoxicity and at least 13 cases of cerebral edema**
- **Cases using CAR T cells with different binders and costimulatory domains, different manufacturing, in different diseases**

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6 additional patients treated were MRD+ at time of treatment. CY, cyclophosphamide; Flu, fludarabine; NE, not evaluable. Sources for MSKCC 09-114: ASCO 2016 presentations; literature search as of October 18, 2017. ROCKET, CR/CRi based on independent review committee (IRC).
Pathophysiology: Autopsy Cases Show BBB Breakdown

• Grade 5 cerebral edema (n = 2):
  - BBB breakdown
    - Endothelial damage
    - Astrocyte damage
    - Microglial activation

  - No significant edema in peripheral tissues [lung, liver, kidney]

• Autopsy findings in phase 1 non-cerebral edema cases (n = 2):
  - T cells present in CNS
  - No evidence of BBB pathology

<table>
<thead>
<tr>
<th>Key Findings in CNS</th>
<th>Pertinent Negatives in CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBB breakdown</td>
<td>No immune cells/leukemia</td>
</tr>
<tr>
<td></td>
<td>No CAR T cells</td>
</tr>
<tr>
<td></td>
<td>No innate immune cells</td>
</tr>
<tr>
<td></td>
<td>No B-ALL, CD19+ cells</td>
</tr>
</tbody>
</table>

- H&E
- Cleaved Casp 3
- GFAP
- CD163
- CD3
- CD68

Perivascular extravasation
Endothelial damage
Astrocyte damage
Microglial activation
Immune cells
Early Peak CAR T Cell Expansion Correlated With Fatal NTX

Serum IL-15 Levels Rose Faster in Those With Early Expansion, and Levels Fell Rapidly With Cell Expansion

Note: Each dot represents the maximum PK measurement for an individual subject and is color coded to show the highest grade of NTX observed. Boxed dots represent median IL-15 levels for group with range bars.
*Prolonged Grade 3 defined as Grade 3, >10 days duration
Exploratory CMC & Clinical Analysis Methodology

• Descriptive, graphical, nonparametric and model-based analyses

• CMC attributes/process variables examined (n ≈ 140) in univariate and multivariate analyses using nonparametric tests and partition-based (decision tree) methods

• Clinical/translational variables examined (n ≈ 500) in univariate and multivariate analyses using nonparametric tests and logistic regression models

• Results should be interpreted as exploratory due to ad hoc nature, lack of power, high-level of multiplicity, and potential confounding variables
Univariate Logistic Regression of CMC Variables Identified CD8 Dose & Cytokine Expression Correlated With Cerebral Edema

<table>
<thead>
<tr>
<th>Attribute Category</th>
<th>Attribute</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose</strong></td>
<td>Annexin V− CD8+CAR+ dose(^1)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td></td>
<td>Annexin V− CD8+CAR+ dose/kg(^1)</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Annexin V− CD3+CAR+ dose(^1)</td>
<td>.012</td>
</tr>
<tr>
<td></td>
<td>Annexin V− CD3+CAR+ dose/kg(^1)</td>
<td>.015</td>
</tr>
<tr>
<td><strong>Function(^2)</strong></td>
<td>% of CD4+CAR+ producing IL-2</td>
<td>.019</td>
</tr>
<tr>
<td></td>
<td>% of CD8+CAR+ producing TNFα</td>
<td>.044</td>
</tr>
<tr>
<td></td>
<td>% of CD4+CAR+ producing TNFα</td>
<td>.044</td>
</tr>
</tbody>
</table>

No association was identified between fatal NTX and T cell differentiation state or other phenotypes

\(^1\) Dose calculation based on viable CAR T cells excluding Annexin V expressing cells; Phase 2 dose calculated based on dye exclusion viability that were not sufficiently sensitive to demonstrate correlation.

\(^2\) 0.25 × 10⁶ cryopreserved CD3+CAR+ cells, 18hrs, E:T Ratio 1:1.
Combination of Total Non-Apoptotic CD8+CAR+ Dose With Antigen-Specific T Cell Function Shows Wide Product Variability that Partitions With Cerebral Edema

1 $0.25 \times 10^6$ cryopreserved CD3+CAR+ cells, 18hrs, E:T Ratio 1:1. Severity 3P, grade 3 lasting > 10 days.
Clinical Factors Associated With Cerebral Edema

Associations Identified Potentially Impacts Product Attributes or Patient Factors at Infusion

<table>
<thead>
<tr>
<th>Demographics/Treatment History</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
<th>Lower</th>
<th>Higher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Received Flu/Cy LD</td>
<td>7.25 (1.14, 53.98)</td>
<td>0.045</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Received high-intensity bridging chemo</td>
<td>4.68 (0.63, 32.64)</td>
<td>0.133</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt;30 years</td>
<td>5.16 (0.83, 55.93)</td>
<td>0.112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 prior lines of therapy</td>
<td>7.24 (0.72, 980.23)</td>
<td>0.208</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No association with higher risk for prior CNS irradiation, prior IT chemo, prior CNS disease, prior AlloTx, higher ECOG performance status, prior blinatumomab
The Relative Risk of Fatal Neurotoxicity (Cerebral Edema) Associated With Both Product and Patient/Clinical Characteristics

**Product Impacts**

Clinical Factors Impacting Dose and/or Function
- Fewer prior therapies
- Young age
- Apheresis CD4:CD8 ratio
- Weight-based dosing of cells

CMC Factors Impacting Variability
- Drug product cell concentration
- Lot-to-lot raw material variability
- Storage/handling of raw materials

**Product Independent Impacts**

Patient Factors Amplifying Expansion
- Disease type
- Disease burden
- Bridging chemotherapy
- Flu/CY lymphodepletion
- CAR T growth factors (IL-15)

Relative Risk of Fatal Neurotoxicity
Lessons Learned for CAR T Therapy

A Three-Pronged Approach to Better Define and Potentially Reduce Risks

1. A defined composition product to reduce product variability and risk
2. Deeper understanding of key patient and clinical factors associated with risk
3. Better pre-clinical models to predict risk and identify targeted interventions
Key Characteristics of Defined Composition Product

Controlling Dose & Variability in Product Manufacturing Platform

Characteristics of Defined Composition CAR

Control CD8 Dose
- Implement flat dosing
- Independent control of CD8 [and CD4] cell doses
- Using small volume closure to control cell concentration
- Optimize cell culture engineering practices

Control T Cell Functional Variability
- Analyze potency of CD4 & CD8 separately
- Control lot-to-lot variability of critical raw materials
- Process controls during cell expansion
- Change potency matrix to increase product control
- Using 4-1BB costimulatory domain may have impact

Note: Panel scales are not intended to reflect the same units given assay differences.
Defining Risk Factors Independent of Product Attributes is Key

Need defined composition product & precise dose to define risks across diseases & line therapy

<table>
<thead>
<tr>
<th>Baseline Factor</th>
<th>Indication</th>
<th>Criterion</th>
<th>Gr ≥ 3 NTX</th>
<th>P Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory Marker: Serum IL-15</td>
<td>B-cell malignancies</td>
<td>high IL-15</td>
<td>26/45 (58%)</td>
<td>&lt; .0001</td>
<td>ROCKET Ph2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>low IL-15</td>
<td>19/109 (17%)</td>
<td></td>
<td>FHCRC Ph1</td>
</tr>
<tr>
<td>Platelet Count</td>
<td>B-cell malignancies</td>
<td>&lt; 120K</td>
<td>60/162 (37%)</td>
<td>.002</td>
<td>ROCKET</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥ 120K</td>
<td>9/60 (15%)</td>
<td></td>
<td>FHCRC Ph1, MSKCC Ph1</td>
</tr>
<tr>
<td>Disease burden</td>
<td>ALL</td>
<td>≥ 5% BM blasts</td>
<td>40/94 (43%)</td>
<td>.02</td>
<td>ROCKET Ph1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 5% BM blasts</td>
<td>9/42 (21%)</td>
<td></td>
<td>FHCRC Ph1, MSKCC Ph1</td>
</tr>
<tr>
<td>Disease Type</td>
<td>B-cell malignancies</td>
<td>ALL</td>
<td>14/47 (30%)</td>
<td>.03</td>
<td>FHCRC Ph1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NHL</td>
<td>8/62 (13%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene Expression Signature</td>
<td>ALL</td>
<td>non-Ph’</td>
<td>12/15 (80%)</td>
<td>.03</td>
<td>ROCKET</td>
</tr>
<tr>
<td></td>
<td></td>
<td>non-Ph’-like</td>
<td>6/16 (38%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lesson Learned for Cell Therapy Across Disease States

De-Risking With Pre-clinical Models to Identify Targeted Interventions

Syngeneic Mouse Model of CAR-Related CRS and Neurotoxicity

RNASEq of brain tissue

300+ genes are differentially expressed in the brain compared to controls

Upregulation of selected genes families in the brain associated with:
- Immune response & inflammation
- Microglia activation
- Antigen processing and presentation
- Cell migration
- Endothelial activation
- Angiogenesis
- Nitric oxide signaling pathway
- Oxidative stress & anti-oxidant defense

Chadwick E, et al. SITC 2017 [abstract P327].
Summary & Conclusions

- Cerebral edema associated with early & rapid CAR-T cell expansion & rise in IL-15 levels

- Cerebral edema associated with endothelial damage & complete BBB breakdown
  - Not associated with CAR-T cell infiltration, CNS leukemia or prior CNS leukemia therapies

- Exploratory analyses: multiple factors were associated with cerebral edema, requiring both:
  - Clinical characteristics, including: young age (< 30 yrs), 2 or fewer prior regimens, intensive bridging chemotherapy and use of high intensity Flu/CY, and
  - CMC variables, including: drug product cell concentration, lot-to-lot raw material variability, and storage/handling of raw materials

- Lessons learned for CAR T therapy
  - Use of defined composition product to reduce variability in dose & function
  - Defining patient risk factors of overall risk as CAR-T therapies move into additional diseases & earlier treatment lines – potentially subsets of disease (eg, Ph’-like ALL)
  - Identify & evaluate potential targeted interventions to better manage toxicities
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