Clinical Applications of Chimeric Antigen Receptor (CAR) – T Cell Therapy in Hematologic Malignancies

Valkal Bhatt PharmD. BCOP, BCPS
Clinical Specialist – Stem Cell Transplant
Memorial Sloan Kettering Cancer Center
New York, NY
Bhattv@mskcc.org
Disclosure:
Nothing to disclose
Objectives

• Describe the rationale and current status of CD-19 CAR therapy in management of hematologic malignancies

• Summarize the features and management of toxicities associated with treatment

• Evaluate the rationale and applications of CD-19 CAR therapy in hematopoietic cell transplant (HCT)

• Discuss current limitations and future directions of CAR therapy
Rationale of Adoptive Immunotherapy

- Cancer cells are constantly escape immune mediated clearance and apoptosis

- Immune mediated clearance of tumor cells has been extensively studied as a mode of cancer treatment

- Autologous adoptive T-cells engineered to express CAR is emerging as a powerful treatment option for chemotherapy refractory hematologic malignancies

Current Adoptive Immunotherapy Treatment Options

History of Adoptive T – Cell Transfer

- Concept of adoptive cellular therapy first reported in mouse models in 1955\(^1\)

- CAR engineering first described 25 years ago as a means of introducing tumor specificity into adoptive cell therapy\(^2\)

- First CARs showed evidence of function in-vitro but had limited response in clinical trials due to poor persistence\(^3\)

- Newer CAR models with additional costimulatory domains have shown impressive results clinical studies

Rationale for CAR-T Cell Engineering

- To overcome immune tolerance
- Circumvent HLA down regulation
- Target both CD4+ and CD8+ T cells to tumor cells
- Broaden T cell reactivity to carbohydrates & glycolipids
- Potential to target cancer stem cells
- Augment T cell potency
- Control T cell longevity
- Exploit alternative (nonautologous) T cell sources

Sadelain M.  J Clin Invest. 2015;125(9):3392-3400
Background: The Chimeric Antigen Receptor

- Initially consisted of variable region of monoclonal antibody & constant region of t-cell receptor (TCR) α & β chains

- Modern design includes ecto domain, hinge, transmembrane domains & several signaling domains

CAR T-Cell Engineering

Mab: Monoclonal antibody
TCR: T cell receptor
ScFv: single chain variable fragment
CAR T-Cell Engineering

First Generation CAR

Second Generation CAR

Third Generation CAR

Cytotoxicity

Proliferation/cytokine production

Survival


VH: Heavy chain variable region
VL: Light chain variable region

Memorial Sloan Kettering Cancer Center
CAR T-Cell Manufacture

Gene Transfer
- Retroviral or Lentiviral or Transposon

Expansion and Stimulation
- Signal 1: anti-CD3 antibody or cognate CAR signal or cognate TCR signal
- Signal 2: agonistic anti-CD28 antibody or anti-41BB antibody
- Signal 3*: exogenous cytokines (IL-2, IL-7, IL-12, IL-15)

Gene Transfer Methodology

Retroviral Vector

Lentiviral Vector

Sleeping Beauty

RNA

Role of lymphodepletion

- Promote homeostatic proliferation
- Depletion of cytokine “sinks”
- Depletion of regulatory T Cells (Tregs)
- Reduction in myeloid derived suppressor cells
- Reduction in dendritic cell access to APC

CD-19 CAR for B cell malignancies

- Expression is restricted to B cells & follicular dendritic cells
- CD19 is not expressed on pluripotent bone marrow stem cells
- CD19 is expressed on the surface of most B cell malignancies
- Antibodies against CD19 inhibit growth of tumor cells

CD-19 CAR - From Bench to Bedside

- Efficacy
  - CLL
  - ALL
  - NHL

- Toxicity

- Where to Improve?
CD-19 CAR in CLL

- Conventional chemo-immunotherapy remains standard of care
- Older patients & those with high risk features have inferior outcomes
- Newly approved agents rarely result in durable complete remissions
- Allogeneic SCT considered potentially curative but associated with high NRM and relapse
- More effective therapies for advanced & high risk CLL are necessary

# CD-19 CAR in CLL – CTL-019

<table>
<thead>
<tr>
<th></th>
<th>CTL-019 (pilot)</th>
<th>CTL-019 (Phase II)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>14</td>
<td>26</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>Rel/Ref: 43% P53, median 5 prior therapies</td>
<td>Rel/Ref: 38% P53, median 3 prior therapies</td>
</tr>
<tr>
<td><strong>CAR Construct</strong></td>
<td>CD-3z &amp; 4-1BB</td>
<td>CD-3z &amp; 4-1BB</td>
</tr>
<tr>
<td><strong>Vector</strong></td>
<td>Lentiviral</td>
<td>Lentiviral</td>
</tr>
<tr>
<td><strong>T cell dose</strong></td>
<td>0.14 – 11.3 x 10^8</td>
<td>5 x 10^7 vs. 5 x 10^8</td>
</tr>
<tr>
<td><strong>Lymphodepletion</strong></td>
<td>Bendamustine, Flu/Cy, Pentostatin/Cy</td>
<td>All Patients</td>
</tr>
<tr>
<td><strong>ORR</strong></td>
<td>8/14 (57%) @ 19 mo (r 6-53)</td>
<td>9/23 (39%) @ 7.3 mo</td>
</tr>
<tr>
<td><strong>CR</strong></td>
<td>4/14 (28%) @ 19 mo (r 6-53)</td>
<td>5/23 (22%) @ 7.3 mo</td>
</tr>
<tr>
<td><strong>Toxicities</strong></td>
<td>• Fever, TLS</td>
<td>• 14/26 (54%) CRS</td>
</tr>
<tr>
<td></td>
<td>• 9/14 (64%) CRS (med 7d {9-14})</td>
<td></td>
</tr>
</tbody>
</table>

CTL-019 CLL Pilot - PFS and OS

Median 8.1 mo
Range (0.9-52.9)

Median 19.2 mo
Range (6.1-52.9)

# CD-19 CAR in CLL – CD19-28z CAR

<table>
<thead>
<tr>
<th>MSKCC – CD19-28z CAR (Relapse/Refractory)</th>
<th>MSKCC -- CD19-28z CAR (First line consolidation)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>8</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>Rel/Ref: 25% P53, 50% del11q Median 2.5 prior therapies (1-4)</td>
</tr>
<tr>
<td><strong>CAR Construct</strong></td>
<td>CD19-28z</td>
</tr>
<tr>
<td><strong>Vector</strong></td>
<td>Retroviral</td>
</tr>
<tr>
<td><strong>T cell dose</strong></td>
<td>0.4-3.0 x 10⁷ 19-28z T cells/kg</td>
</tr>
<tr>
<td><strong>Lymphodepletion</strong></td>
<td>N = 4 none, n = 4 Cy</td>
</tr>
<tr>
<td><strong>ORR</strong></td>
<td>2/8 SD (* no response in non Cy group)</td>
</tr>
<tr>
<td><strong>CR</strong></td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Toxicities</strong></td>
<td>Fevers, rigors, chills, hypotension, CRS</td>
</tr>
</tbody>
</table>


SD: stable disease
PCR: Pentostatin/Cyclophosphamide/Rituximab
CD-19 CAR Therapy in B-ALL
CD-19 CAR in B-ALL -- CTL-019

Detection of CTL019+ cells in Peripheral Blood

Levels of CTL019+ DNA in Peripheral Blood

100,000x in-vivo proliferation

Time to first negative CTL019 in peripheral Blood & Bone marrow

68% persistence @ 6months

# CD-19 CAR in B-ALL -- CTL-019

<table>
<thead>
<tr>
<th></th>
<th>CTL-019 – Rel/Ref Pediatric ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>53</td>
</tr>
<tr>
<td>Population</td>
<td>41/53 (77%) detectable ALL</td>
</tr>
<tr>
<td></td>
<td>(12 pts MRD -) on BMBx 1 day before infusion after lymphodepletion</td>
</tr>
<tr>
<td>CAR Construct</td>
<td>CD-3z &amp; 4-1BB expanded with anti CD3/CD28 via lentiviral vector</td>
</tr>
<tr>
<td>T cell dose</td>
<td>$4.3 \times 10^6$ CTL019 cells/kg (1-17.4x10^6/kg)</td>
</tr>
<tr>
<td>CR</td>
<td>50/53 (94%) { 45/50 (90%) MRD neg at D28 by flow}</td>
</tr>
<tr>
<td>RFS</td>
<td>6mo: 72% (95% CI 59-87%)</td>
</tr>
<tr>
<td></td>
<td>12mo: 44% (95% CI 30-65%)</td>
</tr>
<tr>
<td>OS</td>
<td>78% at 6 &amp; 12 months (95% CI 67-91%)</td>
</tr>
<tr>
<td>Relapse</td>
<td>20 pts in CR at 1 month have relapsed</td>
</tr>
<tr>
<td></td>
<td>• 13 pts with CD-19 neg disease</td>
</tr>
<tr>
<td>Toxicities</td>
<td>• 48/53 (90%) patients developed grade 1-4 CRS</td>
</tr>
<tr>
<td></td>
<td>• 28% treated with Tocilizumab (9 patients required steroids)</td>
</tr>
<tr>
<td></td>
<td>• Encephalopathy: 13/30 (43%) – seizures, aphasia, delirium.</td>
</tr>
<tr>
<td></td>
<td>• B cell aplasia – persistent for 3-39 months</td>
</tr>
</tbody>
</table>

CD-19 CAR in B-ALL – JCAR015

- **Leukapheresis**
- **T cell production**
- **Salvage Chemo**
- **Lymphodepletion**
- **BMBx**
- **Cy → Flu + Cy**
- **19-28z CAR T cells (2 dose levels)**
- **Disease Assessment**

### Disease status

<table>
<thead>
<tr>
<th>Disease status</th>
<th>CAR T Cell Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphologic Disease (≥5% blasts in bone marrow or extmedullary disease)</td>
<td>$1 \times 10^6$ CAR T cells/kg</td>
</tr>
<tr>
<td>Minimal Disease (≤5% blasts in bone marrow)</td>
<td>$3 \times 10^6$ CAR T cells/kg</td>
</tr>
</tbody>
</table>

**CD-19 CAR in B-ALL – JCAR015**

<table>
<thead>
<tr>
<th></th>
<th>JCAR015 – Rel/Ref Adult ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>46</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td></td>
</tr>
<tr>
<td>• n = 24 m (74%) with Median age 45 (22-75)</td>
<td></td>
</tr>
<tr>
<td>• n = 18 (39%) with prior Allogenic HCT</td>
<td></td>
</tr>
<tr>
<td>• n = 25 (54%) with morphologic disease (median 63%, 10-100%)</td>
<td></td>
</tr>
<tr>
<td>• n = 14 (30%) Philadelphia Chromosome (Ph)+</td>
<td></td>
</tr>
<tr>
<td><strong>CAR Construct</strong></td>
<td>CD19 – CD28z via retroviral vector</td>
</tr>
<tr>
<td><strong>T cell dose</strong></td>
<td>1 - 3 x 10⁶ CD19-CD28z CAR T cells/kg</td>
</tr>
<tr>
<td><strong>CR</strong></td>
<td>37/45 (82%) { 30/36 (83%) MRD neg at D28 by flow}</td>
</tr>
<tr>
<td>• Morphologic disease (&gt;5% blasts: 18/24 (75%))</td>
<td></td>
</tr>
<tr>
<td>• Minimal disease (&lt;5% blasts: 19/21 (90%))</td>
<td></td>
</tr>
<tr>
<td><strong>Median time to CR</strong></td>
<td>21 days (8-46)</td>
</tr>
<tr>
<td><strong>Relapse</strong></td>
<td>N = 18 patients relapsed</td>
</tr>
<tr>
<td>• 4/18 relapses occurred post CAR-T allo HCT</td>
<td></td>
</tr>
<tr>
<td>• 3/18 relapses were CD19 negative</td>
<td></td>
</tr>
</tbody>
</table>

CD-19 CAR in B-ALL – JCAR015

CRS and Neurological Toxicities

<table>
<thead>
<tr>
<th></th>
<th>Severe CRS*</th>
<th>Grade 3/4 Neurotoxicity</th>
<th>Grade 5 toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>11 (24%)</td>
<td>13 (28%)</td>
<td>3 (6%)§</td>
</tr>
<tr>
<td>Disease Burden</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphologic disease (n=25)</td>
<td>11(44%)</td>
<td>10 (40%)</td>
<td></td>
</tr>
<tr>
<td>MRD (n=21)</td>
<td>0 (0%)</td>
<td>3 (14%)</td>
<td></td>
</tr>
</tbody>
</table>

* Requiring Vasopressors and/mechanical ventilation for hypoxia

§ All pts received higher dose (3 x 10⁶ CAR T cells/Kg)

- n = 2 sepsis/multi organ failure
- n = 1 seizure, but unknown cause of death

CD-19 CAR in B-ALL – JCAR015

Overall Survival

All Patients
Median OS: 9 months
OS at 6 mos: 65% (47-78)

CR Patients
Median OS: 10.6 months
OS at 6 mos: 71% (51-84)

Survival by MRD Status

MRD – CR
Median OS: Not reached
OS at 6 mos: 80% (57-91)

MRD + CR
Median OS: 6 months
OS at 6 mos: 43% (10-73)

Conclusions CD-19 CAR in B-ALL

- Impressive CR rates seen in rel/ref B-ALL
  - CTL019: 94% (90% MRD neg) – Children
  - JCAR-015: 82% (83% MRD neg) – Adults
  - NCI CD19: 60% overall – Children & young adults

- Relapses still a concern – particularly CD19 negative relapses
  - CTL019: 38%
  - JCAR015: 39%

- MRD negativity seems to improve outcomes
- High rates of CRS & neurologic toxicities

- ROCKET: Multi Center Phase II trial of JCAR015 in adults with Ref B- ALL ongoing (NCT02535364)

- JCAR017: A Pediatric and Young Adult Trial of Genetically Modified T Cells Directed Against CD19 for Relapsed/Refractory CD19+ Leukemia ongoing (NCT02028455)

- ZUMA-4: Multicenter phase II trial to study safety and efficacy of KTE-C19 (CTL019) in pediatric rel/ref B-ALL ongoing (NCT02228096)

- CTL019 Multicenter phase II trial to study safety and efficacy in Adult rel/ref B-ALL ongoing (NCT02167360)
CD-19 CAR in NHL – NCI Pilot

NCI Anti CD-19 CD28-CD3z via gamma retroviral vector
N= 9 with rel/ref DLBCL

↓

Cy (60-120mg/kg)/flu 30mg/m²
lymptodepletion
↓

infusion of anti-CD19 CAR-T cells at dose 1 x
10⁶ T-cells/kg
↓

7 evaluable pts w/ DLBCL/PMBCL:
4 CR, 2 PRs, 1 SD (1-22 months)

Significant Grade 3/4 CRS and neurologic toxicity

NCI Anti CD-19 Pilot Expansion
N= 9 with rel/ref DLBCL

↓

Cy (300mg/m²)/flu 30mg/m²
lymptodepletion
↓

infusion of anti-CD19 CAR-T cells at dose 1 x
10⁶ T-cells/kg
↓

8 evaluable pts w/ chemo-refractory/<1 year relapse post aHCT
1 CR, 4 PRs

## CD-19 CAR in NHL – CTL019 Phase II

<table>
<thead>
<tr>
<th>CTL-019 Rel/Ref  DLBCL, FL or MCL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
</tr>
<tr>
<td><strong>Population</strong></td>
</tr>
<tr>
<td>• Median Prior therapies:</td>
</tr>
<tr>
<td>• History of HCT:</td>
</tr>
<tr>
<td>• Ann arbor: stage III</td>
</tr>
<tr>
<td>• BM involvement:</td>
</tr>
<tr>
<td><strong>CAR / dose</strong></td>
</tr>
<tr>
<td><strong>Lymphodepletion</strong></td>
</tr>
<tr>
<td><strong>ORR (n = 22)</strong></td>
</tr>
<tr>
<td>DLBCL 54% (7/13); FL 100%(7/7); MCL 50%(1/2)</td>
</tr>
<tr>
<td><strong>PFS</strong></td>
</tr>
<tr>
<td><strong>Toxicities</strong></td>
</tr>
<tr>
<td>• CRS occurred in 16 pts</td>
</tr>
<tr>
<td>• n = 14 grade II; n =1 grade III, n=1 grade IV</td>
</tr>
<tr>
<td>• Neurologic Toxicity:</td>
</tr>
</tbody>
</table>

Rad/Cy: Radiation/ cyclophosphamide  
Benda: bendamustine  
Understanding Toxicities Associated with CD-19 CAR therapy
Predictors of CRS – Disease Burden

Predictors of CRS – CRP

CRP post JCAR019

ROC - CRP & sCRS development

AUC=0.968

### Clinical Signs and symptoms of CRS

<table>
<thead>
<tr>
<th>Organ System</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutional</td>
<td>Fever, rigors, malaise, fatigue, anorexia, myalgias, arthralgias, headache</td>
</tr>
<tr>
<td>Skin</td>
<td>Rash</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Nausea, vomiting, diarrhea</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Tachypnea, hypoxemia</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Tachycardia, hypotension</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Elevated D-dimer, hypofibrinogenemia, bleeding</td>
</tr>
<tr>
<td>Renal</td>
<td>Azotemia</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Transaminitis, Hyperbilirubinemia</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Mental status changes, confusion, delirium, aphasia, tremor, altered gait, seizure</td>
</tr>
<tr>
<td>Other</td>
<td>Hemophagocytic lymphohistiocytosis</td>
</tr>
</tbody>
</table>
CRS management Strategies

Does the patient have morphologic residual disease?

No

Monitor 48 hours post CAR T Cells and discharge home if no fevers or other complications

Yes

Does the patient have fevers for at least 2 days?

Yes

Start Levetiracetam

Other Clinical Signs of CRS present?

If CRP ≥ 20mg/dL consider ICU transfer

Grade 1 CRS
Fever, Constitutional Symptoms

Grade 2 CRS
Hypotension: responds to fluids or one low dose pressor
Hypoxia: responds to < 40% O₂
Organ toxicity: grade 2

Grade 3 CRS
Hypotension: requires multiple or high dose pressors
Hypoxia: requires ≥ 40% O₂
Organ toxicity: grade 3 or grade 4 transaminitis

Grade 4 CRS
Mechanical ventilation
Organ toxicity: grade 4 (excluding transaminitis)

• Vigilant supportive care
• Antipyretics, analgesics, adequate hydration, blood pressure support. Broad-spectrum antibiotics

Extensive comorbidities or older age?

NO

• Vigilant supportive care

YES

• Vigilant supportive care
• Tocilizumab 4-8mg/kg
• ± Corticosteroids (dexamethasone 10mg q12 Or Methylprednisolone 2mg/kg /day x 2-4 days)

* Consider monitoring daily Ferritin, CRP, LDH

Encephalopathy

- All groups have reported neurotoxicity after CAR T – Cell infusion
- Significant CSF CAR T cell infiltration reported
- Ranges from confusion to seizures requiring mechanical ventilation
- Mechanism of toxicity is unknown
- IL-6 blockade does not prevent development

B- Cell Aplasia

- An expected on-target toxicity of successful CD19 CAR-T cell therapy
- Provides pharmacodynamic marker of CAR persistence
- IVIg replacement mitigates risk of infectious complications

Role of CD-19 CAR therapy in HSCT
Potential role of CD-19 CAR therapy in HCT

• As a bridge to allogeneic HCT
• Treatment at relapse
• As part of donor lymphocyte infusion
• aHCT consolidative therapy for relapse prevention
CD-19 CAR as Bridge to HSCT

JCAR015
N = 46
Rel/Ref Adult ALL

13/37 (35%) achieving CR
Underwent allogeneic HCT
(2pts – 2nd Allo HcCT)

CD3-CD28z CAR T – Cell

Overall Survival:
By HSCT Status Post CAR T Cells – MRD-CR Patients
Time Since CAR T Cell Infusion (Months)

## CD-19 CAR for treatment of Relapse

The table below summarizes the data from the study:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age (range)</td>
<td>45 (25-63)</td>
</tr>
<tr>
<td>Disease Burden</td>
<td></td>
</tr>
<tr>
<td>Morphologic</td>
<td>12 (66.7%)</td>
</tr>
<tr>
<td>Minimal</td>
<td>6 (33.3%)</td>
</tr>
<tr>
<td>≥ 4 lines of prior therapy</td>
<td>10 (55.6%)</td>
</tr>
<tr>
<td>MRD negative CR</td>
<td>11/12 (91.7%)</td>
</tr>
<tr>
<td>Median OS, months (95% CI)</td>
<td>NR (3.1-NR)</td>
</tr>
<tr>
<td>2nd Allo HCT</td>
<td>2/13 (15%)</td>
</tr>
</tbody>
</table>

**References:**

CD-19 CAR as DLI post Allogeneic HSCT

20 patients CD19 + B-cell malignancies with persistent disease post allogeneic HCT

- Median peak blood CAR T-cell: 39 CAR+ cells/mL in responders vs. 2 CAR+ cells/mL in non responders (P=0.001).

- Higher CD8:CD4CAR+ T cell ratio at the time of peak CAR T-cell level in responders (P=0.007).

- ORR: 8/20 (40%)
  - 6 CR, 2 PR

- 4/5 ALL pts. had MRD – CR

- Longest CR – CLL (1pt – 30+ mo)
  - 1pt Ph+ALL – 15+mo MRD-CR

- NO Acute GVHD seen

- Toxicities: Fever, tachycardia, Hypotension

N = 5 CLL
N = 5 DLBCL
N = 5 MCL
N = 5 ALL
N = 13 MRD
N = 5 MUD
N = 2 MMUD

CD3-CD28 CAR T – Cell
0.4 – 8.2 x 10⁶ cells/kg

- CAR T cell PD1 Expression
  - CD8+ T cell PD1: 12% baseline → 82% at the time of peak blood CAR T-cell levels (P<0.0001)
  - CD4+ T cell PD1: 32% baseline → 91% at the time of peak blood CAR T-cell levels (P<0.0001)


PD1: program death 1 receptor
CD-19 CAR as DLI post Allogeneic HSCT

21 patients with advanced CD19+ ALL(n=18) or NHL(n=3)
*10pts with active disease at HCT

- 10/21 patients (47%) alive and in CR at median 5.2 months (range 0-21.3 months) after CAR T cell infusion
- 7/8 haplo-HCT and CAR recipients remain in CR at 4.2 months
- 3 patients developed grades 2-4 aGVHD (skin, liver, gut; 1 each)
- Rate of CMV re-activation: 24% vs. 41% reported previously
- No acute or late toxicity to CAR+ T cell infusions reported

CD-19 CAR as consolidation Post aHCT for Rel/Ref B-NHL

Adults with Rel/Ref Aggressive B-NHL with either:
* PET + chemosensitive disease after ≥2 cycles of salvage chemotherapy OR
* Bone marrow involvement at time of relapse & not appropriate for Allo HCT

Salvage Chemotherapy
- leukapheresis
- CD-19-28z CAR T cell generation

BEAM Conditioning
-7

0

+1

+2

+3

Dose Level 1
- 5 x 10^6 CAR/kg
Dose Level 2
- 1 x 10^7 CAR/kg
Dose Level 3
- 2 x 10^7 CAR/kg

Pegfilgrastim
-2

ASCT

CD19-28z CAR T cell infusion

Anticipated Engraftment
+10

Dose Level -1 – 2x10⁶ CAR/kg


PET: positron emission tomography
**CD- 19 CAR as consolidation Post aHCT for Rel/Ref B-NHL**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Histology/ #lines of therapy</th>
<th>Status at HDT- ASCT</th>
<th>Dose CAR-T (x10^6/kg)</th>
<th>Clinically Relevant ≥ grade 3 non-heme AE</th>
<th>Cytokine release syndrome [CRS/Rx]</th>
<th>Peak CRP mg/dL (day)</th>
<th>Best Response/ PFS (months)</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>tFL/3</td>
<td>PET(+) PR</td>
<td>5</td>
<td>Gr3 CRS (mental status (MS) changes)</td>
<td>Yes/Toc* x1</td>
<td>27.3 (D4)</td>
<td>CR/26+</td>
<td>CR</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>DLBCL/4</td>
<td>PET(+) PR</td>
<td>5</td>
<td>Gr3 febrile neutropenia, Gr3 MS changes</td>
<td>Yes/None</td>
<td>16.5 (D4)</td>
<td>CR/29+</td>
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<td>3</td>
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<td>tMCL/2</td>
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<td>Gr3 hypophosphatemia</td>
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<td>17.6 (D3)</td>
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<td>4</td>
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<td>tFL/DHL/2</td>
<td>PET(+) PR</td>
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<td>Gr3 hypocalcemia, Gr3 AST/ALT, Gr4 CRS (hypotension, AKI, MS changes)</td>
<td>Yes/Toc* x1+dex</td>
<td>43.1 (D3)</td>
<td>CR/19+</td>
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<td>CR/19+</td>
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<td>CD5+ DLBCL/2</td>
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<td>No</td>
<td>7.9 (D4)</td>
<td>SD/6</td>
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<td>PET(+) PR BM involved</td>
<td>5</td>
<td>Gr3 CRS (MS changes,)</td>
<td>Yes/Toc* x1</td>
<td>11.8 (D7)</td>
<td>CR/2</td>
<td>POD/DOD</td>
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<td>DLBCL/DHL/2</td>
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<td>Gr3 CRS (seizure) Gr3 respiratory failure, Gr 5 infection (mucormycosis)</td>
<td>Yes/Toc* x1</td>
<td>18.1 (D4)</td>
<td>Not-evaluated (NE)</td>
<td>NRM/1 month</td>
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<td>POD/2</td>
<td>DOD</td>
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<td>blastoid MCL/4</td>
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<td>Gr 3 febrile neutropenia, Gr 4 CRS (encephalopathy)</td>
<td>Yes/Toc* x 1+dex</td>
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<td>Gr 2 hypotension Gr 2 seizure</td>
<td>Yes/Toc x1</td>
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<td>Gr 4 aphasia</td>
<td>Yes/Toc x1</td>
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<td>DOD</td>
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<td>POD/3</td>
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<td>PET (+) PR</td>
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<td>Gr 4 encephalopathy</td>
<td>Toc x1/dex</td>
<td>25.7 (D3)</td>
<td>CR/3</td>
<td>Alive</td>
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</tbody>
</table>

Gr: grade  
Toc: tocilizumab  
Dex: dexamethasone  
POD: progression of disease  
DOD: died of disease

The Future of CAR Therapy
Current Challenges with CD-19 CAR therapy

1. CAR Structure
   - KIR TAM
   - 41BB
   - scFV
   - CD3 zeta
   - CD28
   - Zetakine

2. Vector
   - Lentiviral
   - Retroviral
   - Sleeping Beauty
   - RNA

3. Composition
   - T-cell ratio
   - Ideal dose

4. Host Conditioning
   - Ideal lymphodepleting chemotherapy?

5. Toxicity Management
   - CRS minimization
   - Biology of neurotoxicity?
   - Long term toxicities?

Leukemia Resistance after CD-19 CAR Therapy

- Emerging biologies associated with CD-19 epitope loss
  - Isoform switch -- increase in CD19 isoforms specifically lacking exon 2, which binds the scFvs incorporated into CD19-CARs
  - Lineage switch -- global change in leukemia phenotype that is more stem cell like
- Variations in CAR – T Cell persistence
  - CD28 vs. 4-1BB costimulatory domains
  - Retroviral vs. Lentiviral vector utilization

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<th>CTL-019</th>
<th>20/53 pts in CR at 1 month -- have relapsed</th>
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<tr>
<td>Rel/Ref Pediatric ALL</td>
<td>• 13 pts with CD-19 negative disease</td>
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<th>JCAR015</th>
<th>18/46 patients relapsed</th>
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<tr>
<td>Rel/Ref Adult ALL</td>
<td>• 4/18 relapses occurred post CAR-T allo HSCT</td>
</tr>
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<td>• 3/18 relapses were CD19 negative</td>
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</table>

Mackall CL. ASH2015. SCI-24

scFvs: Single chain variable fragment
"Armored" CARs

 targets TILs for reversal of anergy

 IL-12 secretion

 CAR-IRES IL-12

 NK cell recruitment and activation

 IL-12 secretion

 Activated TIL

 Reversal of anergy

 Anergic (TIL)

 Targeted tumor cytotoxicity

The Future

Improving Functionality

Combination Therapy

Enhancing Safety

CART19 + Ibrutinib in MCL

The Future

Improving Functionality

Combination Therapy

Enhancing Safety

iCasp9 suicide GENE “Shut-off Switch”

Conclusion

- Adoptive immunotherapy with CD-19 CAR has provided a novel treatment option for patients with advanced B-cell malignancies

- Excellent initial efficacy data need validation in long term follow up and multicenter clinical trials

- Safety and minimization of toxicities requires further study

- New insights in biology of immunotherapeutics will serve to optimize this very new technology

- Role of CAR therapy in the paradigm of heme malignancies is still yet to be determined
Clinical Applications of Chimeric Antigen Receptor (CAR) – T Cell Therapy in Hematologic Malignancies

Valkal Bhatt PharmD. BCOP, BCPS
Clinical Specialist – Stem Cell Transplant
Memorial Sloan Kettering Cancer Center
New York, NY
Bhattv@mskcc.org