CAR T cell Therapy for Hematologic Malignancies

Nabil Ahmed, MD, MPH
Carlos A. Ramos, MD, et al.
Associate Professor, Baylor College of Medicine, Houston, Texas
Outline

• Adoptive T cell Transfer
• Chimeric Antigen Receptor T cells
• CD19 CAR T cells in the Clinic
• Non-CD19 CAR Trials
• Complications of CAR T cell Therapies
T cell Therapy

1. Blood draw

2. Tumor-specific T-cell Production

3. Delivery to the patient
T cell Therapy: **advantages**

- Δ killing mechanisms ... *conventional Rx*

- **Migrate; extravasate; expand** ... *vs. MAb*

- ↑ frequency; ↓ anergy ... *vs. DC vaccines*

- ↓ **autoimmunity** ... *vs. tumor cell vaccines*

Earliest examples of T cell therapy for hematological malignancies

- Allogeneic BMT GVL (co-infused T cells)
  - Initially unappreciated

*Donor Lymphocyte Infusion
**Post Transplant Lymphoproliferative Disorder
# Treatment and prevention of PTLD with EBV-CTLs

<table>
<thead>
<tr>
<th>Reference</th>
<th>Source</th>
<th>Intent</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heslop 2009</td>
<td>BMT donor</td>
<td>Prophylactic</td>
<td>99% success</td>
</tr>
<tr>
<td>Heslop 2009*</td>
<td>BMT donor</td>
<td>Therapeutic</td>
<td>85% ORR</td>
</tr>
<tr>
<td>Doubrovina 2012</td>
<td>BMT donor</td>
<td>Therapeutic</td>
<td>68% ORR</td>
</tr>
<tr>
<td>Leen 2013</td>
<td>3rd party</td>
<td>Therapeutic</td>
<td>73% ORR</td>
</tr>
<tr>
<td>Tzannou 2015</td>
<td>3rd party</td>
<td>Therapeutic</td>
<td>63% ORR</td>
</tr>
<tr>
<td>Prockop 2015</td>
<td>3rd party</td>
<td>Therapeutic</td>
<td>63% ORR</td>
</tr>
</tbody>
</table>

*and unpublished data

(ORR: overall response rate)
EBV-CTLs work in other malignancies

- **Hodgkin lymphoma** (Bollard et al., JEM 2004)
- **DLBCL** (Bollard et al., Blood 2007)
- **NPC** (Straathof et al., Blood 2005)

Optimization has included:
- Overexpression of weakly immunogenic proteins
- Introduction of resistance to the effects of TGF-β

Type 2 Latency
Hodgkin’s disease/NHL
Nasopharyngeal carcinoma
Making T-cell therapy more broadly applicable...

• Most tumors do not contain **exogenous**, **viral antigens**

• Can we consistently **manufacture** T cells that recognize **weak**, tumor associated antigens?
  
  – One approach: **genetically engineer T cells** to introduce new T-cell receptors

    • αβ (native T-cell receptors)
    • Chimeric Antigen Receptors (CAR)
MHC Restricted Operation

Antigen Presenting Cell

T

CTL

TUMOR

Chimeric Antigen Receptors

T cell Receptor

\( \zeta \)-Chain

\( \nu^H \) \( \nu^L \)

Variable Domain

\( \nu^H \) \( \nu^L \)

Antibody

CAR

Eshhar et al. *PNAS* 1993
MHC UN-Restricted

T cell Therapy: advantages

- MHC-unrestricted
- Customizable
- Reliable & timely manufacturing
- Third Party Product “blood bank model”

Selecting B-cell lymphoma antigens

- **HSC** → **B lymphocyte precursor** → **Naïve mature B lymphocyte** → **Plasma cell**

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Antigen-independent</th>
<th>Antigen-dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD19</strong></td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td><strong>CD20</strong></td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td><strong>CD38</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>CD138</strong></td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>slg (κ/λ)</td>
<td>−</td>
<td>+ (IgM, IgD)</td>
</tr>
</tbody>
</table>

κ/λ: Light chains of immunoglobulins

HSC: Hematopoietic stem cell

IgM, IgD: Immunoglobulin M and D
First vs. later generation CARs

- Ectodomain
  - scFv
  - Spacer
  - Linker
- Transmembrane
- Endodomain

First generation: scFv → ζ (zeta)
Second generation: scFv → CD28 → ζ
Third generation: scFv → CD28 → 4-1BB → ζ
Clinical trials using 1\textsuperscript{st} generation CD19.CAR-T cells

- Feasibility of the approach was established
- Lack of significant anti-tumor effects
- Limited persistence of CAR-modified T cells
Incomplete activation of 1st generation CAR-directed T cells

- Incomplete activation of T cells
- Killing of tumor cells
- Improved T cell activation and proliferation

Diagram: T cell with 1st gen CAR and CD28 interacting with tumor cell, and with 2nd gen CAR interacting with tumor cell through CD28.

Legend:
- 1st gen CAR
- 2nd gen CAR
- CD28
- ζ
- B7
- T cell
- Tumor

Text:
- Incomplete activation of 1st generation CAR-directed T cells
- Killing of tumor cells
- Improved T cell activation and proliferation
Are 2nd gen CAR-T cells superior to 1st gen CAR T cells? (CRETI study)
CAR-T cell manufacture

PBMCs

CD19.CAR Retrovirus

3 days

mAb-stimulated T cells

2 days

CD19.CAR activated T cells

Expansion (1-2 wks)

QC/QA testing & freezing

Infusion
## Patient details

<table>
<thead>
<tr>
<th>Age</th>
<th>Diagnosis</th>
<th>Previous therapy</th>
<th>Disease status</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/53</td>
<td>B-CLL</td>
<td>FCR, FC</td>
<td>Cervical, axillary, RP, inguinal LAD</td>
</tr>
<tr>
<td>M/56</td>
<td>FL→DLBCL</td>
<td>R-CHOP×8, XRT, FCR×6, R-ICE×2, CDDP/Ara-C, TTR×2</td>
<td>Cervical LAD</td>
</tr>
<tr>
<td>M/46</td>
<td>DLBCL</td>
<td>R-CHOP×6, R-ESHAP×4, R-ICE×2, R-IGEV, TTR, R, HyperCVAD×2</td>
<td>Retroperitoneal (RP) lymphadenopathy (LAD)</td>
</tr>
<tr>
<td>M/57</td>
<td>DLBCL</td>
<td>R-CHOP×4, R-ESHAP×2, R-BEAM/ASCT, XRT</td>
<td>Cervical, RP LAD</td>
</tr>
<tr>
<td>F/59</td>
<td>FL→DBLCL</td>
<td>R-CHOP×8, R-ESHAP×3, R-BEAM/ASCT, XRT, R</td>
<td>Muscle and skin</td>
</tr>
<tr>
<td>M/49</td>
<td>DLBCL</td>
<td>MTX×4, ESHAP, temozol., R-ICE×6, R-HyperCVAD×2, R-BEAM/ASCT, XRT×2</td>
<td>Brain &amp; RP LAD</td>
</tr>
<tr>
<td></td>
<td>CNS &amp; systemic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2nd gen CAR-T cells have greater in vivo expansion and persistence

(Savoldo, Ramos et al. JCI 2011)
2nd gen CAR-T cells are detected at tumor sites (Savoldo, Ramos et al. JCI 2011)
Anti-tumor activity: stable disease

Pre-infusion CT scan

Six-week post-infusion CT scan

Pt #3, dose level 2
CD19.CAR-T cell therapy can be highly effective...

### Non-Hodgkin Lymphoma/Chronic Lymphocytic Leukemia

<table>
<thead>
<tr>
<th>Reference</th>
<th>Center</th>
<th>N</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kochenderfer, JCO 2015</td>
<td>NCI</td>
<td>30 (adult/peds)</td>
<td>53% CR, 27% PR</td>
</tr>
<tr>
<td>Porter, Blood (ASH) 2014</td>
<td>UPenn</td>
<td>15 (adult)</td>
<td>29% CR, 29% PR</td>
</tr>
<tr>
<td>Savoldo, JCI 2011</td>
<td>BCM/HMH</td>
<td>6 (adult)</td>
<td>33% SD</td>
</tr>
</tbody>
</table>

### Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Reference</th>
<th>Center</th>
<th>N</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maude, NEJM 2014</td>
<td>UPenn</td>
<td>30 (adult/peds)</td>
<td>90% CR</td>
</tr>
<tr>
<td>Davila, SciTM 2014</td>
<td>MSKCC</td>
<td>15 (adult)</td>
<td>88% CR</td>
</tr>
<tr>
<td>Lee, Lancet 2015</td>
<td>NCI</td>
<td>21 (peds/AYA)</td>
<td>67% CR (ITT)</td>
</tr>
</tbody>
</table>

Reference Center N Efficacy

Maude, NEJM 2014
Davila, SciTM 2014
Lee, Lancet 2015
... but B-cell aplasia occurs after major responses

- CD19 is a universal B marker
- More restricted antigens may leave B-cell subpopulations intact
  - κ and λ light chains are mutually exclusive
  - Malignancies are monoclonal, i.e., κ⁺ or λ⁺
  - Targeting one should spare reciprocal population
κ.CAR-T cells selectively eliminate κ⁺ CLL cells

Control T cells  CD19.CAR-T cells  κ.CAR-T cells

κ⁺ CLL

λ⁺ CLL

T cells co-cultured (5:1 ratio) with CLL cells for 3 days

(Vera et al., Blood 2006)
CHARKALL trial

Peripheral blood draw or apheresis

CD3 CD28

PBMC activation

Transduction

κ.CAR-CD28 retrovirus

κ.CAR-CD28 T cells

Expansion in IL-2

QA/QC testing and freezing

Infusion
<table>
<thead>
<tr>
<th>#</th>
<th>Age/ Sex</th>
<th>Diagnosis</th>
<th>Previous therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53/F</td>
<td>Relapsed lymphoplasmacytic lymphoma</td>
<td>R-CHOP, 2CDA, R-BEAM/ASCT</td>
</tr>
<tr>
<td>2</td>
<td>60/M</td>
<td>Relapsed follicular lymphoma transformed to DLBCL</td>
<td>R-CHOP/XRT, FCR, R-ICE, TTR, CD19.CAR-T cells, R-bendamustine,</td>
</tr>
<tr>
<td>3</td>
<td>71/M</td>
<td>Relapsed DLBCL, leg-type</td>
<td>R-CHOP, ASCT, bortezomib</td>
</tr>
<tr>
<td>5</td>
<td>73/M</td>
<td>Relapsed CLL/SLL</td>
<td>R-bendamustine</td>
</tr>
<tr>
<td>6</td>
<td>59/M</td>
<td>Relapsed lymphoplasmacytic lymphoma</td>
<td>R-CVP, CHOP, bortezomib</td>
</tr>
<tr>
<td>9</td>
<td>55/M</td>
<td>Relapsed follicular lymphoma</td>
<td>R-CHOP, R-IE, R-BEAM/ASCT</td>
</tr>
<tr>
<td>10</td>
<td>69/F</td>
<td>Relapsed CLL/SLL</td>
<td>R-fludarabine, R-bendamustine</td>
</tr>
<tr>
<td>13</td>
<td>74/M</td>
<td>Relapsed MCL</td>
<td>R-hCVAD, bortezomib, carfilzomib/lenalidomide, R-bendamustine</td>
</tr>
<tr>
<td>16</td>
<td>69/M</td>
<td>Relapsed DLBCL</td>
<td>R-CHOP, R-BEAM/ASCT, BVR, R-ibrutinib, R-ESHAP</td>
</tr>
</tbody>
</table>
κ.CAR activated T cells (Pt #2)
Follicular lymphoma → DLBCL
Can we target non-B cell malignancies? (CART CD30)

- Hodgkin lymphoma
- Some non-Hodgkin lymphomas:
  - Anaplastic large T-cell lymphoma
  - CD30+ diffuse large B-cell lymphoma

PBMC activation → Transduction → CD30.CAR-CD28 retrovirus → CD30.CAR-CD28 T cells → Expansion in IL-7/15 → QA/QC testing and freezing → Infusion

Peripheral blood draw or apheresis
2nd generation CD30.CAR T-cells can also be effective
2nd generation CAR-T cell protocols at CAGT/HMH

- CD19.CAR
  - PD, 9
  - CCR, 5
  - PR, 1
  - SD, 5

- k.CAR
  - PD, 8
  - CR, 2
  - SD, 5
  - PR, 1

- CD30.CAR
  - PD, 3
  - CCR, 1
  - PR, 1
  - SD, 3

- Encouraging but far from perfect…
κ.CAR-T cells still have limited persistence... (as CD19/30.CAR-T cells also do have)
Critical issues emerging from clinical trials

• Adequate host lymphodepletion may be necessary
  – Cytokine Release Syndrome

• CAR may need to be expressed in specific T cell subsets
  – Naïve vs. experienced cells

• Different co-stimulatory domains may not be equivalent
  – CD28 vs. others
Critical issues emerging from clinical trials

- Adequate host **lymphodepletion** may be necessary
  - Cytokine Release Syndrome
- CAR may need to be expressed in specific T cell subsets
  - Naïve vs. experienced cells
- Different co-stimulatory domains may not be equivalent
  - CD28 vs. others
Lymphodepletion: $\alpha$ persistence

Copied per ug DNA in Peripheral Blood

Pre-infusion  4hrs  Week 1  Week 2  Week 4  Week

Bone marrow
Allo-SCT & GD2.CAR CTL\textsuperscript{EBV}

pre-transplant

15 weeks post HSCT

9 weeks post CTL
CD19.CAR-T cells in a lymphodepleted patient

- Ferritin (peak): 10,000
- ALT (peak): 450
Cytokine release syndrome and CAR-T cell expansion

![Graph showing plasma IL-6 levels over time (pre, D5, D6, D7) with increasing IL-6 levels from pre to D7.]

![Flow cytometry images showing CAR and CD3 expression on D7, D8, D9, and D10 with increasing CAR expression from D7 to D10.]
Resolution with IL-6R mAb

<table>
<thead>
<tr>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>05/22</td>
<td>05/23</td>
<td>05/24</td>
<td>05/25</td>
<td>05/26</td>
</tr>
<tr>
<td>08-16</td>
<td>16-00</td>
<td>00-08</td>
<td>08-16</td>
<td>16-00</td>
</tr>
<tr>
<td>00-08</td>
<td>08-16</td>
<td>16-00</td>
<td>00-08</td>
<td>08-16</td>
</tr>
<tr>
<td>16-09</td>
<td>00-08</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- CRP
- Temperature (°F)
- Blood Pressure
- SpO₂

= tocilizumab
4 mg/kg IV×1
Critical issues emerging from clinical trials

• Adequate host lymphodepletion may be necessary
  – Lymphocyte homeostasis; Treg removal

• CAR may need to be expressed in specific T cell subsets
  – Naïve vs. experienced cells

• Different co-stimulatory domains may not be equivalent
  – CD28 vs. others
Naïve T cell subset expands better in vivo: IL-7/IL-15 preserve better the naïve subset

(Xu et al. Blood 2014)
Critical issues emerging from clinical trials

• Adequate host lymphodepletion may be necessary
  – Lymphocyte homeostasis; Treg removal
• CAR may need to be expressed in specific T cell subsets
  – Naïve vs. experienced cells
• Different co-stimulatory domains may not be equivalent
  – CD28 vs. others
Rationale for exploring alternative costimulatory endodomains

Early

APC

CD80/CD86

CD28

T cell

PI3K

Late

APC

4-1BBL/OX40L

4-1BB/OX40

TRAF2

T cell
Antitumor activity of κ.CAR.4-1BB T cells

D6

D12

D22

NT

CAR.κ.28

CAR.κ.4-1BB
2\textsuperscript{nd} (CD28) vs. 3\textsuperscript{rd} (CD28-4-1BB) generation CAR-T cells

Peripheral blood draw or apheresis

\begin{center}
\begin{tikzpicture}
  \node [align=center] (a) {PBMC activation};
  \node [align=center, below of=a] (b) {Transduction};
  \node [align=center, below of=b] (c) {CAR.CD19-CD28\(\zeta\) T cells};
  \node [align=center, right of=c] (d) {CAR.CD19-CD28-4-1BB\(\zeta\) T cells};
  \node [align=center, below of=d] (e) {QA/QC testing and freezing};
  \node [align=center, below of=c] (f) {QA/QC testing and freezing};
  \node [align=center, below of=f] (g) {Expansion in IL-7/15};
  \node [align=center, below of=g] (h) {Infusion};

  \draw [->] (a) -- (b);
  \draw [->] (b) -- (c);
  \draw [->] (c) -- (g);
  \draw [->] (g) -- (h);
  \draw [->] (d) -- (e);
\end{tikzpicture}
\end{center}
• 67 yo M, stage IVA follicular lymphoma with transformation to DLBCL
  – R-CHOP: response then progression
  – Lenalidomide/rituximab: no response
  – R-ICE: response then progression
  – Unable to proceed to transplant
• Cytoxan/fludarabine, then CAR-T cells
CRP: 12.2 (d +12) → 6.2 (d +16)
Conclusions

• **Later generation** CAR-T cells can have remarkable activity against B-cell malignancies
  – Especially ALL and CLL, even relapsed/refractory
• **Severe cytokine release syndrome** occurs with major tumor responses
  – Manageable so far with IL-6R antibodies
• CARs can successfully travel **beyond CD19**
  – e.g. κ (and beyond B cells, e.g. CD30)
• **Antigen Escape**
• **Numerous trials** are ongoing…
  – CARs to be incorporated in standard therapy?
    • As consolidation? Bridge to BMT? BMT replacement?
Funding: NCI Lymphoma SPORE, LLS SCOR, V Foundation, ASCO Career Development Award, Celgene Corporation, Bluebird Bio

Acknowledgments

CARLOS RAMOS
Malcolm Brenner
Cliona Rooney
Helen Heslop
Barbara Savoldo
Gianpietro Dotti
Ann Leen
Neeharika Narala
Juan Vera
Soranobu Ninomiya
Yang Xu

Clinical Research
Vicky Torrano
Bambi Grilley
Alicia Brown
Kristal Black
Rachel Kronman
George Carrum
Rammurti Kamble

Statistical analysis
Hao Liu

GMP/GLP Laboratories
Adrian Gee
Oumar Diouf
Huimin Zhang
Joyce Ku
Weili Liu
Pallavi Mahopatra
Enli Liu
Olga Dakhova
Debbie Lyon
Zhuyong Mei