Chimeric Antigen Receptor T Cell Therapy

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Presentation Objectives

- Scientific overview of chimeric antigen receptor (CAR) T cell therapy
- CART Mechanism of action
- Overview of CART clinical trials
- CART patient eligibility considerations
CAR T cells are genetically altered to express CAR on the cell surface.

T Cell Receptor

- APC
- pMHC
- TCR
- CD3ζ
- CD28

Chimeric Antigen Receptor

- scFv: recognize tumor surface proteins
- Costimulatory Signal 2: CD28 or 4-1BB or OX40
- Essential Signal 1: CD3ζ

Activation Independent of MHC Limited to cell surface proteins
Schema of CAR T manufacturing and administration

Chimeric Antigen Receptor T cells (CARTs)

- Lentiviral vector

- T cell
  - Antigen: CD19
  - CAR construct: Anti-CD19
  - Activation
  - Survival and Proliferation

- Tumor cell
  - CTL019 cell
  - Dead tumor cell

References:
- Porter DL et al. NEJM 2011
- Maude et al. NEJM 2014.
First Successful Report of CART19 in CLL (UPenn Trial)

Kalos et al, Sci Transl Med 2011
Porter et al, NEJM 2011

Results from this report
- 3 patients R/R CLL
- 2 → sustained CR, 1 → PR
- Eradicated bulky disease
  - T cells persisted
  - Memory phenotype
- Potent (1 cell killed 1000 tumor cells)
- Cytokine release syndrome
- Tumor lysis syndrome
High Response Rates in ALL

Historic outcomes of patients with relapsed/refractory acute lymphoblastic leukemia

Outcomes of patients with relapsed/refractory acute lymphoblastic leukemia treated with CART19

Grupp et al, ASH Abstract #380
Maude et al, NEJM 2014
## High Response Rates in ALL

<table>
<thead>
<tr>
<th></th>
<th>University of Pennsylvania&lt;sup&gt;31&lt;/sup&gt;</th>
<th>Memorial Sloan Kettering Cancer Center&lt;sup&gt;28&lt;/sup&gt;</th>
<th>National Institutes of Health&lt;sup&gt;30&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target antigen</strong></td>
<td>CD19</td>
<td>CD190</td>
<td>CD190</td>
</tr>
<tr>
<td><strong>CAR generation</strong></td>
<td>2nd</td>
<td>2nd</td>
<td>2nd</td>
</tr>
<tr>
<td><strong>Vector</strong></td>
<td>Lentivirus</td>
<td>Retrovirus</td>
<td>Retrovirus</td>
</tr>
<tr>
<td><strong>Costimulatory domain</strong></td>
<td>4-1BB</td>
<td>CD28</td>
<td>CD28</td>
</tr>
<tr>
<td><strong>Duration of culture</strong></td>
<td>8-12 days</td>
<td>11 days</td>
<td></td>
</tr>
<tr>
<td><strong>No. of ALL patients</strong></td>
<td>30</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td><strong>Conditioning regimen</strong></td>
<td>Individualized, mainly fludarabine based.</td>
<td>Cyclophosphamide 3 g/m&lt;sup&gt;2&lt;/sup&gt; day 2</td>
<td>Fludarabine 25 mg/m&lt;sup&gt;2&lt;/sup&gt; days 4, 3, 2 Cyclophosphamide 900 mg/m&lt;sup&gt;2&lt;/sup&gt; day 2</td>
</tr>
<tr>
<td><strong>Median follow-up</strong></td>
<td>7 months</td>
<td>NR</td>
<td>10 months</td>
</tr>
<tr>
<td><strong>Overall survival</strong></td>
<td>78%</td>
<td>NR</td>
<td>51.6%</td>
</tr>
<tr>
<td><strong>No. of patients undergoing allo-HSCT</strong></td>
<td>3</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td><strong>Response</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphologic CR</td>
<td>90%</td>
<td>88%</td>
<td>70%</td>
</tr>
<tr>
<td>MRD negative CR</td>
<td>73%</td>
<td>75%</td>
<td>60%</td>
</tr>
<tr>
<td><strong>Duration of CAR T-cell persistence</strong></td>
<td>11 months</td>
<td>3 months</td>
<td>68 days</td>
</tr>
</tbody>
</table>
Cancer Immunotherapy
Breakthrough of the Year 2013

2013 Breakthrough
- Cancer Immunotherapy

The Runners-Up
- CRISPR
- CLARITY
- Human Stem Cells from Cloning
- Mini-Organs
- Cosmic Particle Accelerators Identified
- Perovskite Solar Cells
- Why We Sleep
- Our Microbes, Our Health
- In Vaccine Design, Looks Do Matter
Critical Components of CART as a Drug

- CAR construct
- CAR delivery system
- CART phenotype and function
- CART persistence
CAR Construct: What generation is your CAR?

Pioneered by Eshhar et al 1989

First-Generation CAR
scFv-CD3ζ

Second-Generation CAR
scFv-CD28-CD3ζ

Third-Generation CAR
scFv-CD28-4-1BB-CD3ζ
scFv-CD28-OX40-CD3ζ
CAR Construct: CD28 vs 41BB

Kalawekar et al. Immunity 2016

CAR Construct: Antigen Selection

- CD19 expression is generally restricted to B cells and B cell precursors
  - CD19 is not expressed on hematopoietic stem cells or other tissue
- CD19 is expressed by most B-cell malignancies
  - CLL, B-ALL, DLBCL, FL, MCL

CAR Construct: Antigen Selection

- On target, off-tumor toxicity
  - High binding affinity results in recognition of low antigen expression in normal tissue
  - Ex. Liver injury with anti-carbonic anhydrase IX CART
  - Ex. Pulmonary toxicity with anti-Her2 CART
  - Can be fatal

CAR Construct: Delivery System

Viral System
- Lentivirus, retrovirus
- Most commonly used in trials to date
- Permanent genetic modification
- Costly

Non-Viral System
- Transposon/Transposase
  - Permanent genetic modification
  - Less expensive for manufacturing
- RNA transfection
  - Temporary genetic expression
  - Strategy for limiting toxicity
CAR T Phenotype & Function

- Optimize T cell population
  - CD4 to CD8 proportion
  - Central vs effector vs stem memory T cells

- Activated vs exhausted state
  - Duration of culture
  - Cytokines
CAR T Persistence *in vivo*: Clinical Relevance

**CD19 positive relapses (4/30 patients, 13.3%)**
- Poor expansion - CTL019 cells are lost

**CD19 negative relapses (3/30 patients, 10%)**
- Good expansion and persistence of CTL019

Grupp et al, ASH Abstract #380
Maude et al, NEJM 2014
CAR T Persistence in vivo: Conditioning Chemotherapy

Figure 1. CD4 and CD8 CAR-T cell persistence in NHL patients following infusion of $2 \times 10^7$ cells/kg after conditioning with (n=6) or without (n=3) Fludarabine.

Cameron J Turtle et al. Blood 2015;126:184
Strategies to Manage CAR Toxicities

Challice L Bonifant, published online 20 April 2016. doi:10.1038/mto.2016.11
ONGOING CLINICAL TRIALS
## CART Programs at Academic Centers

<table>
<thead>
<tr>
<th>Center</th>
<th>Target</th>
<th>Condition</th>
<th>Construct</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penn</td>
<td>CD19 CAR</td>
<td>ALL</td>
<td>BBz, LV</td>
<td>90% CR</td>
</tr>
<tr>
<td>NIH</td>
<td>CD19 CAR</td>
<td>ALL</td>
<td>28z, RV</td>
<td>70% CR(ITT)</td>
</tr>
<tr>
<td>MSKCC</td>
<td>CD19 CAR</td>
<td>ALL</td>
<td>28z, RV</td>
<td>88% CR</td>
</tr>
<tr>
<td>NIH</td>
<td>CD19 CAR</td>
<td>Lymphoma</td>
<td>28z, RV</td>
<td>85% aggressive lymphomas, 100% indolent lymphomas</td>
</tr>
<tr>
<td>Seattle</td>
<td>CD19 CAR</td>
<td>ALL</td>
<td>BBz, LV</td>
<td>83% CR</td>
</tr>
<tr>
<td>Penn</td>
<td>CD19 CAR</td>
<td>Lymphoma</td>
<td>BBz, LV</td>
<td>50% CR aggressive lymphoma, 100% indolent</td>
</tr>
<tr>
<td>Penn</td>
<td>CD19 CAR</td>
<td>CLL</td>
<td>BBz, LV</td>
<td>25% CR rate</td>
</tr>
<tr>
<td>MDACC</td>
<td>CD19 CAR</td>
<td>CLL/ALL/NHL</td>
<td>28z, SB</td>
<td>23% CR rate</td>
</tr>
<tr>
<td>NIH</td>
<td>CD22 CAR</td>
<td>ALL</td>
<td>BBz, LV</td>
<td>8 patients treated</td>
</tr>
<tr>
<td>NIH</td>
<td>BCMA CAR</td>
<td>Myeloma</td>
<td>28z, RV</td>
<td>6 patients treated</td>
</tr>
</tbody>
</table>

Kenderian et al. BBMT in press.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Antigen</th>
<th>Gene-transfer vector used</th>
<th>Endomains</th>
<th>Cell culture</th>
<th>Cell dose</th>
<th>Conditioning regimen</th>
<th>Cytokine support</th>
<th>No. of patients</th>
<th>Responses to CAR T-cells</th>
<th>Persistence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kershaw 2006 (111)</td>
<td>α-folate receptor</td>
<td>Gammaretrovirus FcRγ</td>
<td>CD3ζ</td>
<td>OKT3 + 600 IU/mL IL-2; 21–56 d</td>
<td>3 × 10^9–1.69 × 10^11 T-cells (1–3 infusions)</td>
<td>None</td>
<td>IL-2 9 (72000 IU/kg) was given i.v. every 12 h in cohort 1</td>
<td>14 patients with ovarian cancer</td>
<td>14 PD</td>
<td>4–21 d</td>
</tr>
<tr>
<td>Park 2007 (71)</td>
<td>CD171</td>
<td>Electroporation CD3ζ</td>
<td>CD3ζ</td>
<td>OKT3 + 50U/mL IL-2 + irradiated PBMC/lymphoblastoid cell line feeders; 14 d (1–3 infusions)</td>
<td>1 × 10^6/m^2</td>
<td>None</td>
<td>None</td>
<td>6 children with neuroblastoma</td>
<td>1 PR, 5 PD</td>
<td>Short (1–7 d)</td>
</tr>
<tr>
<td>Lamers 2013 (108)</td>
<td>CAIX</td>
<td>Gammaretrovirus FcRγ</td>
<td>CD3ζ</td>
<td>OKT3 + 100 IU/mL IL-2; approximately 21 d</td>
<td>0.2 × 10^9–2.1 × 10^9 CAR T-cells (5 infusions)</td>
<td>None</td>
<td>None</td>
<td>12 patients with metastatic renal cell carcinoma</td>
<td>12 NR</td>
<td>Up to 3–5 wk</td>
</tr>
<tr>
<td>Louis 2011 (20)</td>
<td>GD2</td>
<td>Gammaretrovirus CD3ζ</td>
<td>CD3ζ</td>
<td>OKT3 + 100 or 50U/mL IL-2 + irradiated PBMC; 12–18 d and 36–54 d</td>
<td>2 × 10^7/m^2</td>
<td>None</td>
<td>None</td>
<td>19 patients with neuroblastoma</td>
<td>8 NED, 3 CR, 1 PR, 1 SD, 4 PD, 2 tumor necrosis</td>
<td>16 wk</td>
</tr>
<tr>
<td>Morgan 2010 (107)</td>
<td>HER2</td>
<td>Gammaretrovirus CD137−/CD28−CD3ζ</td>
<td>CD3ζ</td>
<td>OKT3 + 300 IU/mL IL-2 (a rapid expansion) procedure: 6000 IU/mL + 50ng/mL OKT3 + irradiated PBMC feeders; 24 d</td>
<td>1 × 10^10 T-cells</td>
<td>None</td>
<td>60mg/kg cyclophosphamide x2 and flurodarebine 25mg/m^2 x5</td>
<td>1 patients with colorectal cancer</td>
<td>Died of cytokine release syndrome</td>
<td>Died 5 d after treatment</td>
</tr>
<tr>
<td>Brown 2015* (70)</td>
<td>IL13Rα2</td>
<td>Electroporation CD3ζ</td>
<td>CD3ζ</td>
<td>OKT3 + 50U/mL IL-2; approximately 63 d</td>
<td>9.6 × 10^6–1.53 × 10^8 CD8+ T (1–17 infusions)</td>
<td>None</td>
<td>None</td>
<td>13 enrolled, 3 treated (glioblastoma)</td>
<td>3 PD</td>
<td>Up to 184 d</td>
</tr>
<tr>
<td>Katz 2015† (106)</td>
<td>CEA</td>
<td>Gammaretrovirus CD28−CD3ζ</td>
<td>CD3ζ</td>
<td>OKT3 + 3000U/mL IL-2; 17–25 d</td>
<td>Cohort 1: 10.1 × 10^6 CAR T; Cohort 2: 3.0 × 10^9 CAR T (3 infusion)</td>
<td>None</td>
<td>None</td>
<td>6 patients with colorectal cancer</td>
<td>5 PD, 1 SD</td>
<td>Approximately 2 wk</td>
</tr>
<tr>
<td>Ahmed 2015 (12)</td>
<td>HER2</td>
<td>Gammaretrovirus CD28−CD3ζ</td>
<td>CD3ζ</td>
<td>OKT3 or CD3/CD28 beads + 100U/mL IL-2; 12–21 d</td>
<td>1 × 10^6/m^2</td>
<td>None</td>
<td>None</td>
<td>19 patients with sarcoma</td>
<td>4 SD</td>
<td>Up to 18 mo</td>
</tr>
</tbody>
</table>
CART Research Directions

Safety
- HSV thymidine kinase
- Inducible caspase 9
- Transient expression
- CCR
- iCAR
- Truncated EGFR

Persistence
- Selection of T-cell subsets for CAR transfer
- IL-15, IL-12 and other cytokines
- Select virus-specific T-cells for transduction
- Off-the-shelf CAR T-cell

Homing
- Chemokine receptors (CXCR2, CCR4, CCR2B)
- VEGFR2 CAR

Overcome immunesuppression
- Expression of survival genes such as Bcl-X(L)
- Treg suppression
- CD25 expression upregulation
- IDO downregulation
- TRUCKs CAR (IL-12)
- Express dominant negative receptors (TGF-β)
- Switch receptor chimeras (PD1-CD28)
- Constitutive CD40L or 4-1BB Expression

Combination therapies
- Checkpoint blockade (PD1, CTLA-4)
- GM-CSF neutralization
- Autologous transplantation
- Radiotherapy
- Chemotherapy

### CAR T-CELL DEALS

<table>
<thead>
<tr>
<th>Institution/Company</th>
<th>Date</th>
<th>Partner</th>
<th>Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of</td>
<td>August</td>
<td>Novartis</td>
<td>Undisclosed</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celgene</td>
<td>March</td>
<td>Bluebird Bio, Baylor College of</td>
<td>Unspecified upfront payment plus up to $225 million per product in</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>Medicine</td>
<td>option fees and milestone payments</td>
</tr>
<tr>
<td>Cellectis</td>
<td>June</td>
<td>Pfizer</td>
<td>$80 million upfront plus up to $185 million per product and royalties</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellectis</td>
<td>January</td>
<td>Ohio State University</td>
<td>Undisclosed</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kite Pharma</td>
<td>January</td>
<td>Amgen</td>
<td>$60 million upfront and up to $525 million per product in milestone</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td></td>
<td>payments, plus royalties on sales and IP licensing</td>
</tr>
<tr>
<td>Md Anderson</td>
<td>January</td>
<td>Ziopharm, Intraxon</td>
<td>$100 million in stock and $15–20 million/year for 3 years</td>
</tr>
<tr>
<td></td>
<td>2015</td>
<td></td>
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</tr>
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</table>

### CAR T-CELL BIOTECH IPOs

<table>
<thead>
<tr>
<th>Company</th>
<th>Date</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kite Pharma</td>
<td>June 2014</td>
<td>$134.1 million</td>
</tr>
<tr>
<td>Bellicum</td>
<td>December 2014</td>
<td>$160 million</td>
</tr>
<tr>
<td>Juno</td>
<td>December 2014</td>
<td>$264.6 million</td>
</tr>
<tr>
<td>Cellectis</td>
<td>March 2015</td>
<td>$228 million</td>
</tr>
</tbody>
</table>
Hematologic Malignancies
- Lymphomas, ALL (n=34)
- Myeloma (n=3)
- AML (n=2)

Solid tumors (n=10)
- Types
  - GBM
  - Neuroblastoma
  - Pancreas cancer
  - Sarcoma
  - NSCLC
  - Triple negative breast cancer
- Antigens
  - EGFRvIII, PSCA, GD2, Her2, ROR1, CD171
Patient Eligibility Considerations

- Adequate blood cell count for leukapheresis
- Relative disease stability
  - CART manufacturing generally 2 – 4 weeks
  - Disease not progressing rapidly through manufacturing period
- Patient ability to tolerate CAR T toxicities
  - Good major organ functions
    - heart, lung, kidney, liver
  - Neurologic considerations
    - Seizure risk, CVA, CNS disease
Conclusion

- Questions from Audience
- Answers from Presenter