Current Status of CAR T Immunotherapy

Don Xu, MD, PhD
VA Healthcare System San Diego
University of California, San Diego

San Diego Society of Hematopathology
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Disclosure of Conflict of Interests

• None
Definition of Immunotherapy

- Immunotherapy: the "treatment of disease by inducing, enhancing, or suppressing an immune response"

- Cancer Immunotherapy (or immuno-oncology): the use of the immune system to treat cancer.
Cancer Immunotherapy named as the breakthrough of 2013
Types of Cancer Immunotherapy

• Antibody-based: anti-CD20 Rituximab

• Cytokine-based: modulating immune system; Interleukin 2, Interferon-α

• Immune checkpoint blockade: CTLA-4 inhibitor - ipilimumab

• Cancer treatment vaccines: sipuleucel-T (Provenge) for meta. prostate cancer

• Virotherapy: oncolytic viruses (reovirus), T-VEC (talimogene laherparepvec) for melanoma

• Adoptive cell transfer: transfer of immune cells into a patient
  • tumor infiltrating lymphocytes (TILs); CAR-T; TCR
Adoptive Cell Therapy (ACT)

- The transfer of immune cells into a patient with or without genetic modification

- One form of cancer immunotherapy.
  - tumour-infiltrating lymphocyte (TIL) therapy: Melanoma
  - T-cell receptor (TCR) therapy: melanoma, colon cancer, synovial sarcoma
  - Chimeric antigen receptor (CAR) therapy: B-ALL

Jensen M, presentation at CIP 2015
Chimeric Antigen Receptor T Immunotherapy

History of CAR T Therapy

- 1989: Initial concept: Dr. Zelig Eshhar from Israel
  - T-body approach

- 1990: Dr. Eshhar collaborated with Dr. Steven Rosenberg at NIH to design CAR-T

- 2006: First clinical trial: renal cancer, ovarian cancer in
  - First generation CAR: CD3-zeta as intracellular domain
  - Limited clinical activity, due to insufficient cell division, activation, and cytokine production

- 2009: Second generation: add one co-stimulatory domain, CD28 or 4-1BB

- Third generation: using two co-stimulatory domains (a combination of CD27, CD28, 4-1BB, ICOS, or OX40)

Chimeric Antigen Receptors (CARs)

1st generation: including activating receptors such as CD8/CD3-ζ fusion receptors) and T-bodies;
2nd generation: dual signaling to direct combined activating and costimulatory signals
3rd generation: comprising more complex structures with 3 or more signaling domains.

CAR Constructs

Domains of CAR Construct

- **scFV**: “single chain variable fragment”
  - a fusion protein of $V_H$ and $V_L$
  - retains the specificity of the original Ig
- **CD3-zeta**: activate and induce proliferation of T cells but can lead to anergy
- **Costimulatory domains (CD28, 4-1BB, OX40)**: improved replicative capacity and persistence of modified T cells
  - 4-1BB is associated with longer persistence
- **Hinge region**
- **Transmembrane domain**
Ideal Features of CAR-T Targets

• Cell surface marker

• Ubiquitously expressed on tumor cells

• No antigen escape: be essential for tumor survival and/or malignant behavior.

• No off-target expression for an essential organ or cell type (i.e., hematopoietic stem cells); minimal off-tumor expression in non-essential organs.
Challenges in Selecting Cancer Specific Antigen

- CD19 works extremely well: An ideal target for CAR-T immune therapy
  - A pan-B cell surface marker
  - Expressed on nearly all B-cell malignancies
  - B cell aplasia is treatable and tolerable
## Gene Transfer Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retroviral</td>
<td>Long-term disease control</td>
<td>risk of transformation, persistent on-target toxicity</td>
</tr>
<tr>
<td>Lentiviral</td>
<td>Long-term disease control; No risk in transformation</td>
<td>persistent on-target toxicity</td>
</tr>
<tr>
<td>RNA expression</td>
<td>decreased on-target toxicity; no risk in transformation</td>
<td>Need multiple infusion for long term control</td>
</tr>
</tbody>
</table>

Production of CAR T cells

Nature 504, S13–S15(12/19/13)
American Association for Cancer Research
B Lymphoblastic Leukemia or B-ALL

- Pediatric cases: Even though with better prognosis, no much improvement in survival rates in relapsed cases in decades.
- Adult cases: poorer prognosis (30-40% 5-year relative survival)
- Relapsed cases are challenging for all age groups.

Genetics of ALL

- Certain genetic abnormalities a/w prognosis: BCR-ABL1, MLL etc
- Inhibitors against genetic abnormalities showed some effects, but has many limitations
- Majority of ALL cases don’t have a driver mutation

### Clinical Trials of CD19 CAR-T

<table>
<thead>
<tr>
<th></th>
<th>CHOP</th>
<th>MSKCC</th>
<th>NCI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong># of patients</strong></td>
<td>30</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td><strong>CR</strong></td>
<td>90%</td>
<td>88%</td>
<td>70%</td>
</tr>
<tr>
<td><strong>CAR Construct</strong></td>
<td>CTL019-BB-ζ</td>
<td>MSKCC19-28z</td>
<td>FMC63-CD28z</td>
</tr>
<tr>
<td><strong>Vector</strong></td>
<td>Lentiviral</td>
<td>Retroviral</td>
<td>Retroviral</td>
</tr>
<tr>
<td><strong>T cell persistence</strong></td>
<td>up to 2 yrs</td>
<td>1-3 mo</td>
<td>&lt;68 days</td>
</tr>
<tr>
<td><strong>B cell aplasia</strong></td>
<td>Correlated with T cell persistence</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Emma Whitehead: The first child to receive CAR T therapy

- Born in 2005
- May 2010: diagnosed B-ALL
- Oct 2011: first relapse
- Feb 2012: second relapse, weeks before BMT
- April 2012: CAR T trial
- May 2012: in remission
- May 2014: 2-years in CR
Complications a/w CAR T Therapy

- Cytokine release syndrome (CRS): related to the massive proliferation of T-cells.
  - most common, potentially severe
  - From mild flu-like symptoms to shock and multisystem organ failure; severe cases have hyperferritinemia, hepatosplenomegaly, and hypofibrinogenemia.
  - Marked increased levels of cytokines (IL-2 receptor α, IL-6, IL-10, and interferon-γ), mirroring HLH
  - correlated with the response to CAR
  - severity correlated with disease burden at infusion
Complications a/w CAR T Therapy

- Encephalopathy:
  - mild and self-limited
  - No specific etiology identified
- B-cell aplasia and hypo gammaglobulinemia
  - on-target toxicity
# The CAR T-Cell Race

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

[http://www.the-scientist.com](http://www.the-scientist.com) article dated April 1, 2015
Active Clinical Trials of CAR T-cell Therapy
Active Clinical Trials of CAR T-cell Therapy

- Total: 56
  - B-cell Lymphoma/leukemia: 29
  - Other lymphoma/leukemia: 7
  - Brain tumor: 5
  - Other solid tumors: 13
  - Other: 2

![Region Name](map) ![Number of Studies](map)

<table>
<thead>
<tr>
<th>Region Name</th>
<th>Number of Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>56</td>
</tr>
<tr>
<td>East Asia</td>
<td>17</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
</tr>
<tr>
<td>Europe</td>
<td>5</td>
</tr>
<tr>
<td>North America</td>
<td>35</td>
</tr>
<tr>
<td>United States</td>
<td>35</td>
</tr>
</tbody>
</table>
Challenges for CAR-T

- Stringent requirements for target antigens
- Clinical trial is the only known way to test efficacy
- Expensive: $300,000 - $500,000 per patient
- Availability: Currently only at a few academic medical centers: NCI, MSKCC, CHOP, MD-Anderson, Seattle Children Hospital, etc
- Need standardized protocol in managing therapy-related complications (CRS)
- Solid Tumors
High Specificity of CAR Targets

- Extremely hard to find other targets as good as CD19
- MAGE-A3 as the target for non-small cell lung cancer (NSCLC) in a NCI clinical trial: two patients died due to the later discovery of low level expression in brain tissue
- T-ALL: hard to find unique surface markers different from normal T cells; T-cell aplasia much harder to manage
Genetically Modified TCR Immunotherapy

From the website of Kite Pharma
TCR Therapy
Genetically Modified TCR

- T-cell response can be improved by increased specificity and affinity for tumour antigens, via genetic modification of the α and β chains of TCR.
- An target peptide sequence needs to be identified.
  - immunise transgenic mice that express the human leukocyte antigen (HLA) with human tumour proteins.
  - allogeneic TCR gene transfer: the reactive TCR sequences from tumour-specific T cells isolated from a patient in remission are transferred to T cells of another patient who shares the disease.
- in vitro technologies to alter the sequence of the TCR and enhance the strength of the interaction (avidity).
- Intracellular antigen: take advantages of somatic mutations of cancer genomes.
Clinical Trials of Modified TCR Therapy

- Demonstrated the overall feasibility and clinical potential
- The first trial is on metastatic melanoma: 4 of 31 patients achieved measurable regression of disease
- Proof of principle for genetically modified TCR therapies

Table 1. Examples of published reports of positive clinical responses to T-cell therapies

<table>
<thead>
<tr>
<th>Target antigen</th>
<th>Target disease</th>
<th>T-cell therapy</th>
<th>No. patients</th>
<th>Responses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP100</td>
<td>Melanoma</td>
<td>TCR</td>
<td>16</td>
<td>1 CR and 2 PR</td>
<td>Johnson et al., 2009</td>
</tr>
<tr>
<td>MAGEA3</td>
<td>Melanoma, oesophageal and synovial sarcoma</td>
<td>TCR</td>
<td>9</td>
<td>1 CR and 4 PR</td>
<td>Morgan et al., 2013</td>
</tr>
<tr>
<td>NYESO-1</td>
<td>Melanoma and sarcoma</td>
<td>TCR</td>
<td>17</td>
<td>2 CR and 7 PR</td>
<td>Robbins et al., 2011</td>
</tr>
</tbody>
</table>
# CAR-T vs TCR

<table>
<thead>
<tr>
<th></th>
<th>CAR-T</th>
<th>TCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>signal amplification</td>
<td>From synthetic biology</td>
<td>Sensitive, derived by evolution</td>
</tr>
<tr>
<td>Target</td>
<td>Cell surface</td>
<td>Intracellular proteome</td>
</tr>
<tr>
<td>MHC dependent</td>
<td>No, “off the shelf”</td>
<td>Yes</td>
</tr>
<tr>
<td>Construct design</td>
<td>Easier to make</td>
<td>Harder to make</td>
</tr>
<tr>
<td>Types of cancers with effects</td>
<td>Leukemias</td>
<td>Solid Tumors</td>
</tr>
</tbody>
</table>
Challenges for Treating Solid Tumors with CAR-T

Solid Tumor CAR
- CAR T cell expansion
- Limited to cells that access immunosuppressive tumor environment:
  - suboptimal prolif
  - functional anergy/exhaustion
  - memory differentiation impairment
  - limited persistence

CD19 CAR/Leukemia (+ Normal B Cells)
- CAR T cell expansion occurs upon engagement of immunostimulatory B cells:
  - lack of immunosuppressive microenvironment
  - acquisition of effector function/tissue & tumor homing
  - memory cell differentiation
  - prolonged persistence
New Ideas and Directions of CAR-T Research

- Controllable CAR-T
- BiSpecific CAR: tandem CAR
- “Armored” CAR-T: express an additional pro-inflammatory cytokine, IL-12
- How to overcome inhibitory immune environments within solid tumors?
- Combination with other therapy, such as immune checkpoint inhibitors?
The Role of Pathologists in CAR-T Immunotherapy

After CD19 CAR-T therapy is approved by FDA:

• How to monitor MRD
  • Identify neoplastic cells
  • and/or monitor persistent CAR-T?
• Follow-up
• What morphologic changes in BM, Lymph node?
  • B-cell aplasia
• Any other long term changes?
Questions Facing the CAR-T Field

- Is it desired to have long-term persistence of CAR T cells?
- Which vector is better: retroviral vs lentiviral?
- How does the type(s) and composition of infused T cells affect the outcome?
- Sorting out the important variables of CAR T cells:
  - tumor heterogeneity, variability of endogenous T-cells,
  - CAR constructs, production protocols, components of infused T-cells (naive, effector, memory)
  - Combining with existing therapeutic regimens
Thank You!