CAR T-CELL THERAPY: PAST, PRESENT AND FUTURE

JOHN R. EDWARDS, MD
CO-MEDICAL DIRECTOR
FRANCISCAN HEALTH INDIANA BLOOD AND MARROW TRANSPLANTATION
CAR T-CELL THERAPY OBJECTIVES

• During this presentation, participants will illustrate how this therapy is being used in a community based setting

• Explain and define CAR T therapy including background, manufacturing, administration and monitoring

• Discuss ongoing developments and future potential of targeted therapy with Adoptive Cell Transfer
IMMUNE SURVEILLANCE: RATIONALE FOR ADOPTIVE CELL TRANSFER

- Mutations and aberrations occur in us all
  - Paul Ehrlich (1909) “in the complicated course of …development, aberrant cells become unusually common”
- Immunodeficiencies are associated with a higher rate of malignancy
- Prognosis of multiple tumor types correlates with lymphocyte count, NK cell count and presence of tumor infiltrating lymphocytes
  - Melanoma, renal, breast, colon prostate, ovary, and glioblastoma
IMMUNE SURVEILLANCE: PROOF OF CONCEPT FOR CLINICAL ADOPTIVE CELL TRANSFER

• Allogeneic stem cell transplant is associated with a powerful graft-versus-leukemia and graft-versus-lymphoma effect
  • Lower relapse rate for allogeneic versus syngeneic donors
  • Relapse rate after allogeneic stem cell transplant correlates with Graft-Versus-Host Disease
  • Donald Thomas won Noble Prize for proof of concept

• Tumor Infiltrating Lymphocytes/Lymphokine-Activated Killer (TIL/LAK) cells
  • Effective in Renal cell, melanoma and other solid tumors
  • Limited production, targeting and effectiveness
CAR T: THE PRESENT
CHIMERIC ANTIGEN RECEPTOR T CELLS

- Define CAR T-cell
- Explain the process and indications for CAR T-cell therapy.
- Describe the risk factors and toxicities of CAR T-cell therapy.
- Describe the clinical data behind CAR T-cell therapy.
**BACKGROUND**

- Chimeric antigen receptor (CAR) is a modular fusion protein comprising extracellular target binding domain usually derived from the single-chain variable fragment (scFv) of antibody, spacer domain, transmembrane domain, and intracellular signaling domain containing CD3ζ linked with zero or one or two costimulatory molecules such as CD28, CD137, and CD134.

- T cells engineered to express CAR by gene transfer technology are capable of specifically recognizing their target antigen through the scFv binding domain, resulting in T cell activation in a major histocompatibility complex (MHC)-independent manner.

* Journal of Hematology & Oncology 2017:10:53
BACKGROUND CONT.
MULTIPLE OPTIONS FOR CAR’S: YUGO VS BMW

- Costimulatory factors enhance the activity of the CAR T-cell.
- There are multiple possible costimulatory molecules and it is not clear which is optimal.
- Animal models may not predict optimal costimulatory molecules in humans.
- There are also molecules that can be inserted as “suicidal genes” that can be used to turn off the reaction by causing cell death of the transplanted CAR T-cells.
The targets of the CAR T-cell have been surface antigens present on malignant cells

- CD 19: Novartis, Kite, Juno target CD 19+ malignancies like ALL, diffuse large B-cell lymphoma, CLL and myeloma. Some tumors have higher number of CD19 targets than others.
- CD20, CD 22: lymphomas and other B cell malignancies
- CD30: Hodgkin lymphoma, T cell lymphoma
- BCMA (B-cell maturation antigen) Juno, Bluebird target in myeloma
- Other myeloma targets: CD 38, CD 138
- AML targets: CD33,
- Solid tumors: epidermal growth factor receptor (EGFR), mesothelin (MSLN), variant III of the epidermal growth factor receptor (EGFRvIII), human epidermal growth factor receptor-2 (HER2), carcinoembryonic antigen (CEA), and prostate-specific membrane antigen (PSMA). The preliminary data in solid tumors suggests poor responses to date
CAR T: THE PROCESS
CAR T: THE PROCESS

- Patient selection: confirming target, disease stage and performance status
- Leukapheresis to collect cells for manufacturing (3-4 weeks)
- Bridging chemotherapy
  - Toxicity versus effectiveness
  - Complete responders?
- Lymphodepleting chemotherapy
- Monitoring
### Patient/treatment related factors

- Underlying disease (ALL > solid tumors)
- Age (worse < 3 yo)
- Prior therapy
  - Significant pre-treatment
  - Post transplant with early collection

### Specific agent

- Cyclophosphamide
- Clofarabine
- Anthracyclines

### Gene transduction

- CD3/CD28 beads

### Optimized culture

### CAR T-cell for infusion

### Patient infusion

### Optimized lympho-depleting therapy

### Potential CAR T-cell recipient

### Expanded cells express genes associated with:

- CD27+ PB-1- CD8+ CAR-T cell with high level IL-6 receptor

### Early loss — T-cell intrinsic

### Early loss — T-cell extrinsic

### Antigen escape

### Persistence

- CD24+ CD45RO- CD8+ T-cells prior to culture

### Failure to respond

### Poorly Expanded cells express genes associated with:

- Effector differentiation
- Glycolysis
- Exhaustion
- Apoptosis
CAR T: MONITORING
CAR T: MONITORING
COMMUNITY PERSPECTIVE

• Identifying Team
  • FACT Accreditation for Effector Cells
  • REMS Requirements from FDA
  • Resource Intense Training
• Responsible Parties
  • Screening and selection
  • Scheduling and coordinating
  • Billing and Collecting
CAR T CELL TOXICITY

- **PANCYTOPENIA FROM THE CONDITIONING TREATMENT**—may last 4 weeks or longer
- **ANAPHYLAXIS**
- **DAMAGE TO NORMAL CELLS THAT HAVE THE TARGET RECEPTOR**
- **TUMOR LYSIS**
  - Treat as appropriate: allopurinol/Rasburicase, fluids, electrolyte management
  - Treat coagulopathy if it develops as appropriate: plasma, cryoprecipitate, platelets
- **Cytokine release syndrome (CRS)**
  - Timing varies by product, as early as in the first 24 hours. Median: KYMRIAH 3 days, (median duration 7 d), YESCARTA 2 days (median duration 7d), JUNO J017 1-5 day (median duration 7d)
  - CAR T cells, or other immune cells e.g. macrophages can produce cytokines including interleukin-6 (IL-6), interferon-γ, tumor necrosis factor, IL-2, IL-2–receptor-α, IL-8, and IL-10.
  - Common symptoms of systemic involvement: fever, tachycardia, hypotension
  - Relation to CAR T cell dose, quantity of disease, specific product characteristics.
**Neurologic:**
- Headaches
- Changes in level of consciousness
- Delirium
- Aphasia
- Apraxia
- Ataxia
- Hallucinations
- Tremor
- Dysmetria
- Myoclonus
- Facial nerve palsy
- Seizures

**Constitutional:**
- Fevers
- Rigors
- Malaise
- Fatigue
- Anorexia
- Arthralgias

**Cardiovascular:**
- Tachycardia
- Widened pulse pressure
- Hypotension
- Arrhythmias
- Decreased left ventricular ejection fraction
- Troponinemia
- QT prolongation

**Pulmonary:**
- Tachypnea
- Hypoxia

**Renal:**
- Acute kidney injury
- Hyponatremia
- Hypokalemia
- Hypophosphatemia
- Tumor lysis syndrome

**Gastrointestinal:**
- Nausea
- Emesis
- Diarrhea

**Musculoskeletal:**
- Myalgias
- Elevated creatine kinase
- Weakness

**Hepatic:**
- Transaminitis
- Hyperbilirubinemia

**Hematologic:**
- Anemia
- Thrombocytopenia
- Neutropenia
- Febrile neutropenia
- Lymphopenia
- B-cell aplasia
- Prolonged prothrombin time
- Prolonged activated partial thromboplastin time
- Elevated D-Dimer
- Hypofibrinogenemia
- Disseminated intravascular coagulation
- Hemophagocytic lymphohistiocytosis
CAR T: MONITORING

• NEUROLOGIC (CAR-related encephalopathy syndrome, CRES): Median time to occur 7 d Kymriah (12 d duration), 4 d Yescarta (17 d duration)
  • Neurologic events may occur at different times than CRS or in the absence of CRS toxicities
  • headaches, confusion, alterations in wakefulness, hallucinations, dysphasia, ataxia, apraxia, facial nerve palsy, tremor, dysmetria, and seizures
  • Neurologic toxicities may also necessitate intubation and mechanical ventilation for airway protection in the absence of respiratory failure

• HEMATOLOGIC
  • Grade 3-4 anemias, thrombocytopenia, leukopenia, neutropenia, and lymphopenia
  • Ofter difficulty in determining the etiology of cytopenias occurring after CAR T-cell infusions (chemotherapy vs. CRS vs infection)
  • Derangements of coagulation following CAR T-cell infusion include prolongation of the prothrombin time and activated partial thromboplastin time (PTT), D-dimer elevation, low fibrinogen, disseminated intravascular coagulation, and macrophage activation syndrome.

• INFECTIONS

• HYPOGAMMAGLOBULINEMIA (CD-19 or CD-20 suppression of B cell function)
Grading of Cytokine Release Syndrome

<table>
<thead>
<tr>
<th>Category</th>
<th>Sign/Symptom</th>
<th>CRS Grade 1(^1)</th>
<th>CRS Grade 2(^2)</th>
<th>CRS Grade 3(^2)</th>
<th>CRS Grade 4(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital signs</td>
<td>Temperature &gt; 38⁰C (100.4⁰F) Yes</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
</tr>
<tr>
<td></td>
<td>SBP &lt; 90 mmHg</td>
<td>No / Responds to IV fluids or low-dose vasopressor / Requires high-dose or multiple vasopressors (See Appendix 2 of CAR-T Clinical Policy)</td>
<td>/ Life-threatening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Needing oxygen to maintain O₂ saturation &gt; 90%</td>
<td>No / FiO₂ &lt; 40%</td>
<td>FiO₂ &gt; 40% and/or requiring BiPAP</td>
<td>Requires ventilator support</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ Toxicity</td>
<td>See CAR-T Clinical Policy</td>
<td>Grade 1</td>
<td>Grade 2</td>
<td>Grade 3 any or grade 4 transaminitis</td>
<td>Grade 4 except grade 4 transaminitis</td>
</tr>
</tbody>
</table>

\(^1\)Grade 1 CRS may manifest as fever and/or grade 1 organ toxicity

\(^2\)For grades 2, 3, or 4 CRS: any one of the criteria other than temperature is sufficient
<table>
<thead>
<tr>
<th>Sign/symptom</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somnolence</td>
<td>Mild drowsiness/ sleepiness</td>
<td>Moderate somnolence, limiting instrumental ADL</td>
<td>Obtundation or stupor</td>
<td>Life-threatening needing urgent intervention or mechanical ventilation</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>Mild symptoms limiting of ADL</td>
<td>Moderate symptoms limiting instrumental ADL</td>
<td>Severe symptoms limiting self-care ADL</td>
<td>Life-threatening consequences, urgent intervention indicated</td>
</tr>
<tr>
<td>Confusion</td>
<td>Mild disorientation/ confusion</td>
<td>Moderate disorientation, limiting instrumental ADL</td>
<td>Severe disorientation, limiting self-care ADL</td>
<td>Life-threatening consequences, urgent intervention indicated</td>
</tr>
<tr>
<td>Dysphasia</td>
<td>Awareness of receptive or expressive characteristics; no impairment in communicating</td>
<td>Moderate receptive or expressive characteristics; impairment in ability to communicate spontaneously</td>
<td>Severe receptive or expressive dysphasia, impairing ability to read, write or communicate intelligibly</td>
<td>-</td>
</tr>
<tr>
<td>Seizure</td>
<td>Brief partial seizure; no loss of consciousness</td>
<td>Brief generalized seizure</td>
<td>Multiple seizures despite medical intervention; new</td>
<td>Life-threatening; prolonged repetitive seizures</td>
</tr>
<tr>
<td>Incontinence or motor weakness</td>
<td>Bowel / bladder incontinence; Weakness limiting self-care ADL, disabling</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tremor</td>
<td>Mild symptoms</td>
<td>Moderate symptoms; limiting instrumental ADL</td>
<td>Severe symptoms; limiting self-care ADL</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: ADL, activities of daily living
CAR T-CELL UNIQUE TOXICITY TREATMENT

• CYTOKINE RELEASE SYNDROME CRS
  • Low grade toxicity use supportive care: aggressive fluids, pressors, oxygen, ventilator
  • Evaluate for infections, heart failure if indicated
  • Anti-Interleukin 6 therapy tocilizumab/Actemra for oxygen-requiring or worse CRS up to 4 doses q 8 hr
  • High dose methylprednisolone 2 mg/kg daily if no improvement with tocilizumab or if life threatening 1000 mg daily X 3 then taper

• NEUROLOGIC TOXICITY CYTOKINE RELEASE ENCEPHALOPATHY SYNDROME CRES
  • For moderate grade 2 symptoms, give dexamethasone 10 mg IV q6h until grade 1 or less then taper over 3 days.
  • Tocilizumab seems to have no effect on CRES
  • Keppra for seizure prophylaxis
  • If severe grade 4 give 1000 mg methylprednisolone X 3 d and taper
CART T-CELL OUTCOMES: DIFFUSE LARGE B CELL LYMPHOMA
Axicabtagene Ciloleucel CART-Cell Therapy in Refractory Large B-Cell Lymphoma (Yescarta Zuma Trial)

Panel A shows the duration of response, (investigator assessment), in the 89 study patients who had an objective response, (complete response and partial response). Patients who had a complete response had a longer duration of response than those with an objective or partial response. Median duration of response was 8.1 months (range, 3.5 to could not be estimated [NE]).

Panel B shows the rate of progression-free survival, and Panel C the rate of overall survival in the 108 patients who were treated in the phase 1 and phase 2 studies.

### Comparison of outcomes for the largest CAR-T trials for patients with DLBCL

<table>
<thead>
<tr>
<th></th>
<th>JULIET Kymriah tisagenlecleucel</th>
<th>ZUMA Yescarta Axicabtagene ciloleucel</th>
<th>TRANSCEND JCAR017</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of enrolled patients</td>
<td>147</td>
<td>111</td>
<td>134</td>
</tr>
<tr>
<td>N of treated patients</td>
<td>99</td>
<td>101</td>
<td>114</td>
</tr>
<tr>
<td>Median time from apheresis to infusion</td>
<td>—</td>
<td>17 d</td>
<td>—</td>
</tr>
<tr>
<td>ORR, %</td>
<td>53</td>
<td>82</td>
<td>75</td>
</tr>
<tr>
<td>CR rate</td>
<td>40</td>
<td>54</td>
<td>55</td>
</tr>
<tr>
<td>Median follow-up, mo</td>
<td>5.6</td>
<td>15.4</td>
<td>—</td>
</tr>
<tr>
<td>Duration of response, mo</td>
<td>Not reached</td>
<td>8.1</td>
<td>—</td>
</tr>
<tr>
<td>Rate of any CRS, %</td>
<td>58</td>
<td>93</td>
<td>39</td>
</tr>
<tr>
<td>Rate of grade ≥3 CRS, %</td>
<td>15</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Rate of any neurotoxicity, %</td>
<td>21</td>
<td>64</td>
<td>23</td>
</tr>
<tr>
<td>Rate of grade ≥3 neurotoxicity, %</td>
<td>12</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>Frequency of tocilizumab use, %</td>
<td>15</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>Frequency of steroid use, %</td>
<td>11</td>
<td>27</td>
<td>9</td>
</tr>
</tbody>
</table>
CAR T: CHALLENGES
CURRENT PROBLEMS/CHALLENGES

• Powerful challenges to the broad application of CAR T-cell therapy
  • (1) antigen loss relapse, an emerging threat to CAR T-cell therapy, mainly observed in anti-CD19 CAR T-cells for B-ALL
  • (2) on-target/off-tumor toxicity resulting from the recognition of healthy tissues by CAR T-cells, especially in the setting of solid tumors
  • (3) less efficacy in solid tumors, mainly due to the hostile tumor microenvironment
  • (4) personalized autologous T cell manufacturing and widely “distributed” approach.
Patient/treatment related factors

Underlying disease (ALL > solid tumors)

Age (worse < 3 yo)

Prior therapy
d ↓ Significant pre-treatment
d ↓ Post transplant with early collection

Specific agent
d ↓ Cyclophosphamide
d ↓ Clofarabine
d ↓ Anthracyclines

Gene transduction

Leukapheresis

Optimized culture

Optimal lympho-depleting therapy

Potential CAR T-cell recipient

Optimal lympho-depleting therapy

Expanded cells express genes associated with:
CD27+ PB-1- CD8+ CAR-T cell with high level IL-6 receptor

→ Early loss — T-cell intrinsic
→ Early loss — T-cell extrinsic — immune mediated rejection
→ Antigen escape
→ Persistence ↑ CD24+ CD45RO- CD8+ T-cells prior to culture

Poorly Expanded cells express genes associated with:
Effecter differentiation
Glycolysis
Exhaustion
Apoptosis
CAR T: THE FUTURE
ACTR: A NEXT-GENERATION T CELL THERAPY

ACTR = Antibody-Coupled T cell Receptor

ACTR087 (CD16-41BB-CD3ζ) is the first ACTR genetically modified autologous T cell in clinical development
- Feasibility of approach previously demonstrated using transient RNA expression of ACTR (Poon, ASH 2016)

Key differences compared with current CAR T-Cell Therapy
- Tumor specificity is derived from an antibody co-administered with ACTR T cells
- ACTR binds tumor-specific antibodies via its CD16 domain
- Targeting of more than 1 tumor antigen possible

Potential to address CAR-T limitations
- ACTR T cell activation and proliferation requires antibody; titration of antibody dosing may optimize therapeutic index
- Facilitates clinical and manufacturing efficiencies through a single T cell product for multiple indications and tumor targets
CAR T-CELL: THE FUTURE

- IS THERE A BEST CONSTRUCT FOR PRODUCING CAR T-CELLS?
- IS THERE A BETTER WAY TO PRODUCE CAR T-CELLS \(\rightarrow\) SEPARATE CD 4 AND CD 8 CELLS?
- CAN YOU MAKE 2 OR THREE DIFFERENT CAR T-CELLS TO ATTACK 2 OR 3 TARGETS
  - BCMA, CD 38, CD 19 in Myeloma)
  - CD19 and CD22 in ALL
- NOVEL METHODS LIKE THE UNUM ACTIVATED CAR T-CELLS USING A TWO STEP BINDING TO A MONOCLONAL ANTIBODY LIKE RITUXIMAB GOING TO BE SUCCESSFUL TO ALLOW COCKTAILS OF MONOCLONALS TO OVERCOME POSSIBLE RESISTANCE MECHANISMS?
- ARE THERE BETTER WAYS TO TREAT CRS AND CRES
  - Cellular mediators of the CAR T-cells are macrophages and monocytes—can they be more specifically inhibited?
  - Blocking IL-6 in animal models does not block the CAR T-cell malignant cell response. Should it be given prophylactically?
  - In mouse models, IL-1 appears to be the main mediator of CRS and CRES. Can anti-IL 1 anakinra/Kinaret be used in prophylaxis or treatment, including for CRES?
- COST MAY ULTIMATELY BE PROHIBITIVE: Yescarta $373,000, Kymriah $475,000 for ALL, $373,000 for NHL just for acquisition!
CAR T-CELL: THE FUTURE

• Natural Killer Cells and T/NK
  • Innate immune effector cells against tumors
  • Potential for “off the shelf” or expanded manufacturing
  • Potential lower toxicity profile

• Modifications of CAR T-cells
  • Improved manufacturing with gene editing (CRISPR/Cas9)
    • Knock out diverse genes such as PD-1 that inhibit immune killing
    • On/Off : switch signaling small molecule dimerization or suicide genes (iCasp9)
CAR T-CELL: THE FUTURE

- Community Based Issues
  - Toxicity management
  - Supportive care team
  - Referral back to Primary Oncologists (15 year gene therapy follow up)

- Cost may be prohibitive:
  - Yescarta $373,000, Kymriah $475,000 for ALL, $373,000 for cell acquisition!
  - Medicare: new and improved reimbursement $265,000 per product
  - Novel reimbursement models (money back guarantee?)