Immunotherapy for Cancer

Kara L. Davis, D.O.
Anne T. and Robert M. Bass Endowed Faculty Scholar in Pediatric Cancer and Blood Diseases
Assistant Professor of Pediatrics
Bass Center for Childhood Cancer and Blood Disorders
Stanford University
Objectives

• Childhood leukemia leads the way for targeted T-cell therapies
  • What are chimeric antigen T cells
  • Use in heme malignancies and solid tumors
  • Limitations in Efficacy
  • Toxicity concerns

• Immune Checkpoint Inhibition for anti-cancer effect
  • Successes and Challenges
Childhood Leukemia was universally fatal 100 years ago.
Now childhood leukemia is a poster child for success in cancer treatment

Pui et al, NEJM 2006
But relapsed leukemia is still a big problem

- Over time, from 1988-2002 studies addressing relapsed ALL have not improved outcomes

Nguyen et al, Leukemia, 2008
The search for surface targets in B cell cancers

Piccaluga et al., Leuk & Lymphoma 2010

Stanford University
What is a CAR-T cell?
How CAR-T cells work…

Viral DNA Insertion

Expression of CAR

CAR enables T cell to recognize tumor cell antigen

Antigen

CAR T cells multiply and release cytokines

Tumor cell apoptosis

Tumor cell

T cell

Antigen

CAR enables T cell to recognize tumor cell antigen

CAR T cells multiply and release cytokines

Tumor cell apoptosis

Tumor cell

T cell

Viral DNA Insertion

Expression of CAR

How CAR-T cells work…

 Courtesy of David Miklos
Timeline of Development

- Concept of CAR-T cells 1989
- Preclinical study of 2nd gen CAR 1998
- 1st trials with 1st gen CAR (ovarian, renal ca) no responses poor persistence 2006
With increased understanding of the critical importance of the intracellular 'second signal' activation for CAR T-cell efficacy, subsequent generations of CARs have optimized ScFv and linker component design and, more importantly, have incorporated additional intracellular costimulatory signaling domains [e.g. CD27, CD28, CD134 (OX40), CD137 (4-1BB)] in efforts to increase the expansion, persistence and potency of CAR T cells, as well as to prevent cellular exhaustion \textit{(Figure 2)}.

Most CARs used in current clinical trials of engineered T cells for patients with B-ALL are derived from second generation constructs with CD3$\zeta$ and another signaling endodomain. Delivery of a second costimulatory signal has indeed appeared to induce significantly greater T-cell expansion and longer-term persistence \textit{in vivo} to date in treated patients.

Third generation CAR constructs comprised of CD3$\zeta$ and two additional co-stimulatory endodomains transduced into T cells are also under clinical evaluation, but have not thus far resulted in greater efficacy than second generation CARs [Davila \textit{et al.} 2012; Sadelain \textit{et al.} 2013; Kenderian \textit{et al.} 2014; Mackall \textit{et al.} 2014].

Given the potential for clinically significant CAR T-cell induced toxicities (discussed below), optimal CAR design thus must carefully balance desired antitumor potency with minimization of hazardous side effects [Gardner and Jensen, 2014]. Various groups have focused intensively in the past decade on targeting the B-lymphocyte antigen, CD19, a phosphoglycoprotein ubiquitously expressed on malignant and nonmalignant B-cells. Based upon the clinical efficacy and apparent tolerability of targeting CD20 (another commonly expressed B cell antigen) with the anti-CD20 monoclonal antibody rituximab in patients with B-cell hematologic malignancies [Cramer and Hallek, 2012], it was hypothesized that the CD19 receptor could be similarly targeted with engineered T cells expressing a CD19-redirected CAR and that treatment with these CD19 CAR T cells would also be ultimately tolerable in patients [Cooper \textit{et al.} 2004; Kochenderfer and Rosenberg, 2013].

In preclinical studies, several research teams observed that co-incubation of CD19 CAR T cells with B-cell leukemia or lymphoma cell lines induced potent T-cell degranulation, cytokine production and tumor cytotoxicity \textit{in vitro}.

\textit{Tasian et al., Ther Adv Hema, 2015}
Design affects Function

Persist less than 3 months in most patients

Persist 6 months or more in most patients
Increased Persistence of CD19.BB.z vs CD19.28.z CAR in Clinical Trials

Days Since Infusion vs Months Since Infusion

CD19.28.z-Bethesda
CD19.BB.z-Penn

Lee, Lancet, 2014
Maude, NEJM, 2014
Efficacy of CAR-T cells
Current strategies for treatment after relapse are suboptimal!
Across centers, excellent outcomes with CAR

Patients achieving MRD-negative remission

NCI, CD19-28z CAR; n=20

Number at risk

0 50 100 150 200 250 300 350 400 450 500 550 600

Leukaemia-free survival (%)

0 20 40 60 80 100

Probability of Event-free Survival

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Survival rate at 6 mo, 67% (95% CI, 51–88)

Months since Infusion

0 3 6 9 12 15 18 21 24

Lee et al. Lancet 2015
Maude et al. NEJM 2015
Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia


Maude et al. NEJM 2018
Hide and seek

Pioneering immunotherapy to find and kill elusive cancer cells in children

Salvador De Leon and his mother, Maria De La Cruz
CD19 CAR in NHL
Refractory LBCL patients do poorly with standard therapies

• SCHOLAR-1, a retrospective, international, patient-level, multi-institution study and the largest reported analysis of outcomes in patients with refractory large B cell lymphoma, demonstrated that these patients have a very poor prognosis1
  – N = 636 (post-rituximab era, 2000-2017)
  – ORR = 26%
  – CR rate = 7%
  – Median OS = 6.3 mo
  – These results provided a benchmark for evaluation of new approaches

• Previous analyses of SCHOLAR-1 standardized to ZUMA-1 with ≥ 6 mo follow-up suggested the benefit of axi-cel in refractory large cell lymphoma2

2. Previous analyses referred to ZUMA-1.
ZUMA1: Study of Kite’s KTE-c19 in NHL

- Axicabtagene ciloleucel (Yescarta) is an autologous CAR T therapy with a CD3ζ/CD28-based signaling that recognizes and eliminates CD19-expressing cells.

- NHL is the most common hematologic malignancy in the US.

- Outcomes in refractory aggressive NHL are poor:
  - SCHOLAR-1 patient level meta-analysis of refractory NHL
    - ORR of 26% (CR of 7%)
    - Median OS of 6.3 months
ZUMA-1 at Median f/u 15.4 Months: 42% Progression-Free and 56% Alive

Progression-Free Survival

<table>
<thead>
<tr>
<th>Landmark</th>
<th>PFS</th>
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<tr>
<td>6-month</td>
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<tr>
<td>12-month</td>
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<td>18-month</td>
<td>41</td>
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Overall Survival

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<tr>
<th>Landmark</th>
<th>OS</th>
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<tr>
<td>6-month</td>
<td>78</td>
</tr>
<tr>
<td>12-month</td>
<td>59</td>
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<tr>
<td>18-month</td>
<td>52</td>
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</table>
F.D.A. Panel Recommends Approval for Gene-Altering Leukemia Treatment

By DENISE GRADY  JULY 12, 2017

F.D.A. Approves Second Gene-Altering Treatment for Cancer

By DENISE GRADY  OCT. 18, 2017

RELATED COVERAGE

Immune System, Loaded With Remade T-cells, Vanquishes Cancer  SEPT. 12, 2011

A Breakthrough Against Leukemia Using Altered T-Cells  DEC. 9, 2012
Limitations in Efficacy

Antigen Escape and T cell Exhaustion
Experience in B-ALL Has Illuminated Mutually Exclusive Causes for CAR T Cell Failures

- Appears to be the most common cause of relapse
- Incidence not truly known
- Emerging data suggests a similar rate in DLBCL
- Addressing this problem is important for B-ALL but also identifies a vulnerability for CAR T cells which will be amplified in AML and solid tumors
Relapse with CD19-ALL was majority of cases

Maude et al, NEJM, 2018
Relapse with Lineage switch

CD19 CAR immune pressure induces B-precursor acute lymphoblastic leukaemia lineage switch exposing inherent leukaemic plasticity

Elad Jacoby1,†, Sang M. Nguyen1, Thomas J. Foultaine1, Kathryn Welp1, Berkley Gryder2, Haiying Qin1, Yimeng Yang1, Christopher D. Chien1, Alix E. Seif3, Haiyan Lei1, Young K. Song2, Javed Khan2, Daniel W. Lee1, Crystal L. Mackall1, Rebecca A. Gardner4, Michael C. Jensen4, Jack F. Shern1 & Terry J. Fry1

Lineage Switch in MLL-Rearranged Infant Leukemia Following CD19-Directed Therapy

Ahmad Rayes, MD,1,† Richard L. McMasters, MD,2 and Maureen M. O'Brien, MD1

Stanford University

 Jacoby et al, Nat Comm, 2015
Immune Escape via Isoform Switch or CD19 mutation

Sotillo et al, Cancer Discovery, 2015
Aligning leukemia cells to developing B cells identifies expanded subpopulations

Good et al., Nat Med 2018
Focus on Failure with CAR-T cells: Who will have CD19-relapse?

- Relapse
- CAR T
- Assess MRD and B cell aplasia
- Months
- ~60% Remission
- ~40% Relapse CD19-
Different mechanisms for CD19 loss could be detected before CAR

<table>
<thead>
<tr>
<th>Condition</th>
<th>CNA analysis by WES (Chr16)</th>
<th>DNA-seq CD19 ex1-14</th>
<th>VAF in DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>CHOP105R</td>
<td>DELETED</td>
<td>N/A</td>
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<tr>
<td></td>
<td>(p13.11p11.1)</td>
<td>W111delinsWPLR</td>
<td>100% (22/22)</td>
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<tr>
<td>Patient</td>
<td>CHOP101R</td>
<td>INTACT</td>
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<tr>
<td></td>
<td>CHOP105R</td>
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<td>CHOP133R</td>
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<tr>
<td></td>
<td>CHOP136R</td>
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<tr>
<td></td>
<td>healthy</td>
<td>INTACT</td>
<td></td>
</tr>
</tbody>
</table>

| Healthy   | CHOP101R                    | INTACT              |            |
|           | CHOP105R                    | INTACT              |            |
|           | CHOP133R                    | INTACT              |            |
|           | CHOP136R                    | INTACT              |            |
|           | healthy                     | INTACT              |            |

<table>
<thead>
<tr>
<th>Multi-Dimensional Scaling (MDS) plot</th>
</tr>
</thead>
</table>

Pablo Domizi, David Barrett
Hypothesis Generation: Focus on MOST Relevant Cell Populations
ALL cells lose CD19 when it ends up on the CAR-T cells

**CAR T cell trogocytosis and cooperative killing regulate tumour antigen escape**

As T-cells gain CD19 or CD22, they demonstrate increased features of exhaustion

Hamieh et al. Nature 2019
Patient #4: 10 yo, refractory B-ALL, High burden disease, Complete response followed by CD19+CD22+ relapse
AP-1 Transcription Factors are part of Exhaustion

Lynn et al, In Revision

c-Jun OE increases IFNg and IL-2 expression in exhausted HA-28z T cells, but has no effect in non-exhausted T cells.
Alternative Targets Have Emerged: CD22

Fry et al., Nature Med, 2017

71% MRD Negative Response Rate at Dose level 2+
Antigen density has emerged as a salient question in the CD22 CAR

Fry et al., Nature Med, 2017
Bispecific Targeting in ALL: CD19 and CD22

- Two trials of CD19/22-bispecific CAR at Stanford have launched: Pediatric and Adult
- First-in-human testing of a bispecific CAR
- Relapsed refractory B cell malignancies
  - Primary objective: safety and feasibility
  - Secondary objectives: Response rate, Incidence of CD19 negative escape
- Heavy correlative science
6 patients infused, 4 in remission at Day 27

<table>
<thead>
<tr>
<th>Age</th>
<th>Disease Indication</th>
<th>Burden</th>
<th>Dose</th>
<th>Toxicity</th>
<th>Day 27 response</th>
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</thead>
<tbody>
<tr>
<td>17 yo F</td>
<td>Isolated BM relapse</td>
<td>MRD+ by NGS at infusion</td>
<td>DL1: 1x10^6 /kg</td>
<td>Gr 1 CRS/ CRES</td>
<td>MRD- at Day 27</td>
</tr>
<tr>
<td>2 yo M</td>
<td>MLLr CNS/BM relapse</td>
<td>MRD+ by NGS at infusion; CNS2</td>
<td>DL1: 1x10^6 /kg</td>
<td>Gr 1 CRS</td>
<td>MRD- at Day 27, s/p HSCT</td>
</tr>
<tr>
<td>16 yo F</td>
<td>Primary refractory</td>
<td>MRD+ 0.15 by flow</td>
<td>DL1: 1x10^6 /kg</td>
<td>Gr 1 CRES (HA)</td>
<td>MRD- at Day 27</td>
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<tr>
<td>8 yo M</td>
<td>BM relapse</td>
<td>6% disease by flow</td>
<td>DL1: 1x10^6 /kg</td>
<td>Gr 2 CRS</td>
<td>Morphologic remission at Day 27</td>
</tr>
<tr>
<td>2 yo F</td>
<td>BM relapse</td>
<td>15% disease</td>
<td>DL1: 1x10^6 /kg</td>
<td>Gr 2 CRS</td>
<td>Non-responder</td>
</tr>
<tr>
<td>12 yo F</td>
<td>2nd BM relapse</td>
<td>7% disease</td>
<td>DL1: 1x10^6 /kg</td>
<td>Gr 1 CRS</td>
<td>TBD</td>
</tr>
</tbody>
</table>
So many questions remain....

**How much disease is best?**

- When to use CAR T cells?
  - Diagnosis?
  - After Chemotherapy for All?
  - Minimal Disease?
  - A lot

**How to use CAR T cells?**

- Only in Patients with Residual Disease?
- Only at relapse?
- Bridge to Transplant?
CAR T cells for Solid Tumors

DIPG and beyond

Mount and Majzner, Nat Med, 2018
DIPG: in need of fresh thinking

Diffuse Intrinsic Pontine Glioma

– Devastating brainstem tumor of childhood, 300 cases/yr in US
– Standard of care: median life expectancy <12mo.
– No improvements in survival since introduction of targeted radiotherapy

Mackay et al, Cancer Cell 2017
Cell surface screen identifies GD2 as immunotherapy target in DIPG

GD2.4-1BBz CAR
R. Majzner; C. Mackall
Single-dose intravenous GD2-CAR T cell therapy clears DIPG xenografts

<table>
<thead>
<tr>
<th>DPT</th>
<th>GD2-CAR</th>
<th>CD19-CAR</th>
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<td>36</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Mount, Majzner et al. Nature Med. 2018
SU-DIPG13P*

DPT

Percent survival

CD19-CAR n=22
GD2-CAR n=23

p <0.0001
GD2 CAR T cells widely infiltrate the brain after intravenous administration
GD2-CAR Trial in Pediatric Solid Tumors Slated to Open later in 2019

- Neuroblastoma, osteosarcoma, DIPG
- GD2-CAR Incorporates a suicide domain that can be “drug activated” in the event of untoward toxicity
- Children with DIPG will be monitored closely by a team of immunotherapists, neurointensivists and neurosurgeons
- We will seek to identify the most appropriate patients in terms of disease burden/life expectancy
## Other CAR T cell therapies in the clinics

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>Target</th>
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<tbody>
<tr>
<td>Multiple Myeloma</td>
<td>BCMA, CD38, CD138</td>
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<tr>
<td>Lung cancer</td>
<td>HER2</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>CEA</td>
</tr>
<tr>
<td>Brain tumors</td>
<td>EGFR, HER2, GD2, IL13ra2</td>
</tr>
<tr>
<td>AML</td>
<td>CD33, CD123</td>
</tr>
<tr>
<td>Hodgkin Lymphoma</td>
<td>CD30, CD20</td>
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<tr>
<td>Pancreatic</td>
<td>Mesothelin</td>
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<tr>
<td>Neuroblastoma</td>
<td>CD171, GD2</td>
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<tr>
<td>T cell ALL, T LL</td>
<td>CD5</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>Glypican 3, mesothelin, ROR1, CD70</td>
</tr>
</tbody>
</table>
Immune Checkpoint Inhibitors in Cancer

Taking the breaks off the immune system

2018 Nobel Prize in Medicine Awarded to 2 Cancer Immunotherapy Researchers

The Nobel Prize for Physiology and Medicine was awarded to James P. Allison, left, and Tasuku Honjo on Monday for their work on cancer research.

Jonathan Nackstrand/Agence France-Presse — Getty Images
Immune Checkpoint Inhibitors have made a huge impact in oncology.
Pediatric tumors have low mutational burden

Checkpoint inhibitors work best when the target malignancy is highly mutated

Le et al, NEJM, 2015
Or in some cancers that upregulate PD-L1 or PD-L2

**B Change in Tumor Burden**

<table>
<thead>
<tr>
<th>Change (%)</th>
<th>Stable Disease</th>
<th>Partial Response</th>
<th>Complete Response</th>
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Individual Patient Data (N=23)

Ansell et al NEJM 2015

Stanford University
Bi-allelic Mismatch Repair Deficiency

- Homozygous germline mutations in one mismatch repair gene (PMS2, MLH1, MSH2, MSH6)
- Most penetrant cancer predisposition syndrome described to date (100% cancer incidence within first two decades of life)
- Not uncommon in populations with high consanguinity
- Estimated 40% of pediatric GBM in Jordan is associated with bMMRD
- High grade bMMRD tumors are “hypermutant”
  - high levels of single nucleotide variants (e.g. neoantigens)
  - > 100 mutations/exome
  - Estimated acquisition of 600 new mutations/cell division

Bouffet et al. JCO 2016
Response of Recurrent GBMs to Nivolumab in Siblings with bMMRD

Sibling #1
~24,000 mutations/exome

Sibling #2
~22,000 mutations/exome

Bouffet et al. JCO 2016
PD-1 inhibition may have limited applicability to pediatric cancers

Majzner et al., Cancer, 2017

Ewing sarcoma (25)
Osteosarcoma (20)
Rhabdomyosarcoma (53)
Medulloblastoma (40)
Neuroblastoma (118)
Glioblastoma multiforme (14)
Burkitt lymphoma (10)
In children, responses seen in Hodgkin’s Disease.
There is more to learn about how the immune system interacts with cancer!

Keren et al, Cell, 2018
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  • What are chimeric antigen T cells
  • Use in heme malignancies and solid tumors
  • Limitations in Efficacy
  • Toxicity concerns

• Immune Checkpoint Inhibition for anti-cancer effect
  • Successes and Challenges
Thank you!

- Cancer Cellular Therapy
  - Crystal Mackall
  - David Miklos
  - Liora Schultz
  - Lori Muffly
  - Tina Baggott
  - Sharon Mavroukakis
  - Katie Kong
  - Cell Therapy Lab
  - LCGM
  - Apheresis
  - Administration

- Correlative Science Unit
  - Sean Bendall
  - Bita Schaff
  - Rohit Gupta
  - Holden Maecker

- Davis Lab: Pablo Domizi, Astraea Jager
- David Barrett
- Monje Lab: Michelle Monje
  - Chris Mount
- Mackall Lab
- Patients and Families