CAR T cell Therapy

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MacMillan Group Meeting

March 25, 2020
Outline

Introduction to CAR T cell Therapy

- current cancer treatment options
- introduction to immunotherapy
- definition of a CAR T cell
- building a CAR

CARs in the Clinic

- how CARs are prepared and administered
- preclinical studies
- clinical trials leading to FDA approvals

Current Limitations and Moving Forward

- toxicity
- difficulties extending to solid tumors
- CARs beyond cancer
**Blood Cancer Survival Rates Over Time**

- Leukaemias: 31%
- Brain and spinal tumor: 26%
- Lymphomas: 10%
- Soft tissue sarcomas: 7%
- Kidney tumors: 6%
- Neuroblastoma: 5%
- Retinoblastoma: 4%
- Liver tumor: 4%
- Bone tumors: 3%
- Germ cell tumor: 3%
- Other: 4%

**Cancer Incidence Rates: Patients 0–14 yrs (2009–2012)**

- Myeloma: 12%
- Hodgkin Lymphoma: 25%
- Non-Hodgkin Lymphoma: 47%
- Leukemia: 34%

**Five Year Survival Rates by Year of Diagnosis**

Significant increases in survival rate over time:

- Better diagnostics and treatment options

**Surveillance, Epidemiology, and End Results (SEER), Cancer Statistics Review, 1975-2015, National Cancer Institute, 2018**
Common Treatment Options for Acute Lymphocytic Leukemia

Typically first course of action

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Radiation Therapy</th>
<th>Bone Marrow Transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>ex: imatinib (Gleevec), dasatinib (Sprycel)</td>
<td>primarily used if cancer spreads to CNS</td>
<td>patients that may require high doses of chemo</td>
</tr>
<tr>
<td>total treatment time: ~2 years</td>
<td>often given in addition to chemo</td>
<td>if nonresponsive to treatment</td>
</tr>
<tr>
<td>poor selectivity → toxicity, including for healthy immune cells</td>
<td></td>
<td>can have severe side effects</td>
</tr>
</tbody>
</table>

While 80–90% of adults go into complete remission, about half of the patient relapse.

Sources: American Cancer Society, Leukemia & Lymphoma Society
Can we use our own immune system to fight cancer?
An (very brief) Overview of Normal Immune Response to “Invader”

Example: Innate and Adaptive Immune Responses in Asthma

a complicated and complex immune response is deployed onto “invaders”

An (very brief) Overview of Normal Immune Response to “Invader”

MHC class I

- present on most cell surfaces
- am I “self” or “invader”?

MHC: Major histocompatibility complex, TCR: T cell receptor

An (very brief) Overview of Normal Immune Response to “Invader”

MHC: Major histocompatibility complex, TCR: T cell receptor

if MHC presents “invader” (antigen) peptide on cell surface
the TCR can recognize as “invader” and create immune response

CD28

MHC class I

present on most cell surfaces
am I “self” or “invader”?

MHC: Major histocompatibility complex, TCR: T cell receptor

Sadelain, M.; Rivière, I.; Riddell, S. Nat. Rev. 2017, 545, 423.
An (very brief) Overview of Normal Immune Response to “Invader”

MHC class I
present on most cell surfaces
am I “self” or “invader”?

MHC class II
present on Antigen Presenting Cells (APC)
display antigen to the immune system

if MHC presents “invader” (antigen) peptide on cell surface
the TCR can recognize as “invader” and create immune response

MHC: Major histocompatibility complex, TCR: T cell receptor

Sadelain, M.; Rivière, I.; Riddell, S. Nat. Rev. 2017, 545, 423.
Ability of Cancer Cell to Evasion the Immune System

Disrupt presentation of tumor antigens to immune system
- Tumor cell
- APC
- Downregulation of MHC expression
- Suppression of APC

Tumor microenvironment
- T cell checkpoint dysregulation
- Recruiting immunosuppressive cells
- Release immunosuppressive factors
- Tregs
- MDSCs

Cancer cells can evade or suppress immune response

Davies, M. Cancer Management and Research, 2014, 6, 63.
Can we use our own immune system to fight cancer?

Yes, by outsmarting the cancer cells
Immunotherapy Overview

Immunotherapy: recruit the body’s own immune system to target and eliminate cancer

Immune Checkpoint Blockade

Adoptive Cell Transfer (ACT)

Anti-Cancer Vaccine

Tumor-Targeting Monoclonal Antibodies

Immunostimulatory Cytokines

Subramanian, K. Targeted Therapies in Oncology, 2019.
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Subramanian, K. Targeted Therapies in Oncology, 2019.
Types of Adoptive Cell Transfer (ACT)

TILs, TCR T cells, and CAR T Cells

T cell receptor (TCR) T cells

- Extract T cells and engineer the receptor to identify antigen (TCR) or design receptor (CAR) to identify (CAR) the antigen, expand, and reinfuse

Chimeric antigen receptor (CAR) T cells

- Extract and identify lymphocytes capable of tumor cell recognition, expand, and reinfuse

Tumor infiltrating lymphocytes (TILs)

- Extract and identify lymphocytes capable of tumor cell recognition, expand, and reinfuse

a “living therapy”: T cells can expand in vivo after administration

Chimeric Antigen Receptor (CAR) T Cells

CAR Concept

Design a receptor that will directly bind to an antigen or cancer biomarker

An advantage of CAR T cells: does not rely on MHC to present antigen

*remember the cancer cells can downregulate MHC expression!

how do we go about designing a receptor?

Chimeric Antigen Receptor (CAR) T Cells: Lessons from Biology

How does nature design functional T cells?

1st generation CARs aim to mimic TCR recognition/activation, though activation isn't sufficient for in vivo persistence.

2nd/3rd generation CARS: add costimulatory elements to promote long-term persistence and proliferation.

Chimeric Antigen Receptor (CAR) T Cells: A Timeline

1989 - design of first chimeric receptor

1990’s - T cell engineering, 1st and 2nd gen CARs (main players:)

Dr. Michel Sadelain
Memorial Sloan Kettering

Dr. Carl June
University of Pennsylvania

Dr. Zelig Eshhar
Weizman Institute

Dr. Zelig Eshhar
Weizman Institute

2000’s

successful preclinical studies
with anti-CD19 CARs

2010’s

successful clinical studies with
anti-CD19 CARs in humans

2017

First CART therapies approved:
Kymriah and Yescarta

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**CAR T Cells: Preclinical Studies with CD19-targeted CARs in mice**

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**CD19 as attractive target**

- expressed on most B cell malignancies: including ALL, CLL, and non-Hodkin’s lymphoma
- also expressed on normal B cells (which are temporarily dispensible)

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**CD19-targeted CARs for treatment of ALL**

Adding co-stimulatory domains conferred persistence and led to increased anti-leukemic efficacy

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Trials Leading to FDA Approval of Kymriah

Pilot Clinical Trial with 3 Patients with Relapsed or Refractory Chronic Lymphoid Leukemia (CLL)

Patient 1:

- diagnosed stage I CLL
  - 1996
- 2 cycles chemo
  - 2002
- 4 cycles chemo
  - 2006
- 4 cycles chemo
  - 2009

- day 1 (baseline)
- day 23
- 6 months

bone marrow biopsy

no evidence of CLL in the bone marrow

CART19 cells (with 41-BB co-stimulatory domain) persisted for at least 6 months

no normal B cells detected after treatment for at least 6 months after treatment

major toxicity: tumor lysis syndrome

Trials Leading to FDA Approval of Kymriah

5 Year Data on Pilot Clinical Study with 14 Patients with Relapsed Refractory CLL with Novartis

June and coworkers use anti-CD19 CARs with 4-1BB co-stimulatory domain

CAR T cell expansion 14– 49 months in patients with CR

B cell aplasia cytokine release syndrome

Trials Leading to FDA Approval of Kymriah

5 Year Data on Pilot Clinical Study with 14 Patients with Relapsed Refractory CLL with Novartis

Trials Leading to FDA Approval of Kymriah

CD19 CARs for Sustained Remission in ALL with Dr. June and Novartis

**trial for relapsed acute lymphoblastic leukemia (ALL)**

- # of patients: 25 patients ages 5–22, 5 patients ages 26–60
- # patients in complete remission: 27 (90%) after one month, 19 remained in remission
- 6 month overall survival: 78%
- B cell aplasia: 73%
- Probability of CAR persistence at 6 months: 68%
- Severe cytokine release syndrome (CRS): 27%

_FDA grants orphan drug designation to Kymriah for treatment of ALL (2014)_

Trials Leading to FDA Approval of Kymriah

Phase 2 Trial: 75 pediatric and young adult patients with relapsed or refractory B cell ALL

Adverse Events within 8 Weeks of Infusion

<table>
<thead>
<tr>
<th>Type of Events</th>
<th>Any Grade</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any adverse event of special interest</td>
<td>67 (89)</td>
<td>26 (35)</td>
<td>30 (40)</td>
</tr>
<tr>
<td>Cytokine release syndrome</td>
<td>58 (77)</td>
<td>16 (21)</td>
<td>19 (25)</td>
</tr>
<tr>
<td>Neurologic event</td>
<td>30 (40)</td>
<td>10 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td>32 (43)</td>
<td>16 (21)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>26 (35)</td>
<td>24 (32)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Cytopenia not resolved by day 28</td>
<td>28 (37)</td>
<td>12 (16)</td>
<td>12 (16)</td>
</tr>
<tr>
<td>Tumor lysis syndrome</td>
<td>3 (4)</td>
<td>3 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

First FDA approved cell and gene therapy (US) approved Aug 30, 2017

for patients up to 25 years old with ALL that is refractory or in second or later relapse

Diffuse Large B-cell Lymphoma (DLBCL) most common subtype of non-Hodkin lymphoma

- chemotherapy (round 1): 20-40% fail initial treatment
- stem cell transplant: limited options for relapsed or refractory patients
- chemo (round 2): 40-60% fail treatment

Overall survival of 112 patients with relapsed DLBCL:
- median overall survival: 6.3 months
- only 10–25% of patients with relapsed or refractory large B-cell lymphoma are cured

636 patient-study with refractory large B-cell lymphoma:
- complete response rate: 7%
- median overall survival: 6.3 months

Blood 2017, 130, 1800.

Trials Leading to FDA Approval of Yescarta

Dr. Rosenberg (NCI) with Kite Pharma using anti-CD19 CAR (with CD28 co-stimulatory domain)

study involved 15 patients with chemo-refractory B-cell malignancies: 8/15 in complete remission, 4/15 partial remission

“Patient No. 2 was diagnosed with PMBCL. She underwent treatment with six cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP)…radiation therapy, which resulted in a CR that lasted 5 months before relapse. Next, she received two cycles of … chemotherapy… Finally, she received a regimen of rituximab, cytarabine, and methotrexate, which also led to SD. Patient No. 2 was treated on the anti-CD19 CAR protocol and entered a CR that is ongoing after 22 months”

Patient with primary mediastinal B-cell lymphoma (PMBLC)

had 10 prior regiments before CAR
ongoing complete response (CR) after 12 months

Patient with primary DLBCL

had two rounds of intensive chemo before CAR
despite CR after treatment, lymphoma recurred after 6 months

acute toxicity occured in some patients, though resolved within 3 weeks
one patient died suddenly after 16 days

Trials Leading to FDA Approval of Yescarta

Follow Up on Long-Duration of Complete Remission from Previous Study

4/5 patients in complete remission had long term duration of remission > 3 years
Trials Leading to FDA Approval of Yescarta

Phase II Trial for Refractory Large B-Cell Lymphoma

54% of the 111 patients had a complete response
overall survival after 18 months was 52%

13% had > Grade 3 cytokine release syndrome, and 28% with neurological events
3 patients died during treatment

Trials Leading to FDA Approval of Yescarta

approved Oct 18, 2017

Yescarta®
(axicabtagene ciloleucel)
Suspension for IV infusion

for adults with large B-cell lymphoma
that is refractory or in second or later relapse

adverse side effect - largely similar after 2 years

- grade 3 or worse adverse events: 98%
- all events were manageable and largely reversible
  - grade 3 or worse CRS: 11%
  - grade 3 or worse neurological events: 32%

two year overall survival: 51%
w/ conventional therapies: 20%

How is CAR T cell therapy administered?
CAR T cell Therapy in the Clinic

**Selection**
- Leukapheresis
- Elutriation/Selection

**Expansion**
- Transduction

**Harvest**
- De-bead
- Wash
- Formulate
- Fill

**Manufacturing Process**
- Collect patient’s white blood cells
- Isolate and activate T cells
- Engineer T cells with CAR gene
- Grow and expand number of T cells
- Infuse same patient with engineered T cells

Leukapheresis: remove cells from patient’s bodies and centrifuge
CAR T cell Therapy in the Clinic

Leukapheresis: remove cells from patient’s bodies and centrifuge

potential issues in this step:

- low lymphocyte blood count for patient treated with chemo
- impure samples may inhibit growth in culture or may contain tumor cells
CAR T cell Therapy in the Clinic

Elutriation and Selection: separation based on cell size and density followed by immunomagnetic bead selection

Fesnak, A. D.; Suhoski Davis, M. M.; Levine, B. L. Nat. Prot. 2017
CAR T cell Therapy in the Clinic

Viral Transduction: using viral vectors from retroviruses or lentiviruses to deliver desired CAR gene

potential issues in this step:

gene modification may lead to some ex vivo cytotoxicity leading to cell loss during manufacturing

Fesnak, A. D.; Suhoski Davis, M. M.; Levine, B. L. Nat. Prot. 2017
Expansion and T cell harvest: activate T cells for proliferation, de-bead, wash and package

overall time: ~ 2 weeks

during this time: patients receive chemotherapy to kill remaining lymphocytes

Fesnak, A. D.; Suhoski Davis, M. M.; Levine, B. L. Nat. Prot. 2017
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Current Limitations to CAR T cell Therapy

Overcoming Treatment-Related Toxicities

Toxicity related to T cell activation:
- Systemic release of high cytokine levels

on-target, off tumor effect
- CAR interact with target on non-malignant cells

Overcoming Treatment-Related Toxicities

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Overcoming Treatment-Related Toxicities

Cytokine release syndrome:
a large number of T cells are activated and release inflammatory cytokines which activate more T cells

Treatments:

IL-6 among core cytokines consistently elevated in CRS
treatment with anti-IL-6 antibody (tocilizumab)

Engineering Solutions: Fine-tuning the CAR

co-stimulatory domain: 4-1BB vs. CD28

CD28: more rapid onset on activity and subsequent exhaustion

4-1BB: lower peak levels of expansion, increased endurance, lower risk CRS

hinge and transmembrane sequence

alterations led to slower proliferation, while retaining potency: 6/11 with complete remission

no CRS > grade 1 observed

Nat. Med. 2019, 25, 947

Overcoming Treatment-Related Toxicities: Engineering Solutions

Developing On/Off switches, suicide genes, and engineering cytokine antagonists


*BMC Biotech.* **2019**, *19*, 44.


knockout cytokine genes or

express cytokine antagonists that neutralize relevant cytokines

Overcoming Treatment-Related Toxicities

Toxicity related to T cell activation:
- Systemic release of high cytokine levels

**on-target, off tumor effect**
- CAR interact with target on non-malignant cells

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Overcoming Treatment-Related Toxicities: On-Target Off-Tumor

Methods for Better On-Target, On-Tumor Efficacy

Affinity Tuning

take advantage of antigen expression levels that are “greater than” those on normal cells
target with a lower affinity CAR

downregulation of target antigen as mechanism of resistance

Overcoming Treatment-Related Toxicities: On-Target Off-Tumor

“AND” Logic: Two Cell-Surface Antigens Required for CAR Activation

**Split CARS**
- Tumour cell
- Antigen A
- CD3ζ linked to receptor for antigen A
- Co-stimulatory domain linked to receptor for B
- Activation only occurs when both CD3ζ and co-stimulatory domains activated

**SynNotch CARs**
- Tumour cell
- Antigen A
- Antigen B
- Synthetic notch receptor engineered to recognize antigen C
- Activates TF encoding a CAR for antigen D

*Cell 2016*, 164, 770.


Overcoming Treatment-Related Toxicities: On-Target Off-Tumor

“NOT” Logic: Adding Inhibitory Signalling

**Inhibitory CART (iCAR)**

- Antigen A
- Antigen B

![Diagram of T cell activation](image)

**CRISPR/Cas9-based approach**

- CD33 is a common target for acute myeloid leukemia (AML)
- CD33 is expressed on most myeloid cells but non-essential if knocked-out
- Knock-out CD33 from stem cells
- Anti-CD33 CARs selectively target CD33+ AML without killing non-malignant cells in a mouse model


antigen B is expressed *only* on non-malignant cells
use inhibitory domains derived from immune-checkpoint proteins, e.g. PD-1
thereby only killing tumor cells

*Cell* 2018, 173, 1439.

Overcoming CAR T Resistance with Multi-Target Strategy

**antigen escape as resistance mechanism**
complete of partial loss of target antigen expression from cancer cells

7–25% of anti-CD19 CAR patients relapse with CD19− disease

targeting multiple antigens: combinatorial strategy
numerous worldwide clinical trials
co-targeting e.g., CD19/CD20, CD19/CD22

CAR.BiTEs
modify CAR to excrete bi-specific T cell engager (BiTE)
which recruit T cells to tumor

Majzner, R. G.; Mackall, C. L. Cancer Discov. 2018, 8, 1219.
Overcoming Hostile Tumor Microenvironment

major challenge in CAR T therapy: developing successful therapy for solid tumors

need to engineer new CARs to successfully move to solid tumors

Targeting Solid Tumors: Overcoming Hostile Tumor Microenvironment

- **overcoming antigen heterogeneity**
  can target multiple antigens: ex: CART.BiTEs

- **bringing to the CAR to the solid tumor**
  direct injection into tumor, (may not always be possible)

- **overcoming T cell inhibitory signalling**
  co-administer anti-PD-1 antibodies or engineer a disruption of PD-1 pathway into CAR

- **penetrating the solid tumor microenvironment**
  program the CARs to secrete tumor extracellular matrix-modifying enzymes

- **altering the milieu of the tumor microenvironment**
  armored CARs and TRUCKS (T cell redirected for universal cytokine killing)
  T cells secrete cytokine or immunomodulatory ligand to alter inflammatory microenvironment

For comprehensive reviews on the area, see:
Global Clinical Trials for CAR T cell Therapy

596/1203 trials targeting solid tumors

CD19 still primary target

CARs: Looking Forward

“off-the-shelf” T cells: allogeneic cell sources (from a healthy donor) to minimize cost of cell manufacturing

CARs beyond cancer therapies: including infection diseases, HIV, autoimmune diseases

Sadelain, M.; Rivière, I.; Riddell, S. Nat. Rev. 2017, 545, 423.
Questions?