1. Why CAR T Therapy?
   - How the normal immune system functions
   - T-cell antitumor responses and mechanisms by which cancer cells escape immune surveillance

2. How CAR T Therapy Fits into the World of Immuno-oncology (…as of 2017)
   - Timeline of Immunotherapy
   - Allogeneic transplant, high-dose IL-2, and donor lymphocyte infusions
   - Monoclonal antibodies and vaccines: INFα and anti-CD20 targeted therapies
   - Immune modulators and checkpoint inhibitors
   - How to optimally harness antitumor immunity: the role of CAR T cells

3. How CAR T Therapy Works
   - CAR T cells: Mechanism of Action
   - Chimeric Antigen Receptors (CARs)
   - Advantages of CAR T Therapy
   - CAR T Therapies: Clinical Data
     - Acute Lymphoblastic Leukemia
     - Non-Hodgkin Lymphoma
     - Multiple Myeloma
   - Bispecific T cell engager (BiTE) antibodies: the difference between CARs and BiTEs
Table of Contents

4. CAR T therapy: Manufacturing to Infusion
   - Apheresis / leukapheresis and current challenges
   - Bridging therapy
   - Manufacturing and vector delivery of CARs
   - Lymphodepletion and infusion
   - 99

5. Clinical Management of the CAR T Therapy Patient
   - Toxicity and Management in CAR T Therapy
   - Best practices
   - 120

6. Institutional Considerations
   - Identification of patients who are appropriate for CAR T therapy
   - Insights into best practices of experienced centers
   - 182

7. CAR T Clinical Development
   - Current targets being investigated
   - Future of CAR T Therapy
   - 192
WHY CAR T THERAPY?

MODULE 1
MODULE 1: Outline

1. How the normal immune system functions
   • CD8 T cells, CD4 T cells, T-cell receptors and the CD3 zeta signaling domain, Co-stimulatory receptors
   • B cell functions, CD19 expression

2. T-cell antitumor responses and mechanisms by which cancer cells escape immune surveillance
   • How the normal immune system functions
   • The tumor microenvironment and mechanisms by which cancer cells escape immune surveillance
How the Normal Immune System Functions: Overview of T Cells
Cell Types of the Immune System

Multipotential hematopoietic stem cell (HSC)

- Common myeloid progenitor
  - Myeloblast
    - Basophil
    - Eosinophil
    - Neutrophil
  - Megakaryocyte-erythrocyte progenitor
    - Megakaryocyte
    - Thrombocyte
  - Granulocytes
- Common lymphoid progenitor
  - Pro-T cell
    - Pre-T cell
  - Pro-natural killer cells
  - Natural killer cells
  - Pro-B cells
    - Pre-B cells
  - B cells

CD8
CD4
Th1
Th2
**T Cell Development**

The thymus generates a naive T cell pool from which the memory repertoire is derived.

**CD4/CD8 T Lineage Decision**

- **Pre T Cell**
- **CD4-8-**
  - Pre TCR
  - Naïve thymic emigrant CD4+ or CD8+ T cell
  - Repeat Exposure to Antigen
  - Antigenic Stimulation
- **Bone marrow**
- **Thymus**
- **Naïve thymic emigrant**
- **Effector T cell** CD4+ or CD8+
- **Memory T cell** CD4+ or CD8+
Naïve T Cell Activation

- Naïve T cells can be either CD4 or CD8 T cells
- Although they are capable of responding to target antigens, they have not yet encountered them
- Naïve T cells become activated by two signals:
  - Signal 1: Recognition: TCR binds to MHC (or HLA):antigen
  - Signal 2: Co-stimulation: CD28 binds to its ligand on APC

T Cell Subsets: CD4 vs CD8

- During T cell development, T cells mature into 1 of 2 types:
  - CD4 helper cells aid the effector functions of other cells
  - CD8 CTLs directly kill target cells that display antigen they are programmed to recognize (i.e., tumor cell or virus-infected cell)
Activated T Cells: Effector CD4 Helper T Cells

- Express CD4 on their surface
- Produce cytokines to impact other immune cells
  - Activate macrophages to destroy intracellular pathogens
  - Stimulate B cells to produce antibodies
  - Promote mast cell and eosinophil growth and differentiation

Activated T Cells: Effector CD8 CTLs

CTLs induce apoptosis through multiple mechanisms, including:

- Release of cytotoxic granules containing perforin and granzyme B
- Surface receptor engagement such as Fas/FasL
Cytotoxic T Lymphocytes are Specific and Potent Effector Cells

Ultrastructure of CTL-mediated apoptosis

The CTL protrudes deeply into cytoplasm of melanoma cell
Activated T Cells: Memory T Cells

- Memory T cells are a repository of antigen-experienced T cells that accumulate over a lifetime
  - Subset of activated T cells
  - CD4 or CD8 T cells
  - Respond faster and more effectively to antigens
  - Persist even in the absence of antigen
  - Part of the biological basis of vaccination

<table>
<thead>
<tr>
<th>Effector Memory T Cells</th>
<th>Central Memory T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Reside in recently infected tissues</td>
<td>• Reside in secondary lymphoid tissues (e.g. lymph nodes)</td>
</tr>
<tr>
<td>• Provide immediate effector response to antigen</td>
<td>• Capable of self-renewal</td>
</tr>
<tr>
<td></td>
<td>• Rapidly proliferate and differentiate into effector memory T cells</td>
</tr>
</tbody>
</table>
Primary and Memory Immune Responses

In reality, the T cell subsets are quite complex...

- **Naïve thymic emigrant CD4+ or CD8+ T cell**
- **Antigenic stimulation**
- **Thymus**
- **Th17 T cell**
  - inflammation
  - IL-17
- **TH1 CD4+ T cell**
  - antiviral immunity
  - IL-2, IFN-γ, TNF-α
- **TH2 CD4+ T cell**
  - parasitic immunity
  - IL-4, IL-5, IL-10
- **CD8+ Cytotoxic T cell**
  - (lyses target cells)
  - Regulatory CD4+ T cell producing IL-10/TGFβ
T Cell Receptors and Co-stimulatory Ligands and Receptors
• TCR binds to antigen-major histocompatibility complex (MHC or HLA) on antigen-presenting cells (APCs), which present intracellular antigen to T cells

• TCR binding to its MHC (or HLA) ligand causes downstream signaling through CD3, a membrane-bound signal transduction molecule, which regulates a number of important cellular functions

• The function of MHC (or HLA) is helping the body differentiate self from foreign primarily to avoid autoimmunity

Peptide Processing, HLA binding, and TCR Recognition

Morris EC, Stauss HJ. Blood 2016;127:3305-3311
Costimulatory Ligands and Cytokines

Signal 1: T cell anergy
- TCR
- Ag
- MHC class II

Signal 2: T cell activation
- CD28
- CD80/CD86

Signal 3: Cytokines
- CD28
- 4-1BB
- OX40
- IL-12
- IL-15
- IL-2

Co-stimulatory and Co-inhibitory Receptors
How the Normal Immune System Functions: Overview of B Cells
B Cell Development
B Cell Receptor (BCR) is a Membrane-Bound Antibody
B Cell Function

• B cells recognize soluble antigen and present it to helper T cells, which promote B cells to produce antigen-specific antibodies

Plasma Cells

• Mature effector B cells
• Do not express CD19 or CD20
• Produce large quantities of antibodies that bind to antigens serving multiple adaptive immunity functions:
  - Antigen inactivation (blocking pathogen binding to host cells)
  - Fixation of complement (leading to cell lysis)
  - Facilitation of phagocytosis (marking for destruction by phagocytes)

# Summary: Similarities and Differences between T cells and B cells

<table>
<thead>
<tr>
<th>Similarities</th>
<th>Differences</th>
</tr>
</thead>
</table>
| • Antigen receptor on surface (TCR or BCR)  
• Recognize single, specific antigen  
• Expand through clonal selection | • BCR directly recognizes and binds to surface antigens. TCR only recognizes new antigens that are bound to MHC (or HLA) and presented by APCs  
• B cells secrete antibodies. T cells secrete cytokines |
T-Cell Antitumor Responses and Mechanisms by which Cancer Cells Escape Immune Surveillance
T Cell Antitumor Responses

1. Cancer antigen presentation (dendritic cells/APCs)
2. Recognition of cancer cells by T cells (CTLs, cancer cells)
3. Priming and activation (APCs & T cells)
4. Trafficking of T cells to tumors (CTLs)
5. Infiltration of T cells into tumors (CTLs, endothelial cells)
6. Release of cancer cell antigens (cancer cell death)
7. Killing of cancer cells (Immune and cancer cells)
The Role of Cytokines in the Antitumor Immune Response
The Three E’s of Cancer Immunoediting

ELIMINATION
The immune system has the ability to eliminate cancer cells. Highly antigenic cancer cells are recognized and eliminated by the immune system.

EQUILIBRIUM
Cancer cells that escape the elimination phase enter the equilibrium phase. These transformed cells are poorly immunogenic and have the ability to coexist with immune cells.

ESCAPE
Cancer cells have acquired resistance by:
- Poor antigenic expression
- Immunosuppressive cytokines
- MDSCs
- Expression of PD-L1

Tumor-Derived Immune Suppression

- Tumors go to great lengths to evade the immune response
- Systematic studies have identified multiple mechanisms cancers employ to defeat the immune response
  - Immunosuppressive cytokines: TGF-β, IL-4, -6, -10
  - Immunosuppressive immune cells: T-regs, macrophage
  - Disruption of immune activation signaling: loss of MHC receptor, IDO production
Mechanisms by which tumors avoid immune recognition

<table>
<thead>
<tr>
<th>Low immunogenicity</th>
<th>Tumor treated as self antigen</th>
<th>Antigenic modulation</th>
<th>Tumor-induced immune suppression</th>
<th>Tumor-induced privileged site</th>
</tr>
</thead>
<tbody>
<tr>
<td>No peptide:MHC ligand No adhesion molecules No co-stimulatory molecules</td>
<td>Tumor antigens taken up and presented by APCs in absence of co-stimulation tolerize T cells</td>
<td>Antibody against tumor cell-surface antigens can induce endocytosis and degradation of the antigen. Immune selection of antigen-loss variants</td>
<td>Factors (e.g., TGF-β) secreted by tumor cells inhibit T cells directly. Induction of regulatory T cells by tumors</td>
<td>Factors secreted by tumor cells create a physical barrier to the immune system</td>
</tr>
</tbody>
</table>

Figure 15-14 Immunobiology, 7 ed. (© Garland Science 2008)
The Hostile Tumor Microenvironment

• The tumor microenvironment contains multiple inhibitory factors designed to potentially suppress effector T cells:
  – CD4^+ CD25^{hi} FoxP3^+ regulatory T cells (Tregs)
  – MDSCs
  – TAMs
  – Expression of inhibitory ligands by tumor (PD-L1)
  – Tumor secretion of T cell suppressive cytokines (TGF-β & IL-10)
CTLA-4 and PD-1/L1 Inhibitory Signals

Primming phase (lymph node)

T-cell migration

Effector phase (peripheral tissue)

CTLA-4 and PD-1 Inhibitory Signals

HOW CAR T THERAPY FITS INTO THE WORLD OF IMMUNO-ONCOLOGY (...AS OF 2018)
MODULE 2: Outline

1. Timeline of Immunotherapy
2. Allogeneic transplant, high-dose IL-2, and donor lymphocyte infusions
3. Monoclonal antibodies and vaccines: INFα and anti-CD20 targeted therapies
4. Immune modulators and checkpoint inhibitors
5. How to optimally harness antitumor immunity: the role of CAR T cells
Timeline of Advances in Immunotherapy

**1950**

*One Hundred Patients With Acute Leukemia Treated by Chemotherapy, Total Body Irradiation, and Allogeneic Marrow Transplantation*

By E. Donald Thomas, C. Dean Buckner, Marcel Bensj, Regional A. Cull, Alexander Pater, Nancy Fournous, Brian W. Goodell, Robert O. Hickman, Kenneth G. Lerner, Paul E. Neiman, George E. Sline, Jean E. Sanders, Jack Singer, Mary Stevens, Robin Duck, and Paul L. Weiden.

**1960**

*Autologous BMT*

**1970**

*Donor Lymphocyte Infusions*

1. *Donor Leukocyte Infusions in 140 Patients With Relapsed Malignancy After Allogeneic Bone Marrow Transplantation*

2. *Donor Leukocyte Transfusions for Treatment of Recurrent Chronic Myelogenous Leukemia in Marrow Transplant Patients*

**1980**

*Tumor Infiltrating Lymphocytes*

- INF-α
- IL-2

**1990**

*Rituximab (Anti-CD20)*

**2000**

*Sipuleucel-T*

**2010**

*Brentuximab Vedotin (Anti-CD30)*

**2015**

*CAR T Therapies*

*Checkpoint Inhibitors*

**Tumor Specificity Increases Over Time**
## Examples of Successful Cancer Immunotherapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Indication</th>
<th>Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allogeneic hematopoietic stem cells</td>
<td>Hematologic malignancies</td>
<td>Curative</td>
</tr>
<tr>
<td>High-dose interleukin-2</td>
<td>Metastatic melanoma, metastatic renal cell adenocarcinoma</td>
<td>Occasionally curative as monotherapy</td>
</tr>
<tr>
<td>Type 1 interferon</td>
<td>Superficial bladder cancer</td>
<td>Curative as monotherapy</td>
</tr>
<tr>
<td>BCG</td>
<td>Superficial bladder cancer</td>
<td>Curative as monotherapy</td>
</tr>
<tr>
<td>Antitumor monoclonal antibodies</td>
<td>Lymphomas (rituximab) HER2+ breast cancer (trastuzumab) Colorectal cancer (cetuximab)</td>
<td>Durable responses; improved cure rates in combination with chemotherapy</td>
</tr>
<tr>
<td>Sipuleucel-T vaccine</td>
<td>Prostate cancer</td>
<td>Improved time to progression</td>
</tr>
<tr>
<td>Anti-checkpoint monoclonal antibodies</td>
<td>Metastatic melanoma (anti-CTLA-4, anti-PD-1, and anti-PD-L1) Basal cell adenocarcinoma (anti-PD-1) Lung (anti-PD-1), bladder (anti-PD-1)</td>
<td>Durable objective responses</td>
</tr>
<tr>
<td>CAR T cells</td>
<td>Leukemia, lymphoma</td>
<td>Responses in refractory disease</td>
</tr>
</tbody>
</table>
Hematopoietic Stem Cell Transplantation

Two Distinct Therapies:

1. High dose chemotherapy with stem cell rescue (Autologous Transplantation)
   - Benefit relies on chemo-sensitivity of cancer

2. Allogeneic HSCT
   - Combines chemotherapy and immunotherapy
   - Can cure patients with chemo-refractory disease
   - Graft-v-tumor (GVT) \(\rightarrow\) Prevents Relapse
   - Graft-v-host disease (GVHD) \(\rightarrow\) Toxicity
Autologous and Allogeneic Transplant Timeline (1957-2007)

Evidence of Graft-versus-Tumor Effect Supporting Allogeneic HSCT

Probability of Relapse after HLA-Identical Sibling Transplants for Early Leukemia

High-Dose Interleukin-2 Provides Benefit to Some Patients with Metastatic Renal Cell Carcinoma

Efficacy of Donor Lymphocyte Infusions Provides Rationale for T Cell Therapy

CLL Progression Responds to Donor Lymphocyte Infusion

![Graph showing VH IgG Quantification and CD3 Donor Chimerism over Months after Transplant]
Donor Lymphocyte Infusions are Associated with Poor Efficacy in ALL

Response to DLI in Patients with Recurrent Leukemia after Bone Marrow Transplant

Hematologic Remissions Demonstrated with Interferon-Alfa in Newly Diagnosed CML

Survival of Patients with CML Treated with Interferon Alfa-2a or Conventional Chemotherapy (Hydroxyurea or Busulfan)

The Structure of Antibodies

© 1996 Mike Clark

The IgG Molecule

Fab arm waving
Fab elbow bend
Fab rotation
Fc wagging

© Mike Clark 1994

Antigen binding sites,
Complementarity Determining Region

Variable antigen binding region
Light chain
Heavy chain

Variable antigen binding region
Light chain
Constant region
Heavy chain

The antibody molecule
Rituximab Targets CD20 Specifically Expressed on The Surface of B Cells
Ibrutinib Inhibits B Cell Receptor Signaling

- Ibrutinib is a BTK inhibitor
  - BTK mediates BCR and chemokine signaling, driving B cell adhesion, migration, proliferation and survival
  - BTK is activated and frequently overexpressed in B cell malignancies
- Ibrutinib is indicated for the treatment of patients with CLL, SLL, MCL, MZL, and WM
- Phase II trial of Ibrutinib alone in relapsed CLL
  - 51 patients @ 420mg & 34 @ 840mg daily
  - BTK occupation >95% @ 2.5mg/kg
  - OR =71%
  - Response independent of risk factors
  - PFS=75% and OS=83% at 26 months

Ibrutinib Depletes TH2 Gata3+ T cells but Preserves TH1 CD8 Cytotoxic T cells
Ibrutinib Selectively Depletes Activated B Cells while Preserving Cytotoxic T Cells and T Regs

Lenalidomide is an Immunomodulating Agent

Lenalidomide Alters Expression of Various Cytokines and Costimulates Immune Effector Cells

Checkpoint Inhibitors Demonstrate Robust Clinical Activity in Melanoma

Combination Therapy with Checkpoint Inhibitors Improves Survival

Paradigm Shift in Oncology

**Target the Tumor**
- Chemotherapy and AutoHCT
- Monoclonal Antibodies
  - Rituximab and Herceptin
- Antibody-Drug Conjugates
  - Brentuximab
- Tumor Checkpoint Blockade – PD-L1

**Target the Host**
- Vaccination
  - Gardasil (anti-HPV16&18)
  - Sipuleucel-T (anti-PSA)
- Immune Modulators
  - Lenalidomide
- Immune Checkpoint Blockade
  - PD1, CTLA4

**Target both Tumor & Host**
- Allogeneic HCT
- Bispecific Antibodies
  - Blinotumumab
- **CAR T Therapy**
How To Optimally Harness Anti-tumor Immunity

1. Physical proximity of polyclonal T cells
   - BITE – Blinatumumab (CD19+ B cells – CD3+ T cells) – ALL*
   - BIKES – Tumor cells and NK cells - AML

2+3. Transfer of antigen-specific T cells
   - CAR – AML*, ALL*, NHL*, MM*, CLL (few solid tumors)

4. Boost Recognition of tumor antigens
   - DC vaccines – prostate*, GBM*
   - Autologous tumor vaccines – AML*, FL*, ovarian*, CRC*

5. Disable the brakes on any activated T cell
   - CTLA-4 inhibition – melanoma*, AML*, ALL*
   - PD-1/PD-L1 blockade – HD*, NSCLC*, bladder*, GU*, HNSCC*

6. Non-specific T-cell activation in situ
   - Cytokines
     - Perleukin-2 – RCC*, melanoma*

7. Transfer of polyclonal T cells
   - HSCT – AML*, ALL*, NHL*

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HOW CAR T THERAPY WORKS

MODULE 3
MODULE 3: Outline

1. CAR T cells: Mechanism of Action
2. Chimeric Antigen Receptors (CARs)
   • Recombinant receptor constructs and pivotal roles of each domain
   • CD19-targeted CARs, comparison of different CAR constructs and different technologies used
3. Advantages of CAR T Therapy
4. CAR T Therapies: Clinical Data
5. Bispecific T cell engager (BiTE) antibodies: the difference between CARs and BiTEs
What is CAR T Therapy?

• CAR T therapy is the name given to chimeric antigen receptor (CAR) genetically modified T cells that are designed to recognize specific antigens on tumor cells resulting in their activation and proliferation eventually resulting in significant and durable destruction of malignant cells.

• CAR T cells are considered “a living drug” since they tend to persist for long periods of time.

• CAR T cells are generally created from the patients own blood cells although this technology is evolving to develop “off the shelf” CAR T cells.
CAR Modified T Cells as Cancer Therapy

1. T cells are isolated from patient
2. T cells are engineered to express CARs that recognize cancer cells
3. Modified T cells are grown and expanded in culture
4. Modified T cells are infused into patient

Source: mskcc.org
CAR T cells: Mechanism of Action

T cell

- Expression of CAR
- Viral DNA Insertion

Tumor cell

- CAR enables T cell to recognize tumor cell antigen
- Antigen
- CAR T cells multiply and release cytokines
- Tumor cell apoptosis

CAR T cells: Mechanism of Action
Chimeric Antigen Receptors (CARs)
**Chimeric Antigen Receptors**

**Antigen Binding Domain**
- **V<sub>H</sub>**
- **V<sub>L</sub>**
- **Hinge region**
- **Antigen binding domain**

**Activation Domains**
- **Costimulatory domain**
- **CD3-zeta chain signaling domain**

**scFv**
Single-chain variable fragment (scFv) bypasses MHC antigen presentation, allowing direct activation of T cell by cancer cell antigens

**Hinge region**
Essential for optimal antigen binding

**Costimulatory Domain: CD28 or 4-1BB**
Enhances proliferation, cytotoxicity and persistence of CAR T cells

**Signaling Domain: CD3-zeta chain**
Proliferation & activation of CAR T cells
CAR T cell-mediated killing of tumor cells
Chimeric Antigen Receptor Design

**ANTIGEN SPECIFICITY**

CAR heavy and light chain chains are components of the B cell receptor

**SIGNAL TRANSDUCTION / ACTIVATION**

CARs integrate key components of intracellular TCR signaling and costimulatory domains
### Variables to be Considered for CAR Therapy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAR design</td>
<td>Use of single-chain variable fragment (scFv); source of scFV monoclonal antibody moiety; murine vs. human</td>
</tr>
<tr>
<td></td>
<td>Use of “stalk” segment to extend scFV from cell surface (ex: Ig Fc region, CD8)</td>
</tr>
<tr>
<td></td>
<td>Trans-membrane domain (CD4, CD8, other)</td>
</tr>
<tr>
<td></td>
<td>One or more intracellular signaling domains: CD3ζ, CD28, 41BB, ICOS, OX40</td>
</tr>
<tr>
<td>CAR target</td>
<td>Lineage-specific antigen, tumor-associated antigen, nonvasculature antigen</td>
</tr>
<tr>
<td>Transgenes co-expressed with CAR</td>
<td>None, cytokine, cytokine receptor, chemokine receptor</td>
</tr>
<tr>
<td>Cell target</td>
<td>PBMCs, CD8-enriched, Treg-depleted, memory subpopulations, viral-specific</td>
</tr>
<tr>
<td>T cell growth conditions</td>
<td>Anti-CD3 + IL-2, anti-CD3 + anti-CD28, IL-7, IL-15</td>
</tr>
<tr>
<td>Gene transfer</td>
<td>Viral transduction (γ-retrovirus, lentivirus), non-viral gene transfer (electrotransfer of DNA, mRNA), transposon/transposase DNA plasmids</td>
</tr>
<tr>
<td>Conditioning chemotherapy</td>
<td>None, lymphodepletion, myeloablation</td>
</tr>
<tr>
<td>Post-cell boost</td>
<td>None, IL-2, dendritic cells</td>
</tr>
</tbody>
</table>
The first second-generation CARs to be used in the clinic were designed at MSKCC (CD28-based CAR) and the St. Jude Children’s Research Hospital (SJCRH) (4-1BB CAR). CD28-based CARs have been utilized at MSKCC, the NCI, and Baylor College of Medicine. 4-1BB-based CARs have been utilized at CHOP/UP and the FHCRC/Seattle Children’s Hospital (FHCRC).
## CD19-Targeted CAR Therapies Approved or Under Investigation in the United States

<table>
<thead>
<tr>
<th>Academic Group</th>
<th>Company (Drug)</th>
<th>Costimulatory Domain</th>
<th>Vector Delivery</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPenn</td>
<td>Novartis (Tisagenlecleucel)</td>
<td>4-1BB</td>
<td>Lentiviral</td>
<td>ALL, CLL, DLBCL, FL</td>
</tr>
<tr>
<td></td>
<td>(CTL019)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Julo (JCAR017)</td>
<td>4-1BB</td>
<td>Lentiviral</td>
<td>ALL, CLL, various B cell malignancies</td>
</tr>
<tr>
<td>NCI (NIH)</td>
<td>Kite, A Gilead Company (Axicabtagene Ciloleucel) (KTE-C19)</td>
<td>CD28</td>
<td>Retroviral</td>
<td>DLBCL ALL, MCL</td>
</tr>
<tr>
<td>MDACC</td>
<td>Ziopharm/Intrexon</td>
<td>CD28 → 4-1BB</td>
<td>Transposon/Transposase</td>
<td>B cell malignancies</td>
</tr>
<tr>
<td>Institute Pasteur</td>
<td>Cellectis/Pfizer (UCART19)</td>
<td>4-1BB</td>
<td>Lentiviral</td>
<td>ALL, CLL, AML, MM</td>
</tr>
<tr>
<td>Baylor</td>
<td>Bellicum (BPX-401)</td>
<td>MyDBB + CD40</td>
<td>Retroviral</td>
<td>Various</td>
</tr>
<tr>
<td>Dartmouth</td>
<td>Cardio3</td>
<td>DAP-10</td>
<td>Retroviral</td>
<td>AML, MDS, MM</td>
</tr>
</tbody>
</table>
## Costimulatory Molecules: CD28 or 4-1BB

<table>
<thead>
<tr>
<th>CD28</th>
<th>4-1BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>• CD28 is a member of the immunoglobulin (Ig) family of costimulatory receptors (^1)</td>
<td>• 4-1BB (CD137) is a member of the TNF receptor superfamily</td>
</tr>
<tr>
<td>• Two ligands: B7-1 (CD80) and B7-2 (CD86) (^1)</td>
<td>• Signaling via 4-1BB upregulates survival genes, enhances cell division, induces cytokine production, and prevents activation-induced cell death in T cells</td>
</tr>
<tr>
<td>• CD28 co-stimulation regulates IL-2 production, enhances T cell survival, and promotes differentiation of T-cell subsets (^1)</td>
<td>• 4-1BB preferentially expands memory T cells (^2)</td>
</tr>
<tr>
<td>• CD28 mostly expands naive cells (^2)</td>
<td></td>
</tr>
</tbody>
</table>

- In one study, both CD28- and 4-1BB–based CAR T cells had the same antitumor activity, but the 4-1BB–based CAR enhanced *in vivo* persistence of T cells \(^3\).
- Another study failed to demonstrate any significant difference between CD28- and 4-1BB–based CARs with regard to proliferation, potency, antitumor efficacy, and persistence of the T cells \(^4\).

---

Costimulation Plays a Major Role in Modulating T Cell Expansion and Persistence

Both CD28– and 4-1BB–containing CAR T cells continue to be investigated. Potential differences between CD28 and 4-1BB may help explain some of the clinical differences that have been observed, including differences in the clinical course.
Kinetics of Axi-Cel (Axicabtagene Ciloleucel) in DLBCL Patients

Evolution in CAR Design

First-Generation CAR
scFv-CD3ζ

Second-Generation CAR
scFv-CD28-CD3ζ

Third-Generation CAR
scFv-CD28-4-1BB-CD3ζ
scFv-CD28-OX40-CD3ζ

Advantages of CAR T Therapy
Advantages of CAR T Therapy

- Infused at a single point in time
- HLA-independent antigen recognition, therefore universal application
- Target antigens may include proteins, carbohydrates and glycolipids
- Active in both CD4\(^+\) and CD8\(^+\) T cells
- Rapid generation of tumor specific T cells
- Capable of rapid proliferation and persistence
- Minimal risk of GvHD
• **Powerful:** Attacks the cancer systemically

• **Specific:** Trains the immune system to recognize & target specific cells, including cancer cells

• **Memory:** Capacity for memory means durability of protection

• **Universal:** Treatment approach applicable to nearly all cancers
CAR T Therapies: Acute Lymphoblastic Leukemia
Poor Prognosis of Relapsed ALL in Adults

MRC UKALL2/ ECOG2993 Study (n=609)

Outcome of patients after 1\textsuperscript{st} relapse
5-yr OS: 7%

LALA-94 Study (n=421)

Outcome of patients after 1\textsuperscript{st} relapse
2-yr OS: 11% & 5-yr OS: 8%


# Anti-CD19 CAR T Cells have Dramatic Activity in Relapsed and Refractory ALL

<table>
<thead>
<tr>
<th>Signaling domain</th>
<th>Gene Transfer</th>
<th>Population</th>
<th>CR Rate</th>
<th>Cytokine Release Syndrome</th>
<th>Neurotoxicity</th>
<th>Site/Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD28-CD3ζ</td>
<td>Gamma-retrovirus</td>
<td>N=16 (ALL) Adults</td>
<td>88%</td>
<td>43% severe</td>
<td>25% Gr3-4</td>
<td>MSKCC Davila et al STM 2014</td>
</tr>
<tr>
<td>CD28-CD3ζ</td>
<td>Gamma-retrovirus</td>
<td>N=21 (ALL) Peds &amp; AYA (ITT)</td>
<td>67%</td>
<td>76% CRS (28% severe)</td>
<td>29%</td>
<td>NCI Lee et al Lancet 2015</td>
</tr>
<tr>
<td>4-1BB-CD3ζ</td>
<td>Lentiviral</td>
<td>N=30 (ALL) 25Peds, 5 adults</td>
<td>90%</td>
<td>100% (27% Severe)</td>
<td>43%</td>
<td>Upenn/CHOP Maude et al NEJM 2014</td>
</tr>
<tr>
<td>4-1BB-CD3ζ</td>
<td>Lentiviral</td>
<td>N=30 (ALL) Adults</td>
<td>93%</td>
<td>83%</td>
<td>50% severe</td>
<td>Seattle Turtle et al JCI 2016</td>
</tr>
</tbody>
</table>

**CTL019 (Tisagenlecleucel, KYMRIAH®)**

- **Indication:** KYMRIAH® is a CD19-directed genetically-modified autologous T cell immunotherapy indicated for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse.

- **Dose:**
  - For patients 50 kg or less: administer 0.2 to 5.0 x 10^6 CAR-positive viable T cells per kg body weight.
  - For patients above 50 kg: administer 0.1 to 2.5 x 10^8 CAR-positive viable T cells.

- **Conditioning Chemotherapy:** Fludarabine (30 mg/m^2 IV daily for 4 days) and cyclophosphamide (500 mg/m^2 IV daily for 2 days starting with the first dose of fludarabine). Infuse KYMRIAH® 2 to 14 days after completion of the lymphodepleting chemotherapy.

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CTL019 (Tisagenlecleucel, KYMRIAH®)

- **Pivotal phase 2 study:**
  - ELIANA (NCT02435849)
- **Evaluable patients: N=63**
  - 10% primary refractory disease
  - 48% one prior stem cell transplantation
  - 8% two prior stem cell transplantations

### Results

<table>
<thead>
<tr>
<th></th>
<th>N = 63</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CR/Cri</strong>¹,² (95% CI)</td>
<td>52 (83%)</td>
</tr>
<tr>
<td></td>
<td>(71%, 91%)</td>
</tr>
<tr>
<td><strong>P</strong>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>CR</strong>³</td>
<td>40 (63%)</td>
</tr>
<tr>
<td><strong>CRi</strong>⁴</td>
<td>12 (19%)</td>
</tr>
<tr>
<td><strong>CR or CRi with MRD-negative bone marrow</strong>⁵,⁶ (95% CI)</td>
<td>52 (83%)</td>
</tr>
<tr>
<td></td>
<td>(71%, 91%)</td>
</tr>
<tr>
<td><strong>P</strong>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

### Duration of Remission⁷

<table>
<thead>
<tr>
<th></th>
<th>N=52</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (months)</strong></td>
<td>Not reached</td>
</tr>
<tr>
<td><strong>(95% CI)</strong></td>
<td>(7.5, NE³)</td>
</tr>
</tbody>
</table>

¹CR/Cri was calculated based on all patients who received KYMRIAH and completed at least 3 months follow-up, or discontinued earlier prior to the data cut-off. Requires remission status to be maintained for at least 28 days without clinical evidence of relapse. ²The null hypothesis of CR/Cri less than or equal to 20% was rejected. ³CR was defined as less than 5% of blasts in the bone marrow, no evidence of extramedullary disease, and full recovery of peripheral blood counts (platelets >100,000/microliter and ANC >1,000/microliter) without blood transfusion. ⁴CRi (complete remission with incomplete blood count recovery) was defined as less than 5% of blasts in the bone marrow, no evidence of extramedullary disease, and without full recovery of peripheral blood counts with or without blood transfusion. ⁵MRD negative was defined as MRD by flow cytometry less than 0.01%. ⁶The null hypothesis of MRD-negative remission rate less than or equal to 15% was rejected. ⁷Duration of remission was defined as time since onset of CR or CRi to relapse or death due to underlying cancer, whichever is earlier, censoring for new cancer therapy including stem cell transplantation (N=52). ³Not Estimable.
## Additional Anti-CD19 CAR T Therapies in Commercial Development for R/R B-ALL

<table>
<thead>
<tr>
<th></th>
<th>KTE-C19</th>
<th>JCAR017</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Trial</strong></td>
<td>ZUMA-3 NCT02614066</td>
<td>ZUMA-4 NCT02625480</td>
</tr>
<tr>
<td><strong>Phase</strong></td>
<td>Phase 1/2</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td><strong>Dose Level</strong></td>
<td>0.5×10^6 CAR T cells/kg</td>
<td>1×10^6 CAR T cells/kg</td>
</tr>
<tr>
<td></td>
<td>1×10^6 CAR T cells/kg</td>
<td>2×10^6 CAR T cells/kg</td>
</tr>
<tr>
<td><strong>Conditioning Chemotherapy</strong></td>
<td>Cyclophosphamide (900 mg/m² x 1 day) + Fludarabine (25 mg/m²/day x 3 days)</td>
<td>Cyclophosphamide (900 mg/m² x 1 day) + Fludarabine (25 mg/m²/day x 3 days)</td>
</tr>
<tr>
<td><strong>Evaluable Patients (N)</strong></td>
<td>R/R Adult ALL (n=24)</td>
<td>R/R Pediatric and Adolescent ALL (N=7)</td>
</tr>
<tr>
<td><strong>Response Rates</strong></td>
<td>CR = 71%</td>
<td>CR = 100%</td>
</tr>
</tbody>
</table>

CAR T Therapy for CD19-negative R/R B-ALL

Anti-CD22 CAR T cells
- Phase 1 dose escalation/expansion study currently sponsored by NCI (NCT02315612)
- Patients: children and young adults with relapsed/refractory CD22+ ALL (N=30)
  • 22 patients treated at expansion dose (1 x 10^6 transduced CAR T cells/kg)
  • Prior transplant: N=18
  • Prior anti-CD19 CAR T cells: N=14
- CR = 76% (16/21 patients evaluable for response)

CAR T Therapy as a Bridge to Allogeneic Transplant?

- In one study, overall survival of ALL patients was similar with or without allogeneic SCT after CAR T cell therapy\(^1\)
- Other studies have shown conflicting results

\(^1\) Park JH et al. ASH 2015. Abstract 682.
CAR T Therapies: Non-Hodgkin Lymphoma
SCHOLAR-1
(Retrospective Non-Hodgkin Lymphoma Research)

- 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 prognosis\(^1\)

  - 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

CR, complete response; ORR, objective response rate; OS, overall survival.

Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma

# Additional Anti-CD19 CAR T Cells in Commercial Development for R/R NHL

<table>
<thead>
<tr>
<th>Clinical Trial</th>
<th>CTL019 (Tisagenlecleucel)</th>
<th>JCAR017</th>
</tr>
</thead>
<tbody>
<tr>
<td>JULIET</td>
<td>NCT02445248</td>
<td>NCT02030834</td>
</tr>
<tr>
<td>NCT02030834</td>
<td>TRANSCEMEND NHL 001</td>
<td>NCT02631044</td>
</tr>
<tr>
<td>Phase</td>
<td>Phase 2a</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Dose Level</td>
<td>$3.1 \times 10^8$ cells</td>
<td>$5 \times 10^8$ cells</td>
</tr>
<tr>
<td>Conditioning Chemotherapy</td>
<td>Low dose Cy/Flu x 3 days (73%) Bendamustine x 2 days (19%)</td>
<td>Varied according to treatment history, blood counts, and organ function</td>
</tr>
<tr>
<td>Evaluable Patients (N)</td>
<td>DLBCL (N=81)</td>
<td>DLBCL (N=14)  \nFL (N=14)</td>
</tr>
</tbody>
</table>
| Response Rates | ORR = 53.1%  
CR = 39.5% | ORR = 64%  
CR = 43% (DLBCL) and 71% (FL) | ORR = 75%  
CR = 56% |

Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas

CAR T Therapies: Multiple Myeloma
## Several Anti–B-cell Maturation Antigen (BCMA) CAR T Therapies are in Development for Multiple Myeloma

<table>
<thead>
<tr>
<th>bb2121</th>
<th>CART-BCMA</th>
<th>LCAR-B38M</th>
<th>CAR-BCMA</th>
<th>KTE-585</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Trial</strong></td>
<td>NCT02658929 (CRB-401 study)</td>
<td>NCT02546167</td>
<td>NCT03090659</td>
<td>NCT02215967</td>
</tr>
<tr>
<td><strong>Phase</strong></td>
<td>Phase 1</td>
<td>Phase 1/2</td>
<td>Phase 1</td>
<td>Phase 1</td>
</tr>
<tr>
<td><strong>Dose Level</strong></td>
<td>Dose escalation: 50, 150, 450, 800, and 1,200 x 10^6 CAR T cells</td>
<td>Cohort 1: 1-5x10^8 CAR T cells alone&lt;br&gt;Cohort 2: Cy + 1-5x10^7 CAR T cells&lt;br&gt;Cohort 3: Cy + 1-5x10^8 CAR T cells</td>
<td>0.17 or 1.05 x 10^6 CAR T cells/kg</td>
<td>4 dose levels, 0.3x10^6, 1x10^6, 3x10^6, and 9x10^6 CAR+ T cells/kg</td>
</tr>
<tr>
<td><strong>Infusion</strong></td>
<td>Single infusion</td>
<td>Split-dose infusions (10% on day 0, 30% on day 1, and 60% on day 2)</td>
<td>Infused on three days (d0, d2, and d6)</td>
<td>Single infusion</td>
</tr>
<tr>
<td><strong>Conditioning Chemotherapy</strong></td>
<td>Fludarabine (30 mg/m^2) and cyclophosphamide (300 mg/m^2) daily for 3 days</td>
<td>Cohort 2 and 3: Cy (1.5 g/m^2) on day -3</td>
<td>Fludarabine (25mg/m^2) and cyclophosphamide (250mg/m^2) daily for 3 days</td>
<td>300 mg/m2 of cyclophosphamide and 30 mg/m2 of fludarabine daily for 3 days</td>
</tr>
<tr>
<td><strong>Response Rates</strong></td>
<td>ORR = 89% (N=18)</td>
<td>Cohort 1: 6/9 patients responded&lt;br&gt;Cohort 2: 2/5 patients responded</td>
<td>ORR = 100% (N=5)</td>
<td>Dose level 4: 9/11 patients responded</td>
</tr>
</tbody>
</table>

Bi-specific T-cell Engagers (BiTEs)
BiTEs

- BiTE antibodies are recombinant proteins with dual specificity for CD3 and a tumor antigen
- BiTE antibodies transiently induce a cytolytic synapse between the cytotoxic T cell and the cancer target cell
- BiTEs replace the need for antigen processing by replacing the MHC/Peptide/T cell receptor complex
- BiTE-activated T cells proliferate, secrete granzymes and perforin, and engage multiple cancer cells
Blinatumomab (B-lineage-anti-tumoral-MoAb)

- Blinatumomab engages the host’s CD3+ T cells to cause direct lysis of CD19+ target/tumor cells
- This type of immunotherapy brings autologous effector cells into direct contact with the target and nothing else

Blinatuximab

- Contains an anti-CD3 arm and anti-CD19 arm joined by a non-immunogenic linker
  - Creates a cytolytic synapse between the T and B cells
  - Engaged cytotoxic T cells release granzymes and perforin via exocytosis into the target cells
  - Blinatuximab-activated T cells secrete inflammatory cytokines

- Short half-life
  - $t_{1/2} \sim 2$ hours
  - Delivered as a continuous infusion for 28 day cycles, then 2 weeks off

- Unclear clearance pathways

- Early evidence shows high response rates, though some cytokine mediated effects must be managed

Blinatumomab Outcomes in Relapsed ALL

CAR T Cells versus Blinatumomab

**CAR T CELLS**
- One time infusion; CAR T cell expansion 1000-10,000 fold amplifies activity; CAR T cells persist
- Time to treatment 2-4 weeks due to manufacturing
- Large international experience
- No randomized trials
- Difficult to compare to other therapies
- CR/CRi in ~90% of patients across many trials
- Sustained remissions
- Toxicity manageable and predictable
  - TRM <10%, may be product, dose, schedule related and improving with experience

**BLINATUMOMAB**
- Prolonged, continuous infusion over several months
- “Off the shelf” product
- Large experience in several hundred patients
- Better than standard chemotherapy (randomized trial)
- Difficult to compare to other therapies
- CR ~43%
- Likely to be an effective bridge to transplant
- DFS 20-30% (after SCT)
- Toxicity manageable and predictable
  - includes CRS and neurologic toxicity
CAR T THERAPY: MANUFACTURING TO INFUSION

MODULE 4
MODULE 4: Outline

1. Apheresis, leukapheresis and current challenges
2. Bridging therapy
3. Manufacturing and vector delivery of CARs
4. Lymphodepletion and infusion
   • Early data that supports use of lymphodepleting chemotherapy
   • Current lymphodepletion regimens
Overview of CAR T Therapy

1. Leukapheresis
2. T cell activation/transduction
3. Modified T cell expansion
4. Quality and release testing: potency checks and infection checks
5. Chemotherapy
6. Modified T cell infusion

Timeline varies by manufacturer

10-28 Days
Patient Journey: Manufacturing to Infusion

1. Apheresis
2. (Manufacturing) Patients Return Home
3. Lymphodepletion
4. Infusion
Patient Journey: Manufacturing to Infusion

1. Apheresis
   - Patients go to the CAR T center or local apheresis center

2. Patients Return Home
   - (Manufacturing)

3. Lymphodepletion

4. Infusion
What is Apheresis?

- Blood is removed through a needle or catheter and circulated into a blood cell separator machine
- The components which may be separated and withdrawn include:
  - Plasma (plasmapheresis)
  - Platelets (plateletpheresis)
  - Leukocytes (leukapheresis)
- Separated cells are transferred to a collection bag, saved and frozen
- The other blood components (ex: red blood cells) circulate back to the patient through a return needle

Leukapheresis

• Separation of white blood cells from the other blood components

• The usual collection goal for hematopoietic cell transplantation is $2 \times 10^6$ CD34+ cells/kg

• Optimal collection for CAR T cell therapy remains unknown

• Current protocols require approximately 12 to 15 liters to be processed with a goal of obtaining approximately $5 \times 10^9$ mononuclear cells
Factors Affecting Lymphocyte Mobilization

- **Absolute lymphocyte count**
  - Minimum required ALC will vary by manufacturer

- **Use of steroids**
  - Steroids reduce the circulating lymphocyte count and are **not** allowed immediately prior to apheresis

- **Duration and time since last exposure to prior therapies, including:**
  - Checkpoint inhibitors
  - Ibrutinib
  - Lymphodepleting chemotherapy

- **Level of disease burden**
  - Active disease in the peripheral blood is generally **not** an exclusion criteria

- **Suitable access for apheresis**
  - Potential need for central venous catheter
## Adverse Events Associated with Leukapheresis

<table>
<thead>
<tr>
<th>Category</th>
<th>Symptom</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypocalcemia</td>
<td>Paresthesias</td>
<td>1.5-9.0</td>
</tr>
<tr>
<td>Hypovolemia</td>
<td>Hypotension</td>
<td>0.4-4.2</td>
</tr>
<tr>
<td></td>
<td>Muscle cramps</td>
<td>0.4-2.5</td>
</tr>
<tr>
<td></td>
<td>Headaches</td>
<td>0.3-5.0</td>
</tr>
<tr>
<td>Anaphylactoid</td>
<td>Urticaria</td>
<td>0.7-12.0</td>
</tr>
<tr>
<td></td>
<td>Rigors</td>
<td>1.1-8.0</td>
</tr>
</tbody>
</table>

Adapted from Kaplan A.A. A practical guide to therapeutic plasma exchange, Blackwell Science Malden MA, 1999.

Patient Journey: Manufacturing to Infusion

1. Apheresis
2. Patients Return Home
3. Lymphodepletion
4. Infusion

Acute Lymphoblastic Leukemia
Many patients receive salvage therapy during this time

Non-Hodgkin Lymphoma
Patients with rapidly-proliferating disease receive bridging therapy

SOC therapy is permitted until CAR T cells are ready for infusion
Bridging Therapy

Practical Guidelines

• Assess whether patients need further therapy to maintain disease control during the period of manufacturing
• Maintain frequent communication with patient and manufacturer
• Choose least toxic bridging therapy, if possible
• Therapies to consider avoiding:
  • Immunosuppressive therapy
  • Checkpoint Inhibitors
  • Blinatumomab

*Patients on clinical trials should follow the trial protocol
T Cells are Isolated and Activated in Commercial Manufacturing Facilities
Cell Therapy and Cell Engineering Facility (CTCEF)

Generation of GMP-grade 19-28z retroviral vector stocks for clinical application

Ex vivo expansion of 19-28z transduced patient T cells under GMP conditions on the Wave® Bioreactor
Viral Vectors are used to Genetically Modify T cells to Express CARs

- Major advantages of viral vectors include:
  - Relative ease of manufacture and production
  - Capacity to stably integrate genetic material into the host genome

- In order to comply with clinical safety standards, viral vector platforms must demonstrate replication incompetence, low genotoxicity, and low immunogenicity

- Due to the potential theoretical risk of secondary leukemogenesis, patients treated with CAR T therapy need to be followed for a minimum of 15 years


## Retroviral versus Lentiviral Vectors

<table>
<thead>
<tr>
<th>SIMILARITIES</th>
<th>DIFFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Structurally similar</td>
<td>• As opposed to retroviral vectors, lentiviral vector transduction is not governed by cell division, allowing effective transduction of a wide range of cell types, including non-cycling terminally differentiated cells</td>
</tr>
<tr>
<td>• Viral genes are replaced with a CAR transgene</td>
<td></td>
</tr>
<tr>
<td>• The viral genome is stably integrated into the host T cell</td>
<td></td>
</tr>
<tr>
<td>• Achieve high rates of transduction and significant transgene expression</td>
<td></td>
</tr>
<tr>
<td>that persists over time</td>
<td></td>
</tr>
<tr>
<td>• Safety must be carefully monitored</td>
<td></td>
</tr>
</tbody>
</table>

There are many different ways to approach manufacturing
Patient Journey: Manufacturing to Infusion

1. **Apheresis**

2. **Patients Return Home**

3. **Lymphodepletion**
   - Blood counts need to recover from any post-apheresis cytotoxicity before patients can return to the CAR T center for lymphodepletion.

4. **Infusion**

(Manufacturing)
Lymphodepletion is Necessary for Expansion of CAR T Cells

- Lymphodepletion creates a “favorable” environment for CAR T cell expansion and survival \textit{in vivo}:
  - Potentially provides disease control
  - Potentially eliminates tumor suppressor cells (T regs)
  - Provides homeostatic cytokines
  - Upregulates tumor immunogenicity and reduces immune suppression

Fludarabine + Cyclophosphamide Increases CAR T Cell Expansion

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.
Current Lymphodepleting Regimens

- Current regimens include:
  - Cyclophosphamide$^{1-3}$
  - Etoposide + cyclophosphamide$^1$
  - Fludarabine + cyclophosphamide$^1$

Cyclophosphamide + fludarabine is the most commonly used regimen$^{1,2}$

Lymphodepletion: Practical Consideration

- Patients are treated on an outpatient basis
- Schedule lymphodepletion 3-5 days prior to infusion of CAR T cells
  - Consider treating patients with lymphodepleting chemotherapy only after it is confirmed that the CAR T cells have arrived at the center
- For patients with low lymphocyte counts (ALC<100) lymphodepletion may not be necessary, since these patients are already “lymphodeplete”
Patient Journey: Manufacturing to Infusion

1. **Apheresis**
2. (Manufacturing) Patients Return Home
3. **Lymphodepletion**
4. **Infusion**
MODULE 5: Outline

1. Toxicity and Management in CAR T Therapy
   • Cytokine Release Syndrome (CRS)
   • CRS grading assessment
   • Management of CRS
   • Neurologic toxicity: diagnosis and management
   • Macrophage Activation Syndrome (MAS) or HLH

2. Best Practices
   • CRS Management: Best Practices
   • What the ICU team needs to know
   • Working in partnership
   • Education
   • What to tell the referring oncologist
CAR T Therapy: Toxicity

• No significant acute infusional toxicity

• Tumor Lysis Syndrome
  - Rarely occurs; effector cell expansion requires time negating massive tumor lysis

• Cytokine Release Syndrome (CRS)
  - Life-threatening if not managed by expert multidisciplinary team
  - May include cardiac events, hepatotoxicity, or renal toxicity

• Neurologic Toxicity
  - 3 subtypes: acute, delayed, idiosyncratic

• Cytopenias
  - Macrophage Activation Syndrome (MAS) or HLH is a very rare and severe form

• B cell aplasia and hypogammaglobulinemia
Cytokine Release Syndrome
Cytokine Release Syndrome (CRS)

- CRS is a condition resulting from the release of cytokines from activated CAR T cells, as well as bystander immune cells such as dendritic cells, monocytes/macrophages, and others.
  - The hallmark of CRS is an elevation in cytokines.

- Most patients who respond to CAR T therapy develop some degree of CRS.
  - In one study, 94% of ALL patients developed some degree of CRS (mostly mild to moderate).

The hallmark presenting sign of CRS is fever which occurs shortly after infusion of CAR T cells.

<table>
<thead>
<tr>
<th>Organ System</th>
<th>Symptoms and Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutional</td>
<td>Fever +/- rigors, malaise, fatigue, anorexia, myalgias, arthralgias, nausea, vomiting, headache</td>
</tr>
<tr>
<td>Skin</td>
<td>Rash</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Nausea, vomiting, diarrhea</td>
</tr>
<tr>
<td>Respiratory</td>
<td>Tachypnea, hypoxemia</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), potentially diminished cardiac output (late)</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Elevated D-dimer, hypofibrinogenemia +/- bleeding</td>
</tr>
<tr>
<td>Renal</td>
<td>Azotemia</td>
</tr>
<tr>
<td>Hepatic</td>
<td>Transaminitis, hyperbilirubinemia</td>
</tr>
<tr>
<td>Neurologic</td>
<td>Headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, altered gait, seizures</td>
</tr>
</tbody>
</table>
CRS Toxicities by Organ System

Neurologic:
- Headaches
- Changes in level of consciousness
- Delirium
- Aphasia
- Apraxia
- Ataxia
- Hallucinations
- Tremor
- Dysesthesia
- Myoclonus
- Facial nerve palsy
- Seizures

Hepatic:
- Transaminits
- 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

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
- Hypotension
- Hypokalemia
- Hypophosphatemia
- Tumor lysis syndrome

Gastrointestinal:
- Nausea
- Emesis
- Diarrhea

Musculoskeletal:
- Myalgias
- Elevated creatine kinase
- Weakness
Multiple cytokines may be responsible for this circulatory cascade including interleukins, interferons, tumor necrosis factor, lymphokines, monokines, and chemokines.

- Promotion or inhibition of cell growth
- Activation of immune effector cells
- Mediation for destruction of cells targeted by monoclonal antibodies
- Mediation of inflammatory response

CRS is a systemic inflammatory disease with a broad range of mediators and/or indicators (ex: ferritin, CRP)
Anti-CD19 CAR T Cells Release IFN-γ, Granzyme B, IL-10, and TNF-α

Cytokine Profiles Observed in Patients Treated with CAR T Cells (Axi-Cel, Axicabtagene Ciloleucel)

<table>
<thead>
<tr>
<th>Cytokine Categories</th>
<th>Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative</td>
<td>IL-15, IL-2</td>
</tr>
<tr>
<td>Inflammatory</td>
<td>IL-6, CRP, SAA, IL-5, Ferritin, IL-1Ra, IL-2Ra</td>
</tr>
<tr>
<td>Immune-modulating</td>
<td>GM-CSF, IFN-γ, IL-10</td>
</tr>
<tr>
<td>Chemokine</td>
<td>IL-8, IP-10, MCP-1</td>
</tr>
<tr>
<td>Effector</td>
<td>Granzyme B</td>
</tr>
</tbody>
</table>

* Analytes shown were elevated in ≥50% of patients with ≥2-fold induction above baseline out of a panel of 44 measured.*
May occur within minutes or hours but generally appears within days or weeks Coincides with maximal T-cell expansion

Decreasing serum CRP may be a clinical indicator of improvement.
Peak Cytokine Levels Correlate with CRS Severity

- CRS grade correlates with peak IL-6 and IL-2RA levels in patients treated with CTL019

Porter DL et al. Sci Transl Med. 2015 Sep 2;7(303):303ra139.
Peak Cytokine Levels Correlate with CRS Severity Requiring ICU Care

Higher peak IL-6 and IFN-γ levels are observed in patients requiring ICU care.

Elevations of serum C-reactive protein (CRP) and ferritin correlate with the occurrence of severe CRS requiring ICU care.

Higher Peak Cytokine Levels Observed in Patients with High Bone Marrow Tumor Burden

Correlation of CRS with Disease Burden

CRS Severity Correlates with Peak Cytokine Levels and Baseline Disease Burden in Pediatric R/R ALL

Peak Cytokine Levels Correlate with Response to Anti-CD19 CAR T Therapy

Decreasing serum CRP may be a clinical indicator of improvement
CRS: Grading Assessment
### CRS Grading by CTCAEv4

- Linked to infusion of a drug. Not applicable to cellular therapy.

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade</th>
<th>Immune system disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine release syndrome</td>
<td>1</td>
<td>Mild reaction; infusion interruption not indicated; intervention not indicated</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Therapy of infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for &lt;=24 hrs</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Life-threatening consequences; pressor or ventilatory support indicated</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Death</td>
</tr>
</tbody>
</table>

Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and shortness of breath; it is caused by the release of cytokines from the cells.
Revised Grading Scales for CRS

<table>
<thead>
<tr>
<th>2014 NCI Consensus Revised Grading Scale¹</th>
<th>Penn Grading Scale (PGS-CRS)²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grade 1</strong></td>
<td><strong>Mild reaction</strong></td>
</tr>
<tr>
<td>• Symptoms are not life threatening</td>
<td>• Treated with supportive care (anti-pyretics, anti-emetics)</td>
</tr>
<tr>
<td>• Symptomatic treatment only (ex: fever, nausea, fatigue, headache, myalgias, malaise)</td>
<td></td>
</tr>
<tr>
<td><strong>Grade 2</strong></td>
<td><strong>Moderate</strong></td>
</tr>
<tr>
<td>• Symptoms require and respond to moderate intervention</td>
<td></td>
</tr>
<tr>
<td>• Hypoxia: responsive to &lt;40% oxygen</td>
<td>• Requires IV therapies or parenteral nutrition</td>
</tr>
<tr>
<td>• Hypotension: responsive to fluids or one low dose vasopressor</td>
<td>• Some signs of organ dysfunction (i.e. grade 2 Cr or grade 3 LFTs) related to CRS</td>
</tr>
<tr>
<td>• Grade 2 organ toxicity</td>
<td>• Hospitalization for CRS-related symptoms including fevers with associated neutropenia</td>
</tr>
<tr>
<td><strong>Grade 3</strong></td>
<td><strong>More severe reaction requiring hospitalization</strong></td>
</tr>
<tr>
<td>• Symptoms require and respond to aggressive intervention</td>
<td></td>
</tr>
<tr>
<td>• Hypoxia: requires oxygen &gt;40%</td>
<td>• Moderate signs of organ dysfunction (grade 4 LFTs or grade 3 Cr) related to CRS</td>
</tr>
<tr>
<td>• Hypotension: requires high dose or multiple vasopressors</td>
<td>• Hypotension treated with IV fluids or low dose pressors</td>
</tr>
<tr>
<td>• Grade 3 organ toxicity</td>
<td>• Coagulopathy requiring FFP or cryoprecipitate</td>
</tr>
<tr>
<td>• Grade 4 transaminitis</td>
<td>• Hypoxia requiring supplemental O₂ (nasal cannula oxygen, high flow O₂, CPAP or BiPAP)</td>
</tr>
<tr>
<td><strong>Grade 4</strong></td>
<td><strong>Life-threatening complications</strong></td>
</tr>
<tr>
<td>• Life-threatening symptoms</td>
<td>• Hypotension requiring high dose pressors</td>
</tr>
<tr>
<td>• Requirement for ventilator support</td>
<td>• Hypoxia requiring mechanical ventilation</td>
</tr>
<tr>
<td>• Grade 4 organ toxicity (excluding transaminitis)</td>
<td></td>
</tr>
<tr>
<td><strong>Grade 5</strong></td>
<td><strong>Death</strong></td>
</tr>
<tr>
<td>Death</td>
<td></td>
</tr>
</tbody>
</table>

### CRS Grading Scale per CARTOX Working Group

**Adapted from 2014 NCI Consensus Revised Grading Scale**

<table>
<thead>
<tr>
<th>Category</th>
<th>Symptom/Sign</th>
<th>CRS Grade 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CRS Grade 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CRS Grade 3&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CRS Grade 4&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital Signs</td>
<td>Temperature ≥38°C</td>
<td>Yes</td>
<td>Any</td>
<td>Any</td>
<td>Any</td>
</tr>
<tr>
<td></td>
<td>SBP &lt;90mmHg</td>
<td>No</td>
<td>Responds to IV fluids or low-dose vasopressor</td>
<td>Needs high-dose or multiple vasopressors</td>
<td>Life-threatening</td>
</tr>
<tr>
<td>Organ toxicity</td>
<td>Needing oxygen for O&lt;sub&gt;2&lt;/sub&gt; sat &gt;90%</td>
<td>No</td>
<td>FiO&lt;sub&gt;2&lt;/sub&gt; &lt;40%</td>
<td>FiO&lt;sub&gt;2&lt;/sub&gt; ≥40%</td>
<td>Needs ventilator support</td>
</tr>
<tr>
<td></td>
<td>See below</td>
<td>Grade 1</td>
<td>Grade 2</td>
<td>Grade 3 or grade 4 transaminitis</td>
<td>Grade 4 (except grade 4 transaminitis)</td>
</tr>
</tbody>
</table>

**Symptoms or signs of organ toxicity**

1. Cardiac – tachycardia, arrhythmias, heart block, low ejection fraction
2. Respiratory – tachypnea, pleural effusion, pulmonary edema
3. Gastrointestinal – nausea, vomiting, diarrhea
4. Hepatic – increased aspartate transaminase, alanine transaminase, or bilirubin
5. Renal – acute kidney injury (increased creatinine), decreased urine output
6. Skin – rash (less common)
7. Coagulopathy – disseminated intravascular coagulation (less common)
8. Neurologic – confusion, disorientation, agitation, dysphasia, aphasia, tremors, seizures, motor weakness, incontinence, increased intracranial pressure, papilledema, cerebral edema

<sup>a</sup>Grade 1 CRS may manifest as fever and/or Grade 1 organ toxicity. <sup>b</sup>For Grades 2, 3, or 4 CRS, any one of the criteria other than temperature is sufficient. <sup>c</sup>Grading of organ toxicities is performed according to CTCAE v 4.03. <sup>d</sup>Definition of high-dose vasopressors provided by Lee DW et al. Blood. 2014;124(2):188-95.
**Definition of High-Dose Vasopressors**

**Table 3. High-dose vasopressors (all doses are required for ≥3 hours)**

<table>
<thead>
<tr>
<th>Pressor</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine monotherapy</td>
<td>≥20 μg/min</td>
</tr>
<tr>
<td>Dopamine monotherapy</td>
<td>≥10 μg/kg/min</td>
</tr>
<tr>
<td>Phenylephrine monotherapy</td>
<td>≥200 μg/min</td>
</tr>
<tr>
<td>Epinephrine monotherapy</td>
<td>≥10 μg/min</td>
</tr>
<tr>
<td>If on vasopressin</td>
<td>Vasopressin + norepinephrine equivalent of ≥10 μg/min*</td>
</tr>
<tr>
<td>If on combination vasopressors</td>
<td>Norepinephrine equivalent of ≥20 μg/min*</td>
</tr>
</tbody>
</table>

*VASST Trial vasopressor equivalent equation: norepinephrine equivalent dose = [norepinephrine (μg/min)] + [dopamine (μg/kg/min) ÷ 2] + [epinephrine (μg/min)] + [phenylephrine (μg/min) ÷ 10].

CRS Grading Assessment: Summary

• CRS grading scale by CTCAEv4 is not sufficient for cellular therapy

• Several revised grading systems for CRS have been developed
  - Consensus grading scale with input from several programs was presented in 2014 and has been used by the NCI to grade CRS
  - PENN/CHOP clinical trial programs also developed a modified grading scale (PGS-CRS)

• When reviewing the literature on CAR T therapy, it may be difficult to compare toxicity across studies, since CRS may be graded differently

Development of a unified CRS grading system is still a work in progress
CRS: Management
CRS Management

• Management of CRS is based on clinical parameters, not laboratory values
  • Ferritin, CRP, serum cytokines should only be used to support the diagnosis

• CRS can be fairly well-managed with high level of clinical surveillance, fluids, and vasopressors
  • CRS requires continuous monitoring

• The IL-6 receptor antibody tocilizumab is the consensus first line treatment for CRS
  • Not currently recommended for prophylactic use as impact on T-cell expansion and persistence is not known. This is currently being investigated

• Second line treatment for CRS varies by protocol and/or institutional guidelines
  • Steroids are effective for treating CRS, however they are lymphotoxic
  • The IL-6 antibody siltuximab stops the production of IL-6 and is currently being investigated
CRS Management: Monitoring and Supportive Care

- Close hemodynamic monitoring is imperative
  - Vital signs should be checked every 2-4 hours
  - CBC with differential and comprehensive metabolic panel should be drawn twice daily
  - Monitor CRP daily
  - Monitor uric acid, lactate and ferritin

- Initiate a full infectious workup and rapid implementation of anti-infective agents upon first signs of fever
  - Fever should be managed with acetaminophen; avoid corticosteroids or NSAIDs
  - If a patient is neutropenic and febrile, blood cultures should be drawn, and broad spectrum antibiotic therapy should be initiated
  - Infectious diagnoses should be aggressively pursued by imaging and cultures to avoid missing infections occurring at the same time as CRS

- Hypotension must be recognized early and managed aggressively
  - Keep MAP>65 and always consider another IVF bolus a liter at a time
  - Patients with hypotension that is not fluid responsive should receive vasopressors and be evaluated for cardiomyopathy by echo

- CRS requires close cardiac monitoring and ICU notification
  - Cardiac events have been associated with CRS including myocardial ischemia and death
  - Patients with CRS should be monitored with ECGs and echocardiograms
  - Tachycardia is common in the setting of CRS and medications to slow sinus tachycardia should be avoided

- Cytopenias should be managed with transfusion support and growth factors
Supportive Care Guidelines for Patients Receiving CAR T Cells at the NCI

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Preventive and supportive care interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutional</td>
<td>• Administer acetaminophen for symptomatic management of fevers in patients with normal hepatic function;</td>
</tr>
<tr>
<td></td>
<td>• Provide cooling blankets for fevers &gt;40°C;</td>
</tr>
<tr>
<td></td>
<td>• Avoid corticosteroids and NSAIDs; and</td>
</tr>
<tr>
<td></td>
<td>• Avoid meperidine</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>• Stop or taper antihypertensive medications prior to cell infusion;</td>
</tr>
<tr>
<td></td>
<td>• Monitor vital signs at least every 4 h on an inpatient unit for at least 9 d following infusion;</td>
</tr>
<tr>
<td></td>
<td>• Monitor vital signs every 2 h in patients with fevers and tachycardia;</td>
</tr>
<tr>
<td></td>
<td>• Initiate replacement IV fluids for patients with poor oral intake or high insensible losses to maintain net even fluid balance;</td>
</tr>
<tr>
<td></td>
<td>• Administer IV fluid boluses for patients with SBP less than their preinfusion baseline:</td>
</tr>
<tr>
<td></td>
<td>• Patients with a SBP &lt;80% of their preinfusion baseline and &lt;100 mm Hg receive a 1 liter normal saline bolus</td>
</tr>
<tr>
<td></td>
<td>• Patients with a SBP &lt;85 mm Hg receive a 1 liter normal saline bolus regardless of baseline blood pressure</td>
</tr>
<tr>
<td></td>
<td>• Patients receiving &gt;1 IV fluid bolus for hypotension or patients in the ICU for toxicity management have a serum troponin drawn, and an ECG and an echocardiogram performed to evaluate for cardiac toxicity; and</td>
</tr>
<tr>
<td></td>
<td>• Patients with hypotension are initiated on vasopressor support. Norepinephrine is the preferred first-line vasopressor</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>• Initiate prophylactic antimicrobials, such as trimethoprim-sulfamethoxazole, for Pneumocystis prophylaxis prior to conditioning chemotherapy;</td>
</tr>
<tr>
<td></td>
<td>• Initiate prophylactic antimicrobials, such as acyclovir or valacyclovir, for herpes virus prophylaxis prior to conditioning chemotherapy; and</td>
</tr>
<tr>
<td></td>
<td>• All patients with fevers and neutropenia have blood cultures drawn and broad-spectrum antibiotic coverage initiated</td>
</tr>
<tr>
<td>Hematologic</td>
<td>• Initiate allopurinol for TLS prophylaxis in patients without a contraindication prior to conditioning chemotherapy;</td>
</tr>
<tr>
<td></td>
<td>• Transfuse packed red cells for goal hemoglobin of ≥8.0 g/dL;</td>
</tr>
<tr>
<td></td>
<td>• Transfuse platelets for a goal platelet count of ≥20 000/mL;</td>
</tr>
<tr>
<td></td>
<td>• Monitor complete blood count with differential twice daily. When ANC decreases to &lt;500/mL, initiate filgrastim support. Continue until ANC increases to ≥1500 mL;</td>
</tr>
<tr>
<td></td>
<td>• Transfuse fresh frozen plasma with a goal of normalization of PTT in patients with a PTT &gt;1.5-fold above the upper limit of normal; and</td>
</tr>
<tr>
<td></td>
<td>• Transfuse cryoprecipitate to maintain fibrinogen of ≥100 mg/dL. If patient is bleeding, a higher level of fibrinogen should be maintained</td>
</tr>
<tr>
<td>Neurologic</td>
<td>• The nursing staff conducts focused neurologic examinations every 8 h in patients experiencing neurologic toxicity;</td>
</tr>
<tr>
<td></td>
<td>• Perform brain MRI in any patient experiencing neurologic toxicity;</td>
</tr>
<tr>
<td></td>
<td>• Perform lumbar puncture to evaluate for infectious pathogens, cytokine levels, and CAR T-cell levels in patients experiencing neurologic toxicity whenever feasible</td>
</tr>
<tr>
<td></td>
<td>• Request a neurology consultation for any patient experiencing neurologic toxicity; and</td>
</tr>
<tr>
<td></td>
<td>• Standard antiepileptic medications are used for patients having active seizures. We do not use prophylactic antiepileptic medications</td>
</tr>
</tbody>
</table>
Prior to CAR T cell infusion
• Baseline brain magnetic resonance imaging to rule out any central nervous system disease
• Central venous access with double or triple lumen catheter
• Cardiac monitoring by telemetry starting on the day of CAR T cell infusion and continued until CRS resolves
• Tumor lysis prophylaxis for patients with bulky tumors
• Seizure prophylaxis with levetiracetam at 750 mg orally q12h for 30 days starting on the day of infusion for CAR T-cell therapies known to cause CRES
• Hospitalization recommended for at least 7 days after CAR T-cell therapy

Notifications and contingency orders
• Notify physician
  • SBP >140 or <90 mmHg
  • Heart rate >120 or <60 / min or arrhythmia
  • Respiratory rate >25 or <12 / min
  • Oxygen Saturation <92% on room air
  • Urine output <1500 mL/24h
  • Upward trends in creatinine or liver function tests
  • Tremors or jerky movements in extremities
  • Change in mental status (alertness, orientation, speech, and ability to write a sentence)
• For temperature > 38.3 °C, send blood cultures (central and peripheral) and urine for urinalysis and culture, obtain portable chest x-ray, and notify physician
• For patients with neutropenia and fever, start empiric broad-spectrum antibiotics
• Do not administer corticosteroids unless approved by physician
• If patient develops CRES, withhold oral intake and notify physician
• PRN medications
  • Acetaminophen (1st choice) or ibuprofen (2nd choice if not contraindicated) for fever > 38.3 °C
  • Cooling blanket prn fever > 38.3 °C
  • Normal saline 500 to 1000 mL bolus prn SBP <90 mmHg; may repeat once if SBP <90 mmHg after 1st bolus
  • PRN tocilizumab or siltuximab to be activated on physician order

Monitoring after CAR T cell infusion
• Vitals q4h, strict input and output, daily weights
• Daily history and physical examination
• Daily blood counts and complete metabolic profile
• C-reactive protein and ferritin levels daily starting on day 0
• Assessment and grading of CRS should be done at least twice daily and whenever there is a change in patient’s status
• Assessment and grading for CRES using the CARTOX 10-point neurological assessment should be done at least every 8 hours and should include writing a sentence twice daily
• Maintenance IV fluids with normal saline to ensure adequate hydration

CRS – cytokine release syndrome; CRES – CAR-Related Encephalopathy Syndrome; CARTOX – CAR T-cell therapy-associated TOXicity; IV – intravenous; SBP – systolic blood pressure; PRN – pro re nata (as needed)
Tocilizumab

- IL-6 receptor inhibitor
- Blocks IL-6 mediated effects
- Monoclonal antibody with $t_{1/2}$ ~21 days
- Indicated for the treatment of rheumatologic disorders

**Dosing for CRS management is based on clinical parameters**

- Dosing of tocilizumab varies by protocol and/or institutional guidelines
  - Most common doses: 4 mg/kg or 8 mg/kg
  - Maximum dose: 800 mg
- Timing of second dose of tocilizumab also varies by protocol and/or institutional guidelines
  - Range: every 2 – 12 hours
CRS Response to Tocilizumab is Biphasic

First phase is highly responsive to tocilizumab
Second phase may be less responsive to tocilizumab

Temperature Response to Tocilizumab

![Graph showing temperature response to Tocilizumab over days after CAR T cell infusion.](image)

David L Porter, unpublished
Response to Tocilizumab

Patient is a 20-year-old woman with a history of ALL. She experienced CRS toxicity with fevers, tachycardia, tachypnea, hypoxia, left ventricular systolic dysfunction, prolonged activated PTT, and increased creatine kinase. She received tocilizumab on day 4 following CAR T-cell infusion. Her respiratory rate and heart rate decreased following tocilizumab, and intubation was avoided. Following tocilizumab, CRP decreased over a period of days.

Brudno JN and Kochenderfer JN. Blood 2016; 127(26):3321-3330
Ferritin Response to Tocilizumab
CRS Treatment Algorithms: Summary

• Guidelines for CRS management vary by protocol and/or institution
• The overall goal of these guidelines is to avoid life-threatening grade 4 toxicity
• Tocilizumab is the consensus first line treatment for grade ≥2 CRS
• There is also general consensus that if CRS has not improved with initial tocilizumab administration, an additional dose of tocilizumab should be given or another immunosuppressive agent such as corticosteroids should be considered
Treatment Algorithm for Management of CRS based on Lee Criteria

**Grading Assessment**

- **Grade 1 CRS**
  - Fever, constitutional symptoms

- **Grade 2 CRS**
  - Hypotension: responds to fluids or one low dose pressor
  - Hypoxia: responds to <40% O₂
  - Organ toxicity: grade 2
  
  - **Extensive co-morbidities or older age?**
    - No → **Vigilant supportive care**
    - Yes → **Vigilant supportive care**

- **Grade 3 CRS**
  - Hypotension: requires multiple pressors or high dose pressors
  - Hypoxia: requires ≥ 40% O₂
  - Organ toxicity: grade 3, grade 4 transaminitis
  
  - **Extensive co-morbidities or older age?**
    - No → **Vigilant supportive care**
    - Yes → **Vigilant supportive care**

- **Grade 4 CRS**
  - Mechanical ventilation
  - Organ toxicity: grade 4, excluding transaminitis

**Treatment**

- **Vigilant supportive care**
  - Assess for infection
  - (Treat fever and neutropenia if present, monitor fluid balance, antipyretics, analgesics as needed)
  
  - **Vigilant supportive care**
    - Monitor cardiac and other organ function closely
  
  - **Vigilant supportive care**
    - Tocilizumab ± corticosteroids

Foundation for the Accreditation of Cellular Therapy (FACT): CRS Management Guidance

<table>
<thead>
<tr>
<th>Cytokine Release Syndrome Grading assessment</th>
<th>Treatment</th>
</tr>
</thead>
</table>
| **Grade 1:** Fever (defined as >38.3°C)  
  • Constitutional Symptoms |  
  • Vigilant supportive care  
  • Assess for infection  
  • Treat fever and neutropenia if present  
  • Provide antipyretics & analgesics PRN |
| **Grade 2:** Hypotension that responds to fluid or low dose vasopressor, ie dopamine  
  • Hypoxia: responds to <40% O2  
  • Organ toxicity: grade 2 |  
  • As above for grade 1 and notify PI  
  • Cardiac monitor  
  • If patient has received prophylactic tocilizumab earlier in CAR-T course then consider steroids |
| **Grade 3:** Hypotension unresponsive to IVF bolus or requires high-dose vasopressors  
  • Hypoxia: requires >40% O2  
  • Organ toxicity: grade 3 or grade 4 transaminitis |  
  • Cardiac monitor, care as above, notify PI  
  • Consider Tocilizumab (8 mg/kg q 4-6 hr) (max 800 mg infused over 30 minutes)  
  • Consider dexamethasone 10 mg q6hrs or mg/kg solumedrol IV BID |
| **Grade 4:** Mechanical Ventilation  
  • Organ toxicity: grade 4 excluding transaminitis |  
  • Give 1 gram IV solumedrol daily x3 |
# Recommendations for CRS Management per CARTOX Working Group

<table>
<thead>
<tr>
<th>CRS Grade</th>
<th>Symptom or Sign</th>
<th>Management</th>
</tr>
</thead>
</table>
| Grade 1   | Fever or grade 1 organ toxicity | • Acetaminophen and hypothermia blanket for fever  
• Ibuprofen may be used as second option for fever if not contraindicated  
• Assess for infection with blood and urine cultures, and chest x-ray  
• Empiric broad-spectrum antibiotics and filgrastim if neutropenic  
• Maintenance IV fluids for hydration  
• Symptomatic management of constitutional symptoms and organ toxicities  
• Consider tocilizumab 8 mg/kg or siltuximab 11 mg/kg IV for persistent (>3 days) and refractory fever |
| Grade 2   | Hypotension              | • IV fluid bolus of 500 – 1000 mL normal saline  
• May give a second IV fluid bolus if SBP remains <90 mmHg  
• Tocilizumab 8 mg/kg<sup>b</sup> IV or siltuximab 11 mg/kg IV for hypotension refractory to fluid boluses; may be repeated if needed  
• If hypotension persists after two fluid boluses and anti-IL-6 therapy, start vasopressors, consider transfer to ICU, obtain echocardiogram and initiate other methods of hemodynamic monitoring  
• In patients at high-risk<sup>c</sup> or if hypotension persists after 1-2 doses of tocilizumab/siltuximab, may use dexamethasone 10 mg IV every 6h  
• Manage fever and constitutional symptoms as in grade 1  
| Hypoxia (FiO<sub>2</sub>&lt;40%) | Supplemental oxygen  
Tocilizumab/siltuximab +/- corticosteroids and supportive care as in hypotension  |
| Grade 2 organ toxicity | Symptomatic management of organ toxicities as per standard guidelines  
Tocilizumab/siltuximab +/- corticosteroids and supportive care as in hypotension  |

<sup>a</sup>All medication doses indicated are for adults. <sup>b</sup>Tocilizumab – maximum per DOSE is 800 mg  
<sup>c</sup>High risk patients include subjects with bulky disease, co-morbidities, and those who develop early onset CRS within three days of infusion
## Recommendations for CRS Management per CARTOX Working Group (continued)

<table>
<thead>
<tr>
<th>CRS Grade</th>
<th>Symptom or Sign</th>
<th>Management</th>
</tr>
</thead>
</table>
| **Grade 3** | **Hypotension** | • IV fluid boluses as needed as in grade 2  
• Tocilizumab/siltuximab as in grade 2 if not administered previously  
• Vasopressors as needed  
• Transfer to ICU, echocardiogram and hemodynamic monitoring as in grade 2  
• Dexamethasone 10 mg IV every 6h; increase to 20 mg IV every 6h if refractory  
• Manage fever and constitutional symptoms as in grade 1 |
| **Hypoxia (FiO2≥40%)** | | • Supplemental oxygen including high flow oxygen delivery and non-invasive positive pressure ventilation  
• Tocilizumab/siltuximab + corticosteroids and supportive care as above |
| **Grade 3 organ toxicity or grade 4 transaminitis** | | • Symptomatic management of organ toxicities as per standard guidelines  
• Tocilizumab/siltuximab + corticosteroids and supportive care as above |
| **Grade 4** | **Hypotension** | • IV fluids, anti-IL-6 therapy, vasopressors, and hemodynamic monitoring as in grade 3  
• Methylprednisolone 1 gram/day IV may be used in place of dexamethasone  
• Manage fever and constitutional symptoms as in grade 1 |
| **Hypoxia** | | • Mechanical ventilation  
• Tocilizumab/siltuximab + corticosteroids and supportive care as above |
| **Grade 4 organ toxicity excluding transaminitis** | | • Symptomatic management of organ toxicities as per standard guidelines  
• Tocilizumab/siltuximab + corticosteroids and supportive care as above |

*All medication doses indicated are for adults.  
*Tocilizumab – maximum per DOSE is 800 mg  
*High risk patients include subjects with bulky disease, co-morbidities, and those who develop early onset CRS within three days of infusion
Clinical Management of Less Common CRS-Associated Events

• Decrease in Cardiac Ejection Fraction/Stroke Volume
  • Transient; requires cardiac monitoring and ICU notification

• Renal dysfunction
  • Related to hypotension; may require dialysis; generally reversible

• Respiratory failure, ARDS

• DIC with clinical bleeding, low/undetectable fibrinogen
  • Seen especially in the setting of severe CRS
  • Requires blood product support per institutional practice

• Cytopenias >28 days
  • May require G-CSF; avoid GM-CSF during the time of possible CRS as may exacerbate symptoms
  • Hemophagocytic lymphohistiocytosis (HLH) is a very rare but severe form
Neurologic Toxicity
Concurrent with CRS and high fevers - Result of elevated cytokines - Common; some degree of neurotoxicity occurs in nearly all CAR T patients - Symptoms include decreased attention, confusion, disorientation, delirium and ataxia - Effectively resolved with tocilizumab

Result of elevated cytokines

Common; some degree of neurotoxicity occurs in nearly all CAR T patients

Symptoms include decreased attention, confusion, disorientation, delirium and ataxia

Effectively resolved with tocilizumab

### Acute

- Concurrent with CRS and high fevers
- Result of elevated cytokines
- Common; some degree of neurotoxicity occurs in nearly all CAR T patients
- Symptoms include decreased attention, confusion, disorientation, delirium and ataxia
- Effectively resolved with tocilizumab

### Delayed

- Occurs within days to weeks; following CRS; often on resolution of CRS
- Range of symptoms: confusion, mental status changes, encephalopathy, seizures, hallucinations, aphasia, and coma
- Generally reversible: typical duration ~ 3 days

### Cerebral Edema

- Rare
- Idiosyncratic
- Rapid acute onset
- Requires immediate ICU transfer and intervention with mannitol with or without anti-seizure medications
- May be fatal

---

Each type of neurological toxicity is likely due to different manifestations of CAR T therapy (different underlying physiologies), responds to different mechanisms, and has a different likelihood of reversibility.
Grading of Neurologic Toxicity

- CTCAE grading may not adequately quantify the acute neurologic deficits unique to CAR T therapies
- CARTOX Working Group has proposed the following grading scale for CAR-related encephalopathy syndrome (CRES):

<table>
<thead>
<tr>
<th>Symptom/Sign</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurological assessment score (see below)</td>
<td>Mild (7-9)</td>
<td>Moderate (3-6)</td>
<td>Severe (0-2)</td>
<td>Critical / Obtunded</td>
</tr>
<tr>
<td>Raised intracranial pressure</td>
<td></td>
<td>Stage 1 or 2 papilledema(^a); or CSF opening pressure &lt;20 mmHg</td>
<td>Stage 3, 4, or 5 papilledema; CSF opening pressure ≥20 mmHg; or cerebral edema</td>
<td></td>
</tr>
<tr>
<td>Seizures or motor weakness</td>
<td>Partial seizure; non-convulsive seizures on EEG responding to benzodiazepine</td>
<td>Generalized seizures; convulsive or non-convulsive status epilepticus; new motor weakness</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CARTOX 10-point neurological assessment**  
(Assign one point for each task performed correctly; Score of 10 = normal)  
• Orientation to year, month, city, hospital, President: 5 points  
• Name 3 objects (point to clock, pen, button): 3 points  
• Ability to write a standard sentence (e.g. Our national bird is the bald eagle): 1 point  
• Count backwards from 100 by ten: 1 point  

\(^a\)Papilledema grading is performed according to Modified Frisén scale
Neurotoxicity Management

**MONITORING**

- All patients with grade ≥ 2 neurologic toxicity should be evaluated by the neurology consult service
  - Neurological examination q 4 hours, wake the “sleeping patient”
  - Brain MRI
  - Continuous electroencephalogram (EEG)
  - Examination of the cerebrospinal fluid (CSF)

**TREATMENT**

- Severe neurologic toxicities are frequently treated with systemic corticosteroids
  - Dexamethasone is commonly used because of its excellent CNS penetration
- For patients with seizures, standard anti-epileptic therapy is given
  - At some institutions, anti-epileptic therapy is used prophylactically
- Response to tocilizumab remains unclear
  - Tocilizumab is a monoclonal antibody, so its size makes efficient BBB penetration unlikely

Guidelines for management of neurotoxicity vary by protocol and/or institution
Foundation for the Accreditation of Cellular Therapy (FACT): Neurotoxicity Management Guidance

- At the onset of ≥ grade 2 neurotoxicity
  - Levetiracetam (1000mg PO or IV BID)
  - Levetiracetam continues >750mg BID thru day 28 once neurotoxicity develops

- Tocilizumab infusion should be considered in grade 2 Neurotoxicity
  - Involve the PI
  - Tocilizumab at a dose of 8mg/kg (IV) over 1 hour (not to exceed 800mg)
  - If patient has received tocilizumab earlier in CAR-T therapy then consider steroids (dexamethasone 10mg IV every 6 hours)

- Subjects with ≥ grade 3 neurotoxicity:
  - Monitor continuous cardiac telemetry /pulse oximetry as clinically indicated
  - Tocilizumab should be given 8mg/kg (not to exceed 800mg)
  - Corticosteroids should be given to life-threatening neurotoxicity (methylprednisolone 1g/day x3)
# CARTOX Working Group: Recommendations for Management of Neurotoxicity

<table>
<thead>
<tr>
<th>Grade</th>
<th>Management</th>
</tr>
</thead>
</table>
| Grade 1   | • Vigilant supportive care; aspiration precautions; IV hydration  
• Withhold oral intake of food, medicines, and fluids and assess swallowing  
• Convert all oral medications and/or nutrition to IV if swallowing is impaired  
• Avoid medications that cause central nervous system depression  
• Low doses of lorazepam (0.25-0.5 mg IV every 8h) or haloperidol (0.5 mg IV every 6h) may be used for agitated patients with careful monitoring  
• Neurology consultation  
• Fundoscopic exam to assess for papilledema  
• MRI brain with and without contrast; diagnostic lumbar puncture with opening pressure; MRI spine if focal peripheral neurological deficits; CT scan of brain may be performed if MRI brain is not feasible  
• Daily 30 min EEG until toxicity symptoms resolve; if no seizures on EEG, continue levetiracetam 750 mg every 12h  
• If EEG shows non-convulsive status epilepticus, treat as per algorithm for management of non-convulsive status epilepticus after CAR T-cell therapy  
• Consider tocilizumab 8 mg/kg or siltuximab 11 mg/kg IV if associated with concurrent CRS                                                                 |
| Grade 2   | • Supportive care and neurological work-up as per grade 1  
• Tocilizumab 8 mg/kg\(^b\) or siltuximab 11 mg/kg IV if associated with concurrent CRS  
• Dexamethasone 10mg IV every 6h or methylprednisolone 1 mg/kg IV every 12h if refractory to anti-IL-6 therapy or for CRES without concurrent CRS  
• Consider ICU transfer if associated with grade 2 or greater CRS                                                                 |

\(^a\) All medication doses indicated are for adults.  
\(^b\) Tocilizumab – maximum per DOSE is 800 mg

CRES – CAR-Related Encephalopathy Syndrome  
CARTOX Working Group: Recommendations for Management of Neurotoxicity (continued)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Management</th>
</tr>
</thead>
</table>
| Grade 3 | • Supportive care and neurological work-up as per grade 1  
• ICU transfer is recommended  
• Tocilizumab/siltuximab if associated with concurrent CRS as per grade 2 and if not administered previously  
• Corticosteroids as above for worsening symptoms despite anti-IL-6 therapy or for CRES without concurrent CRS; Continue corticosteroids until improvement to grade 1 and then taper  
• Stage 1 or 2 papilledema with CSF opening pressure < 20 mmHg, treat as per algorithm for management of cerebral edema after CAR T-cell therapy  
• Consider repeat neuro-imaging (CT or MRI) every 2-3 days if persistent ≥ grade 3 CRES |
| Grade 4 | • Supportive care and neurological work-up as per grade 1  
• ICU monitoring; Consider mechanical ventilation for airway protection  
• Tocilizumab/siltuximab and repeat neuro-imaging as per grade 3  
• High-dose corticosteroids (e.g. methylprednisolone IV 1 g/day x 3 days followed by rapid taper at 250 mg every 12h x 2 days, 125 mg every 12h x 2 days, and 60 mg every 12h x 2 days); Continue corticosteroids until improvement to grade 1 and then taper  
• For convulsive status epilepticus, treat as per algorithm for management of convulsive status epilepticus after CAR T-cell therapy  
• Stage 3, 4, or 5 papilledema, CSF opening pressure ≥ 20 mmHg, or cerebral edema, treat as per algorithm for management of cerebral edema after CAR T-cell therapy |

aAll medication doses indicated are for adults. bTocilizumab – maximum per DOSE is 800 mg

CRES – CAR-Related Encephalopathy Syndrome
CARTOX Working Group: Additional Recommendations for Management of Status Epilepticus After CAR T Therapy

<table>
<thead>
<tr>
<th>Non-convulsive status epilepticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Assess airway, breathing, and circulation; check blood sugar</td>
</tr>
<tr>
<td>• Lorazepam 0.5 mg IV × 1 with additional 0.5 mg IV every 5 min up to a total of 2 mg to control electrographical seizures</td>
</tr>
<tr>
<td>• Levetiracetam 500 mg IV bolus</td>
</tr>
<tr>
<td>• If seizures persist, transfer to ICU and add phenobarbital loading dose 60 mg IV</td>
</tr>
</tbody>
</table>
| • Maintenance doses after resolution of non-convulsive status epilepticus  
  • Lorazepam 0.5 mg IV every 8h × 3 doses  
  • Increase levetiracetam to 1000 mg IV every 12h  
  • Phenobarbital 30 mg IV every 12h |

<table>
<thead>
<tr>
<th>Convulsive status epilepticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Assess airway, breathing, and circulation; check blood sugar</td>
</tr>
<tr>
<td>• Transfer to ICU</td>
</tr>
<tr>
<td>• Lorazepam 2 mg IV × 1 with additional 2 mg IV to a total of 4 mg to control seizures</td>
</tr>
<tr>
<td>• Levetiracetam 500 mg IV bolus</td>
</tr>
<tr>
<td>• If seizures persist, add phenobarbital loading dose 15 mg/kg IV</td>
</tr>
</tbody>
</table>
| • Maintenance doses after resolution of convulsive status epilepticus  
  • Lorazepam 0.5 mg IV every 8h × 3 doses  
  • Increase Levetiracetam to 1000 mg IV every 12h  
  • Phenobarbital 1-3 mg/kg IV every 12h  
  • Continuous EEG monitoring, if seizures are refractory |

All medication doses indicated are for adults
CARTOX Working Group: Additional Recommendations for Management of Cerebral Edema After CAR T Therapy

Stage 1 or 2 papilledema\(^b\) with CSF opening pressure < 20 mmHg without cerebral edema

- Consider acetazolamide 1000 mg IV followed by 250 mg to 1000 mg IV every 12h (adjust dose based on renal and acid/base balance)

Stage 3, 4, or 5 papilledema\(^b\), any cerebral edema on imaging studies, or CSF opening pressure ≥ 20 mmHg

- Use high-dose steroids as per grade 4 CRES along with the following measures for management of cerebral edema
- Elevate head end of bed to 30 degrees
- Hyperventilation to achieve target PaCO\(_2\) of 28-30 mmHg for no greater than 24h
- Hyperosmolar therapy with either mannitol 20% or hypertonic saline (3% or 23.4%)
  - Mannitol: initial dose 0.5 to 1 g/kg, maintenance at 0.25 to 1 g/kg every 6h while monitoring metabolic profile and serum osmolality every 6h; hold mannitol if serum osmolality ≥ 320 mOsm/kg or osmolality gap ≥40
  - Hypertonic saline: initial 250 mL of 3% hypertonic saline, maintenance at 50-100 mL/h while monitoring electrolytes every 4h; hold infusion if serum Na ≥155 mEq/L
- Imminent herniation: Initial 30 mL of 23.4% hypertonic saline (may repeat in 15 min)
- If patient has ommaya reservoir, drain CSF to target opening pressure < 20 mmHg
- Consider neurosurgery consultation, IV anesthetics for burst-suppression EEG
- Metabolic profile every 6h, daily computed tomography scan of head and adjust above medications to prevent rebound cerebral edema, renal failure, electrolyte abnormalities, hypovolemia, and hypotension

All medication doses indicated are for adults

\(^b\)Papilledema grading is performed according to Modified Frisén scale
Macrophage Activation Syndrome (MAS or HLH)
HLH Laboratory Abnormalities

Elevated ferritin
  Likely secreted by activated macrophages

Elevated triglycerides
  Increased levels of TNF-alpha suppress activity of lipoprotein lipase

Elevated LDH
  • Depressed fibrinogen
    – Increased levels of plasminogen activator secreted by activated macrophages
  • Impaired NK cell activity
  • Elevated soluble IL-2 receptor (sCD25)
  • Transaminitis
Current Diagnostic Criteria for HLH

Fulfill 5 of the following clinical/laboratory criteria*:

1. Fever
2. Splenomegaly
   - Cytopenias (at least 2 cell lines) HGB < 9; PLT < 100,000; ANC< 1000
   - Hypertriglyceridemia and/or hypofibrinogenemia
     - Fasting Trig > 265 mg/dL
     - Fibrinogen < 150 mg/l
3. Hemophagocytosis in bone marrow, CSF, or lymph nodes
4. Decreased/absent NK cell activity *
5. Ferritin > 500 ug/l - Diff dx for ferritin > 10,000: histiocytic malignancies, adult-onset Still’s disease
6. Soluble CD25 > 2400 U/ml *

Many of the traditional diagnostic criteria for HLH are not specific and are frequently present in patients with even low-grade CRS and among patients with advanced hematologic malignancies in the absence of CAR T therapy

*Current diagnostic criteria from the Histiocyte Society; HLH – hemophagocytic lymphohistiocytosis
Proposed Diagnostic Criteria for CAR-related HLH or MAS per CARTOX Working Group

If a subject that had peak ferritin >10,000 ng/mL during the cytokine release syndrome phase developed any two of the following organ toxicities after CAR T-cell therapy, the subject may have HLH/MAS

- ≥ Grade 3 increase in bilirubin, aspartate transaminase, or alanine transaminase
- ≥ Grade 3 oliguria or increase in creatinine
- ≥ Grade 3 pulmonary edema
- ≥ Presence of hemophagocytosis by morphology and/or CD68 immunohistochemistry in bone marrow or organs

Grading as per Common Terminology Criteria for Adverse Events, version 4.03

HLH – hemophagocytic lymphohistiocytosis; MAS – macrophage activation syndrome
Recommendations for Management of CAR-related HLH or MAS per CARTOX Working Group

HLH – hemophagocytic lymphohistiocytosis; MAS – macrophage activation syndrome
Best Practices
Best Practices for CRS Management

✓ Maintain close observation and open line of communication
  • Detection and management of CRS requires teamwork: Clinicians, Nurses, Cell Pharmacists
  • Confirm regular and frequent assessment of patients
  • Establish a process for rapid escalation of care, increased intensity of monitoring, and relevant workup
  • Ensure timely communication between clinical staff, ICU, emergency departments, and pharmacies

✓ Ensure rapid availability of ICU support, neurology and/or infectious disease consults

✓ Develop written guidelines for management of complications, including the use of anti-cytokine directed therapy and corticosteroid administration

✓ Ensure rapid availability and infusion of anti-cytokine directed therapy
  • Goal: infusion within one hour of order

✓ Fever: ID workup and empiric gram-negative coverage
### Fluids
- It is important to avoid aggressive fluid administration in patients at risk of pulmonary edema from cytokine release syndrome

### Tocilizumab
- Tocilizumab should be administered within 1-2 hours of the drug being ordered
- Tocilizumab should be given at full dose despite blood or platelet counts
- It is not uncommon to re-dose every 6-12 hours

### Corticosteroids
- The judicious use of steroids is recommended
- Only give steroids if a patient has CRS symptoms and no response to first dose of tocilizumab to prevent limiting the benefits of the immunotherapy
Working in Partnership: Best Practices

Neurology
- Ensure availability of neurology staff who are trained to manage complications associated with CAR T therapy
- Patients should receive frequent neurologic exams
- As appropriate, full neurology work-up may be needed to rule out other etiologies

Pharmacy
- Ensure rapid availability of formularies adequate to treat CRS and other expected complications
- Tocilizumab should be kept in pharmacy and added to hospital formulary
- In addition to having medications available, there should always be a pharmacist available on-site or on-call who has been trained to manage complications associated with CAR T therapy
Education: CRS Management

• Training must include care interventions to manage complications
• All appropriate staff should receive education, including:
  - Physician staff: cellular therapy, ICU, neurology and infectious disease consultants
  - Nursing: cellular therapy, inpatient, ICU
  - ER
  - Pharmacy
  - Admissions
What to Tell the Referring Oncologist

- Patients may be sent back to their primary oncologist after 28 days
  - Long-term CRS (post-day 28) is very rare

- Primary oncologist should closely monitor patients:
  - Blood counts
  - IgG levels
  - Signs of infection

- Hypogammaglobulinemia and prolonged B-cell aplasia is common
  - The utility of intravenous Ig (IVIg) replacement therapy needs to be further understood
  - The long-term sequelae of B-cell aplasia and IVIg replacement remain unknown

- Patients should follow-up with the CAR T center monthly
INSTITUTIONAL CONSIDERATIONS

MODULE 6
1. Identification of patients who are appropriate for CAR T therapy
   • Patient journey and logistics
   • Common eligibility criteria

2. Insights into best practices of experienced centers
   • Building a multidisciplinary CAR T team
   • What does good look like?
Due to the characteristics of patients who are treated with CAR T therapy, the time pressure from patient identification to apheresis is expected to be a significant constraint.
Patients who are Appropriate for CAR T Therapy

Factors to consider when selecting patients for CAR T therapy:

1. Age
2. Organ function
3. ECOG PS
4. Underlying neurological disorders, including seizures
5. Active infections
   • Uncontrolled infections may exacerbate certain toxicities, such as CTCAE grade 5 infections
6. CNS disease
   • Exclusion varies by CAR T therapy and indication

Many of the perceived barriers to CAR T therapy are generally not real barriers for patients
# Common Eligibility Criteria for CAR T Clinical Trials

## Key Inclusion Criteria

- Life expectancy ≥12 weeks
- ECOG performance status of 0 - 1 at screening
- Adequate bone marrow reserve
  - ANC ≥ 1000/µL
  - ALC >100-300/µL
  - Platelet count ≥ 50,000-75,000/µL
  - Hemoglobin > 8.0 g/dl
- Adequate renal function
  - Serum creatinine ≤1.5 x ULN
  - eGFR ≥ 60 mL/min/1.73 m²
  - Creatinine clearance (as estimated by Cockcroft Gault) > 60 mL/min
- Adequate hepatic function
  - Serum ALT/AST <2.5-5 x ULN
  - Total bilirubin <1.5-2 mg/dl, except in subjects with Gilbert's syndrome
- Adequate cardiac function
  - Cardiac ejection fraction >45-50%, no evidence of pericardial effusion as determined by an ECHO
- Adequate pulmonary function
  - Baseline oxygen saturation >91-92% on room air
- Adequate vascular access for leukapheresis procedure

## Key Exclusion Criteria

- History of allogeneic stem cell transplantation
- Prior CAR therapy or other genetically modified T cell therapy
- Active CNS involvement by malignancy
- Active hepatitis B, hepatitis C, or HIV infection
- Uncontrolled acute life threatening bacterial, viral or fungal infection (e.g. blood culture positive ≤ 72 hours prior to infusion)
- Cardiovascular disease
  - Unstable angina and/or myocardial infarction within 6 months
  - Cardiac arrhythmia not controlled with medical management
  - Patients on oral anticoagulation therapy
- Previous or concurrent malignancy with the following exceptions:
  - Adequately treated basal cell or squamous cell carcinoma
  - In situ carcinoma of the cervix or breast, treated curatively and without evidence of recurrence for at least 3 years prior to the study
  - A primary malignancy which has been completely resected and in complete remission for ≥ 5 years
- History or presence of CNS disorder such as seizure disorder, cerebrovascular ischemia/hemorrhage, dementia, cerebellar disease, or any autoimmune disease with CNS involvement
Insights into Best Practices of Experienced Centers
Best Practices of Experienced Centers: Logistics

• Provide information sheets for referring oncologists and patients/caregivers that clearly describe eligibility requirements

• Calls for referrals should be directed to experienced coordinators and nurse navigators

• During consultation, patients should undergo consenting process and be clearly educated on the risks of therapy

• Create a dedicated team to manage the logistics of patient intake and billing / financial services

• Maintain close and frequent communication with the manufacturers of CAR T therapy
Best Practices of Experienced Centers: Building a Multidisciplinary Team

Key Stakeholders

- Special team of physicians dedicated to CAR T therapy
  - Some institutions will have a separate clinical unit (e.g., cellular therapy) that administers CAR T cells
  - Other institutions will administer CAR T cells through the transplant program
  - Best practice is to limit the number of physicians/investigators that administer CAR T cells
- Dedicated coordinators and nurse navigators
- Dedicated MICU physician and nursing service for CAR T therapy
- Pharmacy
- Collection facilities and processing facilities
Best Practices of Experienced Centers: What Does Good Look Like?

• Ensure weekly multidisciplinary team meetings
  - Ongoing education and communication is required to maintain a high level of vigilance across the whole system
  - Educational programs, protocol in-services, inpatient unit education, and toxicity management guidelines may be included
  - Consider weekly grand rounds that include lymphoma and leukemia teams, transplant, neurology, radiology, and MICU

• Ensure communication to MICU of all CAR T therapy patients admitted to cellular therapy or transplant service

• Develop SOP for communication between teams relative to nighttime and weekends
Best Practices: Ensure Crosstalk between Clinical, Nursing, Financial, and Coordination Teams
MODULE 7: Outline

1. Current targets being investigated
   - Clinical trials in hematologic malignancies
   - Ongoing investigations in extending CAR T therapy to solid tumors

2. Future of CAR T Therapy
   - Next-generation CARs
CAR T Therapy is a Rapidly Growing Technology

CAR T Cell Trials Are Now Global

Clinical trials.gov search term “chimeric antigen receptor”
265 trials ongoing as of March 6, 2018
### Characteristics of Ideal Tumor Antigen Targets

1. Not expressed in normal tissue
2. No epitope sharing with normal molecules
3. Good affinity for the receptors
4. Not easily shed or down regulated

#### Table 1: Examples of human cancer antigens

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Class I-restricted antigens recognized by CD8+ lymphocytes</strong></td>
<td></td>
</tr>
<tr>
<td>Melanoma–melanocyte</td>
<td></td>
</tr>
<tr>
<td>differentiation antigens</td>
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<tr>
<td>MART-1 (Melan-A)</td>
<td>42</td>
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<tr>
<td>gp100 (pmel-17)</td>
<td>43</td>
</tr>
<tr>
<td>Tyrosinase</td>
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<tr>
<td>Tyrosinase related protein-1</td>
<td>45</td>
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<tr>
<td>Tyrosinase related protein-2</td>
<td>46</td>
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<tr>
<td>Melanocyte-stimulating hormone receptor</td>
<td>47</td>
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<tr>
<td><strong>Cancer-testes antigens</strong></td>
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<td>48</td>
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<td>HLA-A2-R1701</td>
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<td><strong>Non-mutated shared antigens overexpressed on cancers</strong></td>
<td></td>
</tr>
<tr>
<td>α-Fetoprotein</td>
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<td>Telomerase catalytic protein</td>
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<td>Carcinoembryonic antigen</td>
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<td>Her-2/neu</td>
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<tr>
<td><strong>II. Class II-restricted antigens recognized by CD4+ lymphocytes</strong></td>
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<tr>
<td>Epitopes from non-mutated proteins</td>
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<td>gp100</td>
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<td>NY-ESO-1</td>
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<td>Epitopes from mutated proteins</td>
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<tr>
<td>Triosephosphate isomerase</td>
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<td>CDC-27</td>
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<tr>
<td>LDLR-FUT</td>
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</table>

## Ongoing CAR Trials in Hematologic Malignancies

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Targets Currently Being Investigated</th>
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</thead>
<tbody>
<tr>
<td><strong>Lymphoma</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cell Lymphoma</td>
<td>56</td>
<td>47</td>
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<td>CD19, CD20, CD22, CD30</td>
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<tr>
<td>ALL</td>
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Challenges with Incorporating CAR T Cells in AML

• No tumor-specific target identified but mostly overexpression of normal myeloid antigens on malignant cells
  – Off-tumor, on-target toxicity
  – Prolonged or permanent myelosuppression
  – Concern with persisting T cells
  – Bridge to transplant or as conditioning therapy prior to HSCT

• Lack of clinical efficacy in high burden of disease, at least in mouse models
# Ongoing CAR Trials in Solid Tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Number of Clinical Trials</th>
<th>Targets Currently Being Investigated</th>
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<tbody>
<tr>
<td>Astrocytoma</td>
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<td>HER2, EGFRvIII, IL13Ra2</td>
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<tr>
<td>Glioblastoma</td>
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<td>Breast</td>
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Requirements for Successful CAR T Cell Activity

1. trafficking
2. accumulation
3. Recognition, stimulation & activation
4. effector function
5. Avoidance of inhibitory signals & suppression
6. persistence of effectors until tumor elimination

Future of CAR T Therapy
Potential Modifications to CAR T Therapy

- Different antibody variable chain designs
- Different hinge regions and costimulatory domains
- Different viral vectors
- Multiple infusions
- Humanized constructs
- Modifying lymphodepleting or debulking chemotherapy
- Modifying cellular composition
Evolution in CAR Design

First-Generation CAR
scFv-CD3ζ

Second-Generation CAR
scFv-CD28-CD3ζ

Third-Generation CAR
scFv-CD28-4-1BB-CD3ζ
scFv-CD28-OX40-CD3ζ

Future Modification Capability

• Intracellular domains can be modified to increase efficacy and durability of CAR T cells

• CARs can be engineered to express chemokines and cytokines that further enhance function and migration

• CARs can be modified to express suicide genes that limit CAR T population if toxicity occurs
Strategies to Overcome Limitations of Current CARs

- OFF/ON Switch
- Affinity-tuned CAR T cells
- Split receptor design: dual antigen recognition
- Inducible CAR expression
- TCR-like Ab-based CAR: targeting intracellular Ag
- Armored CARs
Strategy for Design of ON-Switch CARs

Conventional CAR design

INPUT 1: tumor antigen

scFv

co-stim. domain

CD3ζ domain (TCR ITAMs)

T cell response

Suicide switch

Small molecule

ACTIVE

DEAD

ON-switch

Small molecule

INACTIVE

ACTIVE

Split CAR design

INPUT 1: tumor antigen

scFv

INPUT 2: small molecule

Part I

CD3ζ domain (TCR ITAMs)

Part II

T cell

Split-Receptor Designs

(A) Normal tissue A

Attenuated Signal 1

No activation

(A) Normal tissue B

Signal 2

No activation

(A) Tumor cell

Full activation

(B) Normal tissue

-nove

No activation

(B) Tumor cell

Full activation

Key:

Antigen 1

Target for Inhibitory signal

Inducible CAR T Cells

1. IF antigen A THEN synNotch activates CAR transcription
2. IF antigen B THEN CAR activates T cell

two antigen tumor recognition circuit:

synNotch primes CAR expression

antigens A B
AND
T CELL ACTIVATION
Armored CARs

Figure 1.

<table>
<thead>
<tr>
<th>First generation CAR</th>
<th>Second generation CAR</th>
<th>Third generation CAR</th>
<th>“Armored” Fourth generation CAR</th>
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<tr>
<td>scFv (V_1 + V_2)</td>
<td>ζ signaling domain</td>
<td>41B8 costimulatory domain</td>
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<td>Costimulatory ligand</td>
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</table>

1  2
Proposed Mechanism of IL-12 Secreting Armored CAR T cells

- Enhanced CM phenotype
- Enhanced cytotoxicity
- Enhanced persistence
- Resistance to Treg and TGFβ inhibition

**NK cell**
- Recruitment and activation

**Tumor cell**
- Targeted tumor cytotoxicity

**Activated TIL**
- Reversal of anergy

**Anergic TIL**
- Targeted tumor cytotoxicity

**CAR-RES IL-12**
- IL-12 secretion
- Enhanced CM phenotype, enhanced cytotoxicity, enhanced persistence
- Resistance to Treg and TGFβ inhibition
CD40L genetically modified T cells: Armored CAR T cells v2.0

Proposed mechanism of CD40L immune stimulation within the tumor microenvironment. (1) CAR/CD40L modified T cells activate tumor cells directly to upregulate CD80/86, CD54, CD95, and MHC, while (2) inducing auto or trans-costimulation of the modified T cells. (3) Further CD40L and sCD40L may modify tumor and recruit/activate endogenous NK cells. (4) CD40L induces maturation of DCs which in turn have increased APC function to stimulate endogenous T cells, as well (5) release IL-12 which (6) inhibits Tregs and reverses TIL anergy, further enhancing the anti-tumor immune response.