Neurotoxicity in CAR-T Therapy
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Learning Objectives

• To provide a brief overview of chimeric antigen receptor T cell therapy (CAR-T) and side effects
• To review neurotoxicity associated with CAR-T therapy
• To use case-based vignettes to discuss the current evaluation and treatment of CAR-T related neurotoxicity

American Society for Transplantation and Cellular Therapy Practice Guidelines

• There is an APP for that!

Chimeric Antigen Receptor T cells (CAR T) are Reprogrammed T cells Used to Target and Eliminate Cancer Cells

• Customized receptor
  - Extracellular antigen-binding domain
  - Intracellular signaling domain of T cells
• Recognize cell surface antigens independent of MHC, have co-stimulatory signals integrated
• Retains the functionality of a T-cell with the antigen recognition properties of antibody
Making a CAR-T Cell

1. Apheresis
2. Stimulation and Transduction
3. Expansion
4. Lymphodepletion
5. Infusion

CD19 CAR Therapy is Effective in ALL and Lymphoma

<table>
<thead>
<tr>
<th>Trial</th>
<th>CAR Construct</th>
<th>Name</th>
<th>Disease</th>
<th>Age</th>
<th>Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eliana</td>
<td>CD19-41BB</td>
<td>Tisagenlecleucel®</td>
<td>ALL</td>
<td>Peds</td>
<td>81% CR</td>
</tr>
<tr>
<td>FLAT-G2</td>
<td>CD19-41BB</td>
<td>ALL</td>
<td>Peds</td>
<td>89% CR</td>
<td></td>
</tr>
<tr>
<td>NCI</td>
<td>CD19-28z</td>
<td>ALL</td>
<td>Peds</td>
<td>87% CR</td>
<td></td>
</tr>
<tr>
<td>Zuma-1</td>
<td>CD19-28z</td>
<td>Axicabtagene Ciloleucel®</td>
<td>DLBCL</td>
<td>Adult</td>
<td>84% ORR</td>
</tr>
<tr>
<td>JULIET</td>
<td>CD19-41BB</td>
<td>Axicabtagene Ciloleucel®</td>
<td>DLBCL</td>
<td>Adult</td>
<td>53% ORR</td>
</tr>
<tr>
<td>MSKCC</td>
<td>CD19-28z</td>
<td>Axicabtagene Ciloleucel®</td>
<td>ALL</td>
<td>Adult</td>
<td>83% CR</td>
</tr>
</tbody>
</table>

* Represents FDA approved products

CD22 CAR Therapy is Effective as a Salvage Therapy After CD19 Directed Therapy

• NCI Phase I experience
• 51/58 patients infused had prior CD19 targeted therapy
• 40/57 patients had complete response

FDA Approval Granted for First Gene Therapy in Pediatric Patients in US

• Kymriah™ (tisagenlecleucel, Novartis):
  - For children up to age 25 with ALL (August 2017)
  - For adults with diffuse large B cell lymphoma (May 2018)
• Yescarta™ (axicabtagene ciloleucel, KITE):
  - For adults with diffuse large B cell lymphoma (October 2017)
  - CD22 CAR T cell (NCI):
    - Breakthrough Drug Designation by FDA in August 2019
    - For pediatric patients that are CD19 negative or relapsed/refractory to CD19 directed therapy

  Complete remission rates: +/- 50-80%
Cytokine Release Syndrome is the Most Common Side Effect

- Constellation of symptoms due to supraphysiologic cytokine production.
- Onset occurs within hours to days post infusion
- Severity of CRS correlates with:
  - Disease burden
  - Lymphodepleting chemotherapy
  - Cell dose

Hypotension

Fever*

Severity of CRS correlates with:
- days post infusion
- Onset occurs within hours to supraphysiologic cytokine

Constellation of symptoms due to cytokine release syndrome.

Cytokine Release Syndrome is the Most Common Side Effect

- Hypotension
- Fever*

Severity of CRS correlates with:
- days post infusion
- Onset occurs within hours to supraphysiologic cytokine

ASTCT CRS Consensus Guidelines

<table>
<thead>
<tr>
<th>CRS Parameter</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever*</td>
<td>Temp ≤ 38 C</td>
<td>Temp ≤ 38 C</td>
<td>Temp ≤ 38 C</td>
<td>Temp ≤ 38 C</td>
</tr>
<tr>
<td>Hypotension</td>
<td>None</td>
<td>Not requiring vasopressors</td>
<td>Requiring a vasopressor with or without vasopressor</td>
<td>Requiring multiple vasopressors (including vasopressor)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>None</td>
<td>Requiring low-flow nasal cannula or blow by</td>
<td>Requiring high-flow nasal cannula, face mask, non-rebreather</td>
<td>Requiring positive pressure (CPAP, BiPAP, intubation and mechanical ventilation)</td>
</tr>
</tbody>
</table>

New Guidelines:
- CRS Severity is determined by hypotension and hypoxia
- Grade is determined by the more severe event
- Notably, organ toxicities are not included in new CRS definitions and do not affect grading
- Once anti-cytokine treatment is given, fever is no longer applicable

Discrepancies Existed Amongst Published CRS Grading Scales (2009-2017)

<table>
<thead>
<tr>
<th>CRS Grading Scale</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Cytokine (Lee Criteria)</td>
<td>Symptoms require and respond to early supportive interventions.</td>
<td>Symptoms require and respond to moderate interventions.</td>
<td>Symptoms require and respond to aggressive interventions.</td>
<td>Symptoms requiring hospitalization for supportive care including mechanical ventilation and vasopressors.</td>
</tr>
<tr>
<td>Fever*</td>
<td>Life-threatening</td>
<td>Life-threatening</td>
<td>Life-threatening</td>
<td>Life-threatening</td>
</tr>
</tbody>
</table>

None
None
None
None

None
None
None
None

None
None
None
None

None
None
None
None

Grade 1 organ toxicity
Grade 2 organ toxicity
Grade 3 organ toxicity
Grade 4 organ toxicity

Treatment of CRS

- Tocilizumab
- FDA approval August 31, 2017

- Anakinra
- Used to block pro-inflammatory effects of IL-1

Recommended Timing of Initial Intervention With Tocilizumab and Corticosteroids Based on Grade of Cytokine Release Syndrome.

<table>
<thead>
<tr>
<th>Grade CRS (as per ASTCT consensus criteria)</th>
<th>Tocilizumab</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1—no comorbidities</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2—no comorbidities</td>
<td>Yes</td>
<td>Consider</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Consider</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Lee DW, et al 2019
Severe CRS May be Effectively Prevented with Early Treatment

- Increasing use of pre-emptive tocilizumab and/or steroids to prevent more severe CRS
- Early treatment with tocilizumab and/or steroids demonstrated equipotent efficacy of CAR with reduced severity of CRS (Gardner et al)
- New ASTCT guidelines support use of early tocilizumab to reduce severity of CRS

Neurotoxicity

Reported Incidence of Neurotoxicity is Variable and Depends on CAR construct

- Incidence ranges from 0-64% depending on specific trial, CAR construct, and target
- Severe neurotoxicity (sNT defined as CTCAE ≥ grade 3, and/or seizures) occurs in 5-50% of patients

Immune effector Cells Associated Neurotoxicity Syndrome (ICANS)

- Similar to CRS grading, no uniform grading existed prior to ASTCT ICANS
- ICANS: A disorder characterized by a pathologic process involving the central nervous system following any immune therapy that results in the activation or engagement of endogenous or infused T cells and/or other immune effector cells

Gardner, RD. Blood 2019
Neelapu, S. Nat Rev Clin On - 2018
Lee, D.W. Blood Marrow Transplant 2018
Lee et al, Bio Blood Marrow Transplant 2018
Risk Factors Associated with More Severe Neurotoxicity

- Severity of CRS
- Neurologic co-morbidities
- Disease burden
- Lymphodepletion with Fludarabine/Cyclophosphamide
- Higher CAR T cell dose

Etiology of Neurotoxicity is an Area of Active Research

- Cytokines have been implicated
- Endothelial activation can cause disruption of blood brain barrier, increasing permeability
- Endothelial disruption activates angiopoetin (ANG) leading to a proinflammatory/prothrombotic state
- Astrocyte/microglial injury → cerebral edema

Clinical Presentations Vary Amongst Patients

<table>
<thead>
<tr>
<th>Mild</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Tremor</td>
<td>• Seizures in ALL patients receiving CD19 CAR T cells</td>
</tr>
<tr>
<td>• Dysgraphia: inability to write</td>
<td>• CD19-1BB ranges 3-14%</td>
</tr>
<tr>
<td>• Expressive aphasia</td>
<td>• CD19-28z ranges 0-50%</td>
</tr>
<tr>
<td>• Apraxia: difficulty following commands</td>
<td>• Global toxic-metabolic encephalopathy*</td>
</tr>
<tr>
<td>• Confusion</td>
<td>• Tremors</td>
</tr>
<tr>
<td>• Headache</td>
<td>• Peripheral neuropathy</td>
</tr>
<tr>
<td>• Weakness</td>
<td>• Cerebral edema</td>
</tr>
</tbody>
</table>

*Gust J, Cancer Disc 2017
*Santomasso BD, Cancer Disc 2018
Non-Specific Symptoms & Signs are Now Excluded from ICANS Definition

- Headache
- Tremors
- Weakness
- Hallucinations
- Myoclonus
- Intracranial Hemorrhage

Encephalopathy Grading Scales

<table>
<thead>
<tr>
<th>Grade</th>
<th>Patient unarousable and unable to perform ICE</th>
<th>Patient unremarkable or requires vigorous stimulation</th>
<th>ICU</th>
<th>Seizure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>Patient unable to perform ICE</td>
<td>Patient unremarkable or requires vigorous stimulation</td>
<td>ICU</td>
<td>Any clinical seizure focal or generalized that resolve rapidly; or non-convulsive seizures on EEG that resolve with intervention</td>
</tr>
<tr>
<td>Grade 1</td>
<td>ICU Score &gt;12</td>
<td>&lt;9</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Grade 2</td>
<td>ICU Score &lt;9</td>
<td>&lt;9</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Grade 3</td>
<td>ICU Score &lt;9</td>
<td>&gt;9</td>
<td>N/A</td>
<td>Any clinical seizure focal or generalized that resolve rapidly; or non-convulsive seizures on EEG that resolve with intervention</td>
</tr>
<tr>
<td>Grade 4</td>
<td>ICU Score &lt;9</td>
<td>&gt;9</td>
<td>Any clinical seizure focal or generalized that resolve rapidly; or non-convulsive seizures on EEG that resolve with intervention</td>
<td></td>
</tr>
</tbody>
</table>

Bedside Assessments are Critical in Monitoring Patients for Neurotoxicity

- Clinical Exam is Key
- History of Events from Family members
- Mini Mental Status Exam/Review of Symptoms
Further Evaluation of Patients with Neurotoxicity Depends on Degree of Impairment

- Neurology Consult
- CNS Imaging
  - MRI preferred
  - CT for acutely ill
- Lumbar puncture to evaluate CSF:
  - Leukocytosis, lymphocyte predominant
  - Increased protein
- EEG
  - Diffuse slowing, non-specific for encephalopathy

Prevention of CAR-associated Neurotoxicity

- Seizure prophylaxis
- Tocilizumab
  - To prevent severe CRS
  - Leads to increased IL-6 levels which can cross blood brain barrier potentially worsen neurotoxicity

Treatment of CAR T cell Associated Neurotoxicity

- Corticosteroids
  - First line therapy for CNS neurotoxicity
  - Excellent penetration into CNS
  - Steroids can affect T cell function → decrease efficacy of CAR
  - Dexamethasone is most routinely used
- Role for IT steroids
  - Anecdotal evidence suggests its safe and can decrease severity of NT

Can We Identify Patients Prospectively Who are at Risk of Developing Severe Neurotoxicity?

- Multiple studies are exploring whether biomarker-based stratification can identify patients who are at significant risk of developing severe neurotoxicity
- Early fever and several elevated serum cytokines (MCP-1, IL-10, IL-15, IL-2) have been correlated with more severe NT
- Elevated levels of ANG2:ANG1 pre-lymphodepletion correlated with patients who had ≥grade 4 NT

Gust, Cancer Disc 2017
Santomasso BD, Cancer Disc 2018
Shalabi H, J Immunother 2018
No irreversible neurotoxicity was seen.

Prospective Neurocognitive Studies Were Feasible in an Acutely Ill Population

- Serial Neurocognitive evaluations were performed testing:
  - Working memory
  - Attention
  - Processing Speed
  - Executive Function
  - Neurosymptom Checklist

Neurocognitive Assessments Demonstrated Stable to Improved Scores Post CD22 CAR T Therapy

- No irreversible neurotoxicity was seen
- Cytokine levels were significantly higher in patients with hallucinations or disorientation

Patient Reported Outcomes Demonstrated Improved Quality of Life Post CAR therapy

- Patients treated with tisagenlecleucel who prospectively assessed for quality of life outcomes using 2 questionnaires
- Results demonstrated rapid improvement in broad aspects of quality of life among all patients who had treatment response
QUESTIONS

Clinical Vignettes
ASTCT app has CRS and ICANS guidelines and algorithms at your fingertips

Patient 1

• 20 yo with primary refractory ALL

• Enrolled on CD19 CAR T cell trial @ dose level 1

• High dose lymphodepletion

• Disease status:
  • 85% ALL (M3 marrow)
  • CNS1 (flow+ disease)

What are his risk factors for CRS? Neurotoxicity?

• Clinical Manifestation:
  • Day+2 developed fevers, hypotension, and tachycardia prompting ICU transfer
  • Required multiple fluid boluses for hypotension
    • Started on broad spectrum antibiotics for fever and neutropenia
    • Received Tocilizumab
  • Ejection Fraction dropped to 25%

https://tct.confex.com/tandem/2020/audiencepoll/ask.cgi?password=151925&Entryid=14249&EntryTable=Paper&Questionid=482#ask
Patient 1 Continued

- D+11 developed delirium and agitation
  - He was able to name three objects, follow commands, and count backwards
  - What was his ICE score? 5
- D+12 had generalized seizure and was intubated due to neurologic status

Work up, Findings, and Treatment

Work up:
- CT head: normal
- MRI brain: normal, no white matter changes
- EEG: no epileptiform activity noted
- Lumbar puncture
  - LP showed NO disease and 77% CAR T cells

Treatment:
- Dexamethasone for neurotoxicity
- IT hydrocortisone

Patient neuro status was back to baseline 48 hrs after administration of steroids, and he achieved an MRD negative remission

Patient 2

- 5 yo with primary refractory ALL
- Enrolled on CD19 CAR T cell trial @ dose level 2
- Standard Lymphodepletion
- Disease status
  - 3% ALL (M1 marrow)
  - CNS1- no evidence of leukemia

What are his risk factors for CRS? Neurotoxicity?

Clinical Manifestation:
- Day+3 developed fevers
  - Started on broad spectrum antibiotics for non-neutropenic fever
Work up, Findings, Treatment

Work up:
- CT was normal
- Lumbar puncture
- LP showed no disease and 81% CAR T cells
- MRI showed non-specific diffuse white matter injury

Treatment:
Patient received IV dexamethasone for neuro symptoms and within 12 hours of dexamethasone, symptoms resolved. He had an MRD negative response to CAR therapy and went on to receive a curative bone marrow transplant.

Patient 3

- 13 yo male with relapsed/refractory ALL
- Enrolled on CD22 CAR T cell trial @ dose level 1
- Standard Lymphodepletion
- Disease status: 20% ALL (M2 marrow)
- CNS1: no evidence of leukemia

What are his risk factors for CRS?
Neurotoxicity?

Patient 3 Continued

- Day +12 patient developed altered mental status with confusion
  - He was able to name three objects, state the month and year, follow commands, and count backwards
  - What was his ICE score? 7

Clinical Manifestation:
- Day+8 developed fevers
  - Started on broad spectrum antibiotics for neutropenic fever
- Day+10 he developed hypotension and coagulopathy and was transferred to ICU
  - Received multiple fluid boluses and was started on epinephrine
  - Tocilizumab was administered

ICE score:

https://tandem.com/2020/audiencepoll/ask.cgi?password=151925&Entryid=14249&EntryTable=Paper&Questionid=484#ask

Patient 3 Continued

- Day + 12 patient developed altered mental status with confusion
  - He was able to name three objects, state the month and year, follow commands, and count backwards
  - What was his ICE score? 7

https://tandem.com/2020/audiencepoll/ask.cgi?password=151925&Entryid=14249&EntryTable=Paper&Questionid=495#ask
Work up, Findings, Treatment

Work up:
• CT head was normal

Treatment:
Patient did not receive any therapy for neuro symptoms and recovered over the course of 3 days. He had a complete response to CAR therapy.

Future Directions

• Active areas of research in CAR-associated neurotoxicity:
  • Cytokine evaluations in serum and CSF
  • Identification of blood brain barrier permeability biomarkers
  • Imaging studies
  • Neurocognitive assessments
  • Quality of life measures

• Consortium amongst CAR pediatric centers has been established to share data and work more closely towards assessing later effects post CAR therapy.

Thank you for your attention!

Questions/Comments:
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