Chimeric antigen receptor (CAR) T cells

Lilian Tang-Dizon, PA-C
Fred Hutchinson Cancer Center
Seattle, WA

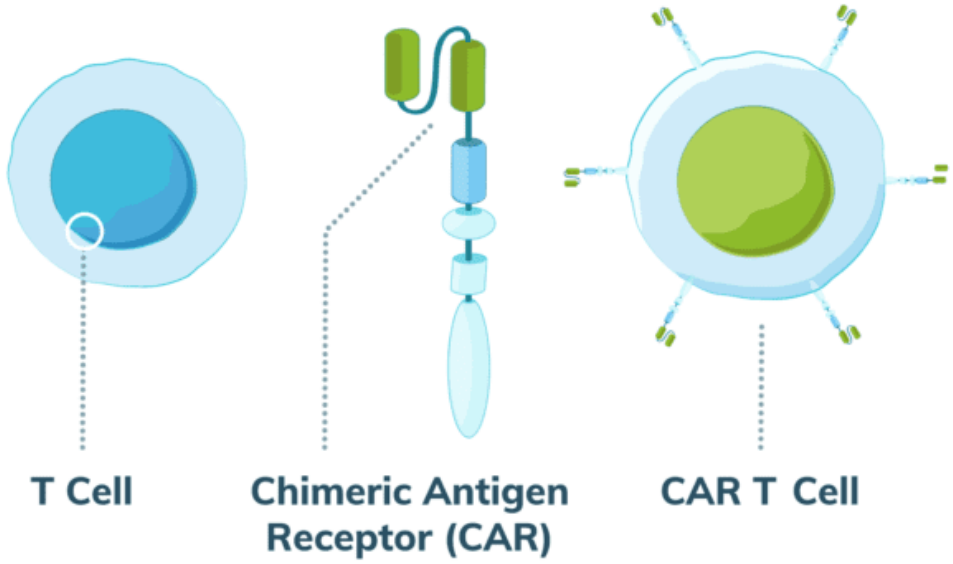
FRED HUTCH
CURES START HERE®

Objectives:

- Understand the mechanism of action of CAR T cells
- Identify toxicities of CAR-T cell therapy
- Identify appropriate prophylaxis and treatment of Cytokine Release Syndrome (CRS) and Immune Effector Cell Associated Toxicities (ICANs)

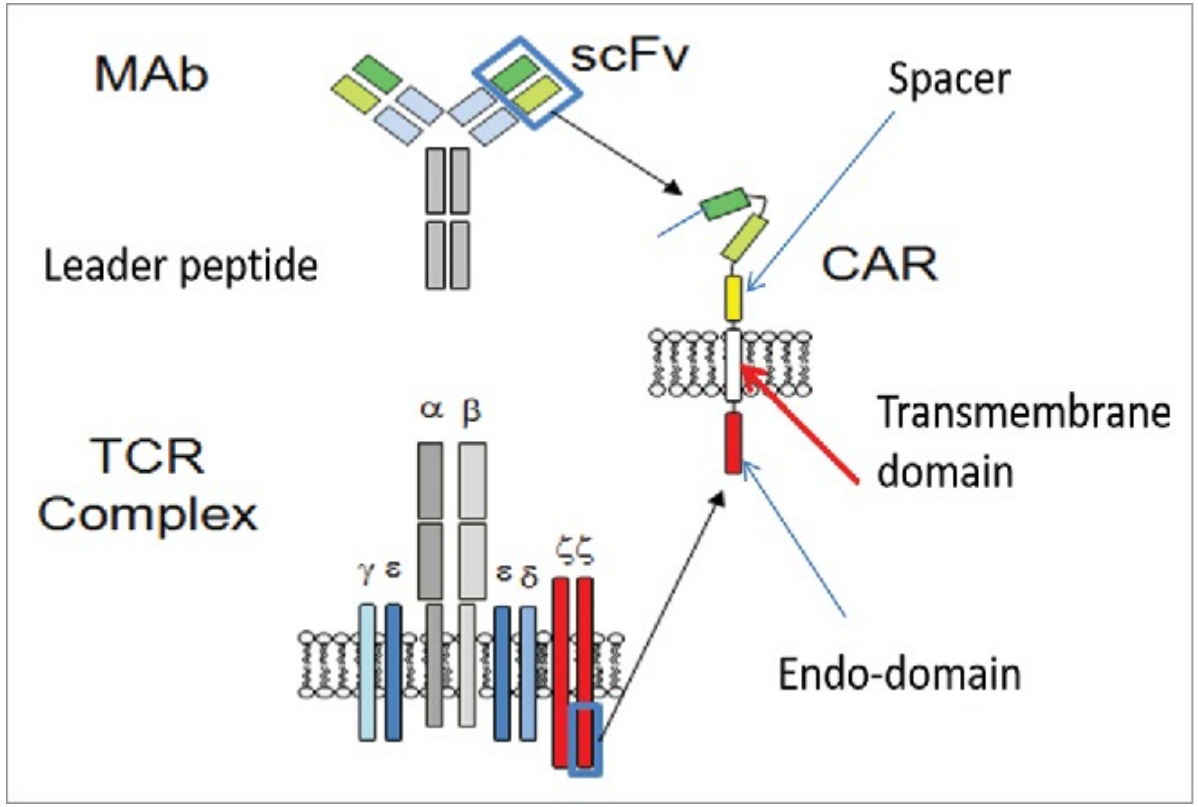
OVERVIEW OF CAR T CELLS

- T cells are a type of lymphocyte
- Vital part of our immune system
- T cells have receptors that recognize antigens of foreign and abnormal cells in the body
- Cancer cells have developed mechanism to evade killing by T cells
- CAR-T cells are genetically engineered T cells with new receptors that recognize and target specific antigen expressed by cancer cells



CAR T CELLS – A CLOSER LOOK

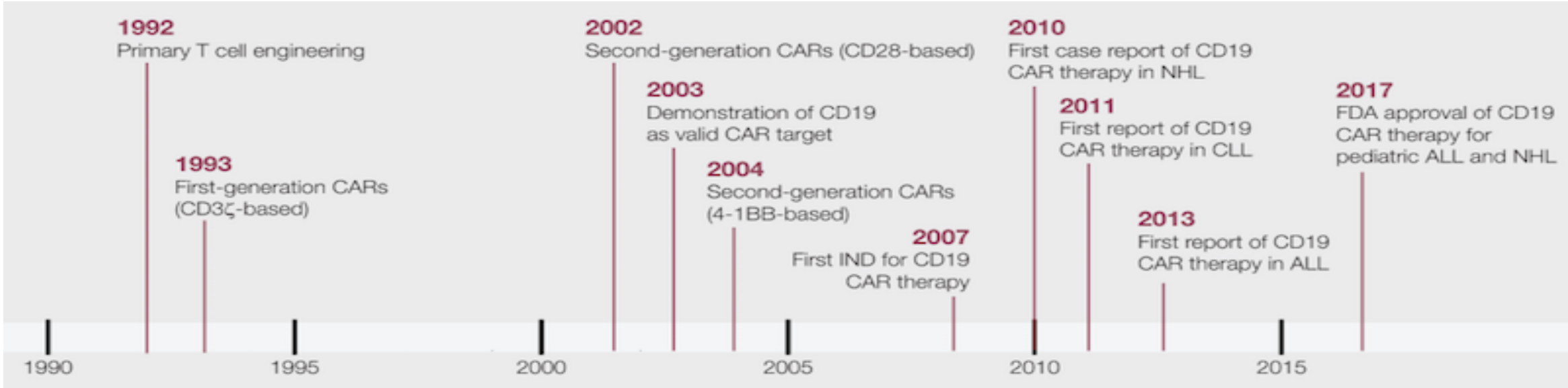
- Main construct: a single chain fraction variable (scFV), a signaling pathway molecule (TCR zeta), and co-stimulatory domain.
- Different cancers have different antigen expression
- Challenge to find an antigen target present on cancer cells and not on non-cancer cells



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5512759/

DEVELOPMENT OF CAR-T CELLS

- Activates and utilize patient's own immune system to attack cancer cells
- Four generation of CAR T cells
- Success in blood cancers, now research in solid tumors and other hematologic malignancies



https://www.labiotech.eu/in-depth/car-t-therapy-cancer-review/

TYPES OF CAR T CELLS

- Two FDA approved CAR T cells
  - CD19 directed
    - Expressed on normal and malignant B cells
      - Target for B cells malignancies
  - B cell maturation antigen (BCMA) directed
    - Expressed on subset of B cells and on mature plasma cells
    - Target for multiple myeloma

CAR T CELL INDICATIONS

- Relapsed/Refractory:
  - B cell malignancies
    - Diffuse large B cell lymphoma, Follicular lymphoma, high grade B cell lymphoma, primary mediastinal large B cell lymphoma
    - Mantle Cell lymphoma
    - Acute lymphoblastic leukemia
- Relapsed/Refractory Multiple Myeloma after four lines of therapy

FDA approved CAR T cells

KYMRIAH®
(tisagenlecleucel) Suspension for IV infusion

August 2017: B cell ALL, age 2-25 years refractory or in second or later relapse
May 2018: Adults with DLBCL

YESCARTA®
(axicabtagene ciloleucel) Suspension for IV infusion

October 2017: Relapsed or refractory DLBCL, high grade B cell lymphoma, primary mediastinal large B cell lymphoma, follicular lymphoma after 2 or more lines of therapy

FDA approved CAR T cells

TECARTUS™
(brexucabtagene autoleucel) Suspension for IV infusion

July 2020: Relapsed or refractory MCL
October 2021: 18 yo + primary refractory ALL with first relapse following remission lasting < 12 months or relapsed/refractory after 2nd line or more therapy

Breyanzi™
(lisocabtagene maraleucel) SUSPENSION FOR IV INFUSION

February 2021/June 2022: Relapsed or refractory DLBCL, high grade B cell lymphoma, primary mediastinal large B cell lymphoma, follicular lymphoma after 2 or more lines of therapy; Refractory to first-line chemotherapy or relapse within 12 months of first-line chemotherapy OR Refractory disease to first-line chemotherapy or relapse after first-line chemotherapy and are not eligible for hematopoietic stem cell transplantation (HSCT) due to comorbidities or age.

FDA approved CAR T cells

March 2021: Relapsed or refractory MM after 4 or more prior lines of therapy including immunomodulatory agent, a protease inhibitor, and an anti-CD38 monoclonal antibody.

February 2022: Relapsed or refractory MM after 4 or more prior lines of therapy including immunomodulatory agent, a protease inhibitor, and an anti-CD38 monoclonal antibody.

How are CAR T cells made?

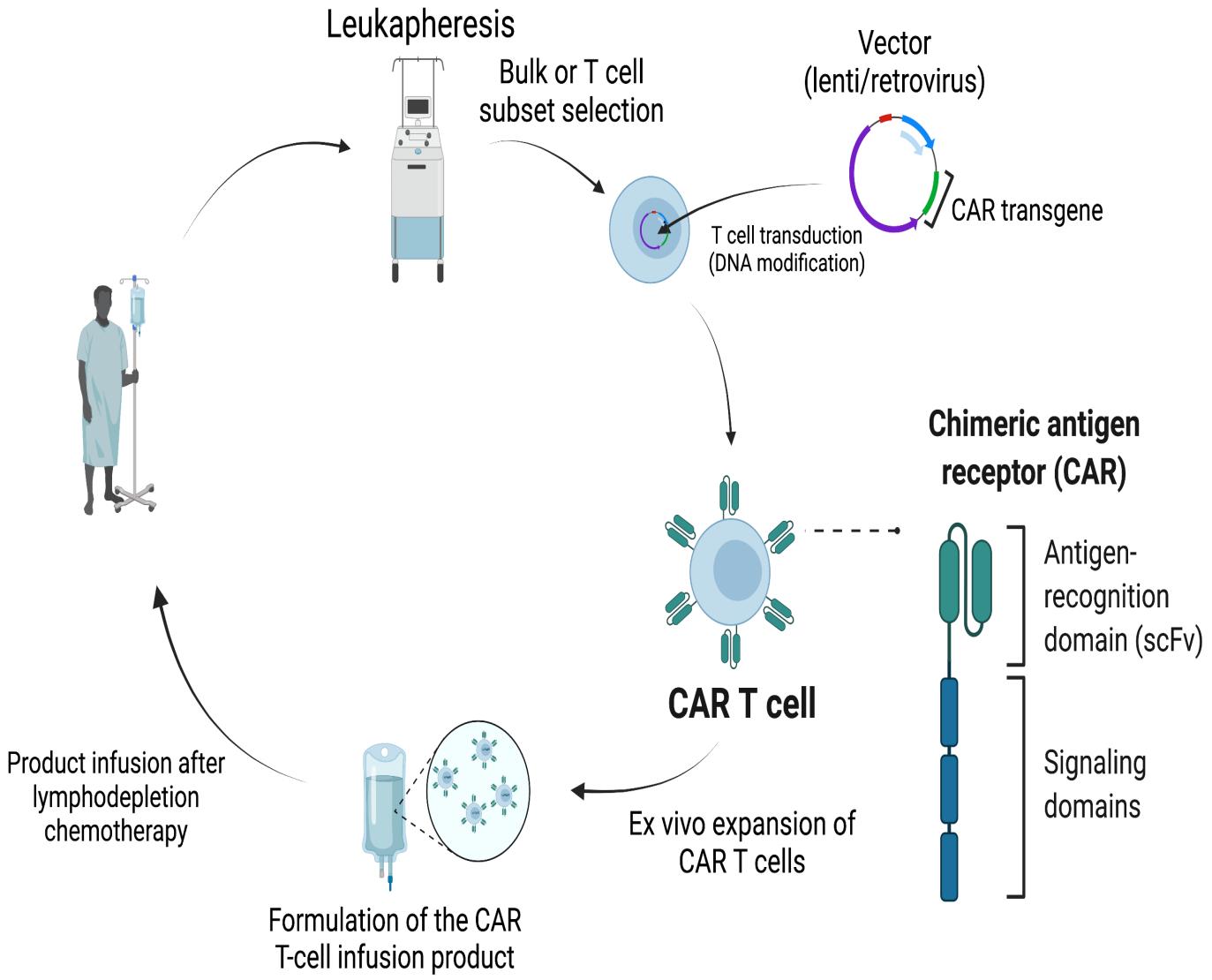
STEP 1: Leukapheresis

STEP 2: Manufacturing

STEP 3: Lymphodepletion

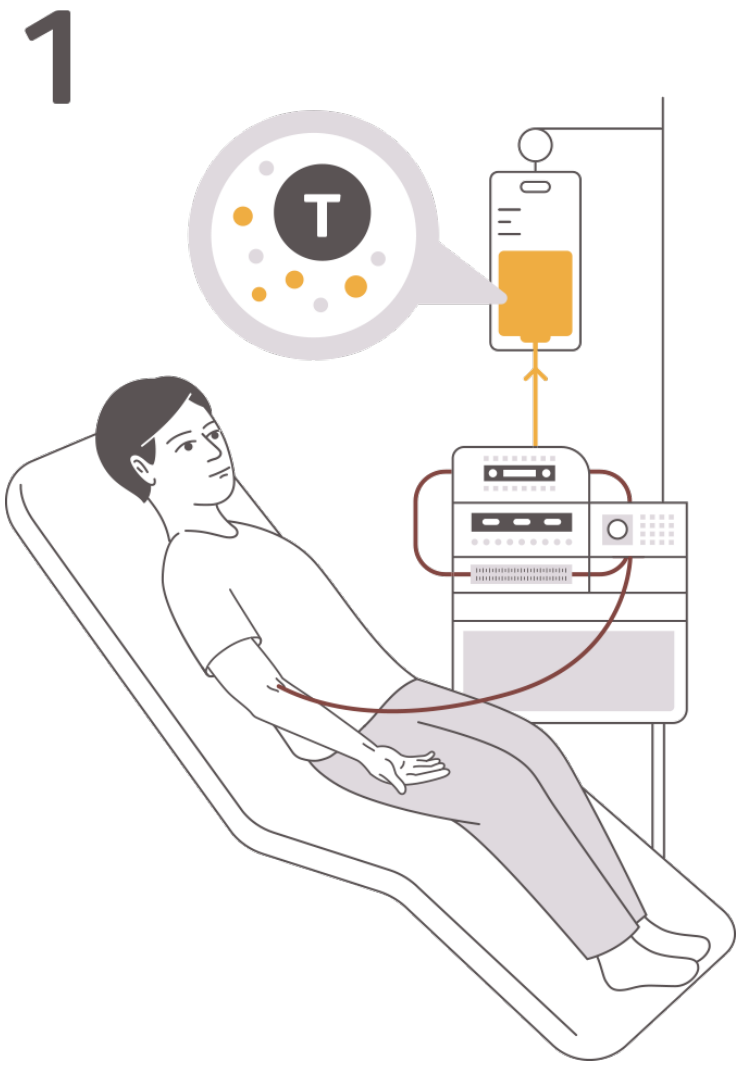
STEP 4: Cell infusion

STEP 5: Monitoring



STEP 1: Leukapheresis

- Adequate absolute lymphocyte count
- Require large bore double lumen catheter
- Centrifuge that filters out naïve T cells
- Cryopreserved and shipped to manufacturing facility



https://www.cartcellscience.com/car-t-cell-process/

STEP 2: Manufacturing

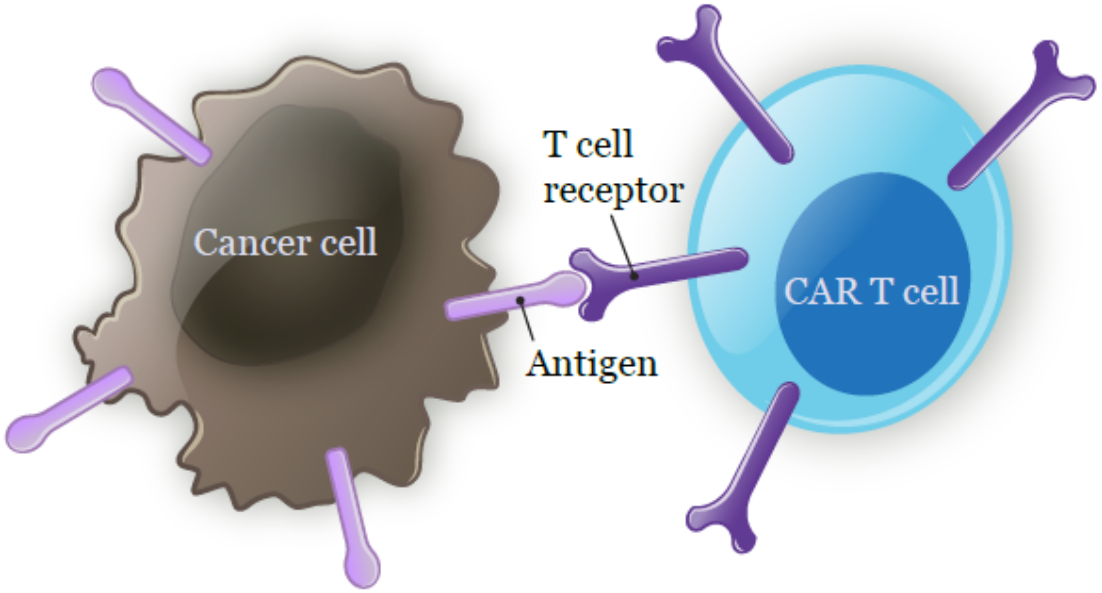
- Manufacturing time varies between products
  - Ranging between 3-6 weeks
- T cells are activated and genetically transduced ex-vivo with lentiviral vector that carries the gene for chimeric antigen receptor
- Modified T-cells expansion.
  - Sample for quality assurance.
- Cryopreserved and shipped back
- Rate limiting factor in number of patients being treated

STEP 3: Lymphodepletion

- Chemotherapy over 3 days, 2 days of rest, followed by cell infusion
  - Cyclophosphamide
  - Fludarabine
- Improve survival of transferred T cells and therapeutic efficacy

STEP 4: Cell infusion

- Similar to a autologous stem cell infusion or RBC transfusion
- Depending on the product – infused outpatient vs. inpatient
- Await expansion and monitoring



https://www.mskcc.org/cancer-care/patient-education/car-cell-therapy-guide-adult-patients-caregivers

STEP 5: Monitoring

- Day 0 = date of cell infusion
  - Remain 30 days locally
- Monitoring for toxicities
  - Cytokine release syndrome (CRS)
  - Immune effector cell-associated neurotoxicity syndrome (ICANS)
  - Peak within 2 weeks

CAR T CELL TOXICITIES

Range of CAR-T cells toxicities

- **Cytokine release syndrome (CRS)**
- **Neurotoxicity (immune effector cell-associated neurotoxicity syndrome; ICANS)**
- Hematologic toxicities
- Infection risk
- Tumor lysis syndrome
- Chemotherapy related toxicities

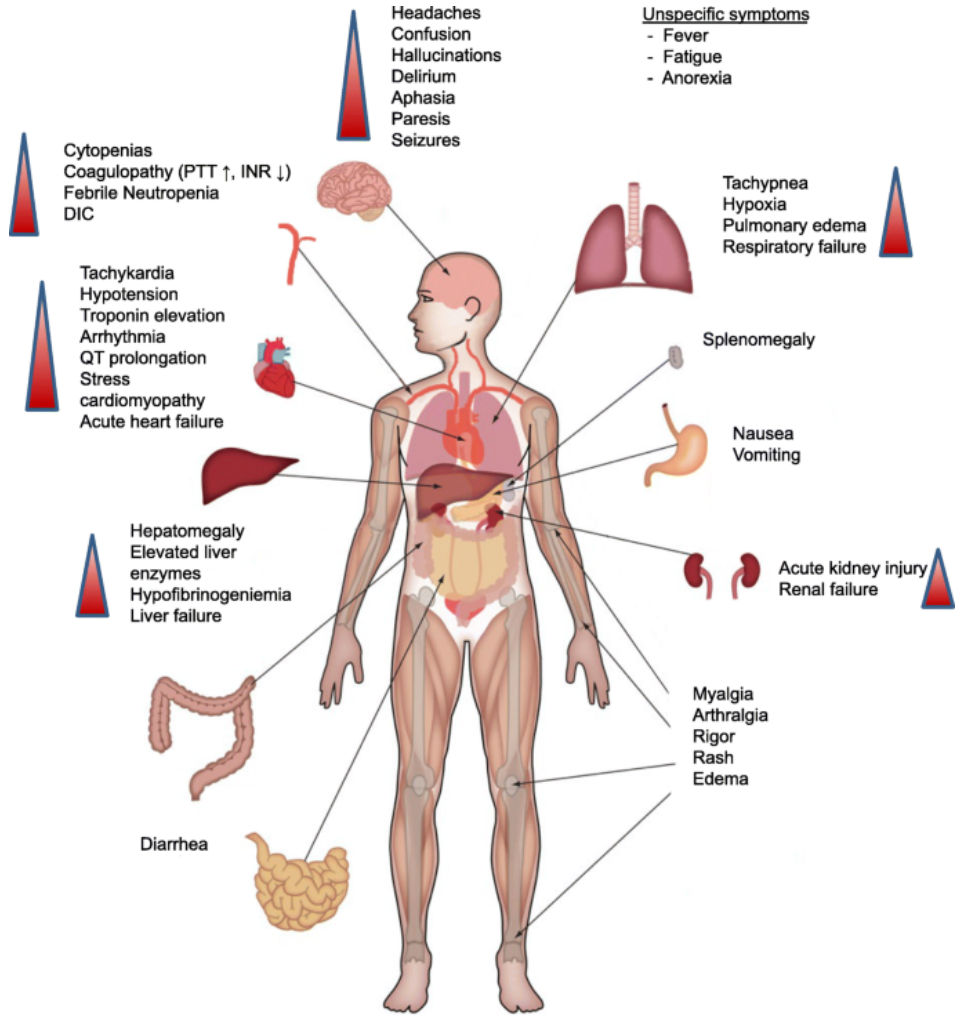
CYTOKINE RELEASE SYNDROME (CRS)

PATHOPHYSIOLOGY

- Over activation of the immune system
- Activated T cells release cytokines and chemokines
  - IL-2, soluble IL-2Ra, IFNy**, IL-6**, soluble IL-6R, and GM-CSF
- Recruit bystander immune cells (monocytes and/or macrophages) to release cytokines and chemokines, creating a feedback loop
  - IL-1RA, IL-10, **IL-6,** IL-8, CXCL10, CXCL9, IFNa, CCL3, CCL4, and soluble IL-6R
- Lead to widespread organ dysfunction

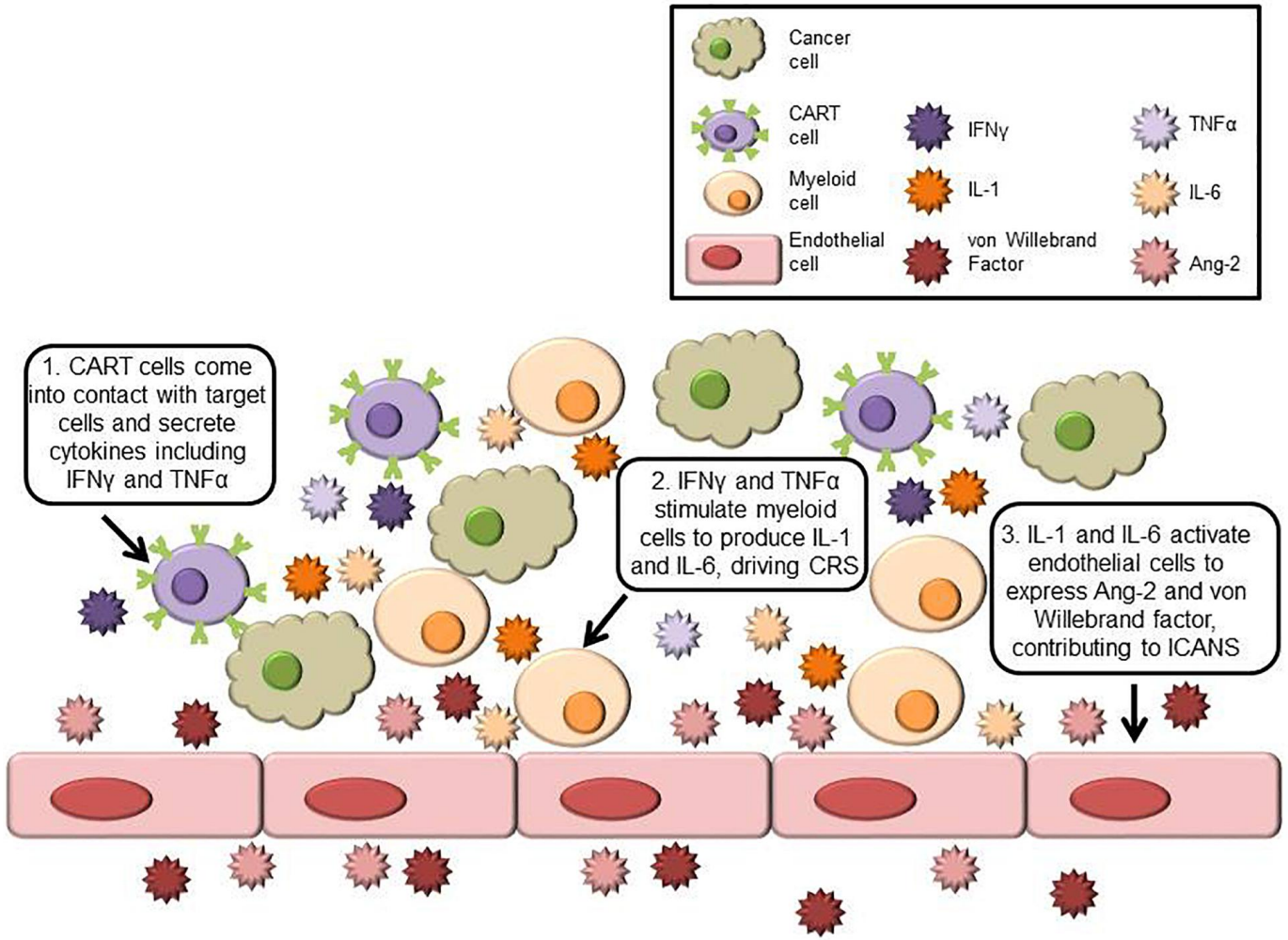
SIGNS/SYMPTOMS

- Fever
- Tachycardia
- Hypotension
- Hypoxia
- Capillary leak
- Coagulopathy
- Shock/organ failure



https://jitc.biomedcentral.com/articles/10.1186/s40425-018-0343-9

- CRS onset coincides with peak CAR T cell expansion and cytokine production
- Varies depending on CAR T construct
  - CD28: Yescarta/Tecartus
  - 4-1BB: Breyanzi, Kymirah, Abecma, Caryvkyti



https://www.frontiersin.org/articles/10.3389/fimmu.2020.01973/full

LABORATORY MONITORING

- CBC
- Renal/hepatic
- DIC Panel
  - Prolonged PT/PTT
  - Decrease Fibrinogen
  - Elevated D-dimer
- Inflammatory markers
  - Elevated CRP, Ferritin, **IL6**

GRADING SYSTEM

- Number of different grading systems
- The American Society for transplantation and cellular therapy (ASTCT)came up with CRS consensus grading

Table 1
Published CRS Grading Systems

Grading System	Grade 1	Grade 2	Grade 3	Grade 4
CTCAE version 4.03 [11]	Mild reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (antihistamines, NSAIDs, narcotics, i.v. fluids); prophylactic medications indicated for ≤24 h	Prolonged (eg, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (eg, renal impairment, pulmonary infiltrate)	Life-threatening consequences; pressor or ventilatory support indicated
CTCAE version 5.0 [13]	Fever, with or without constitutional symptoms	Hypotension responding to fluids. Hypoxia responding to <40% FiO_2	Hypotension managed with one pressor. Hypoxia requiring ≥40% FiO_2	Life-threatening consequences; urgent intervention needed
Lee criteria [14]	Symptoms are not life-threatening and require symptomatic treatment only (fever, nausea, fatigue, headache, myalgias, malaise)	Symptoms require and respond to moderate intervention: • Oxygen requirement <40% FiO_2 OR • Hypotension responsive to i.v. fluids or low dose of one vasopressor OR • Grade 2 organ toxicity*	Symptoms require and respond to aggressive intervention: • Oxygen requirement ≥40% FiO_2 OR • Hypotension requiring high-dose or multiple vasopressors OR • Grade 3 organ toxicity* or grade 4 transaminitis	Life-threatening symptoms: • Requirement for ventilator support OR • Grade 4 organ toxicity* (excluding transaminitis)
Penn criteria [17]	Mild reaction: Treated with supportive care, such as antipyretics, antiemetics	Moderate reaction: Some signs of organ dysfunction (grade 2 creatinine or grade 3 LFTs) related to CRS and not attributable to any other condition. Hospitalization for management of CRS-related symptoms, including neutropenic fever and need for i.v. therapies (not including fluid resuscitation for hypotension)	More severe reaction: Hospitalization required for management of symptoms related to organ dysfunction, including grade 4 LFTs or grade 3 creatinine, related to CRS and not attributable to any other condition Hypotension treated with multiple fluid boluses or low-dose vasopressors Coagulopathy requiring fresh frozen plasma, cryoprecipitate, or fibrinogen concentrate Hypoxia requiring supplemental oxygen (nasal cannula oxygen, high-flow oxygen, CPAP, or BiPAP)	Life-threatening complications such as hypotension requiring high-dose vasopressors Hypoxia requiring mechanical ventilation
MSKCC criteria [16]	Mild symptoms requiring observation or supportive care only (eg, antipyretics, antiemetics, pain medication)	Hypotension requiring any vasopressors <24 h Hypoxia or dyspnea requiring supplemental oxygen <40%	Hypotension requiring any vasopressors ≥24 h Hypoxia or dyspnea requiring supplemental oxygen ≥40%	Life-threatening symptoms Hypotension refractory to high dose vasopressors Hypoxia or dyspnea requiring mechanical ventilation
CARTOX criteria [12]	Temperature ≥38°C Grade 1 organ toxicity†	Hypotension responds to i.v. fluids or low-dose vasopressor Hypoxia requiring FiO_2 <40% Grade 2 organ toxicity†	Hypotension needing high-dose or multiple vasopressors Hypoxia requiring FiO_2 ≥40% Grade 3 organ toxicity† or grade 4 transaminitis	Life-threatening hypotension Needing ventilator support Grade 4 organ toxicity† except grade 4 transaminitis

ASTCT CRS CONSENUS GRADING

ASTCT CRS Consensus Grading

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C
		With		
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
		And/or†		
Hypoxia	None	Requiring low-flow nasal cannula‡ or blow-by	Requiring high-flow nasal cannula‡, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg, CPAP, BiPAP, intubation and mechanical ventilation)

Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.

* Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

† CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.

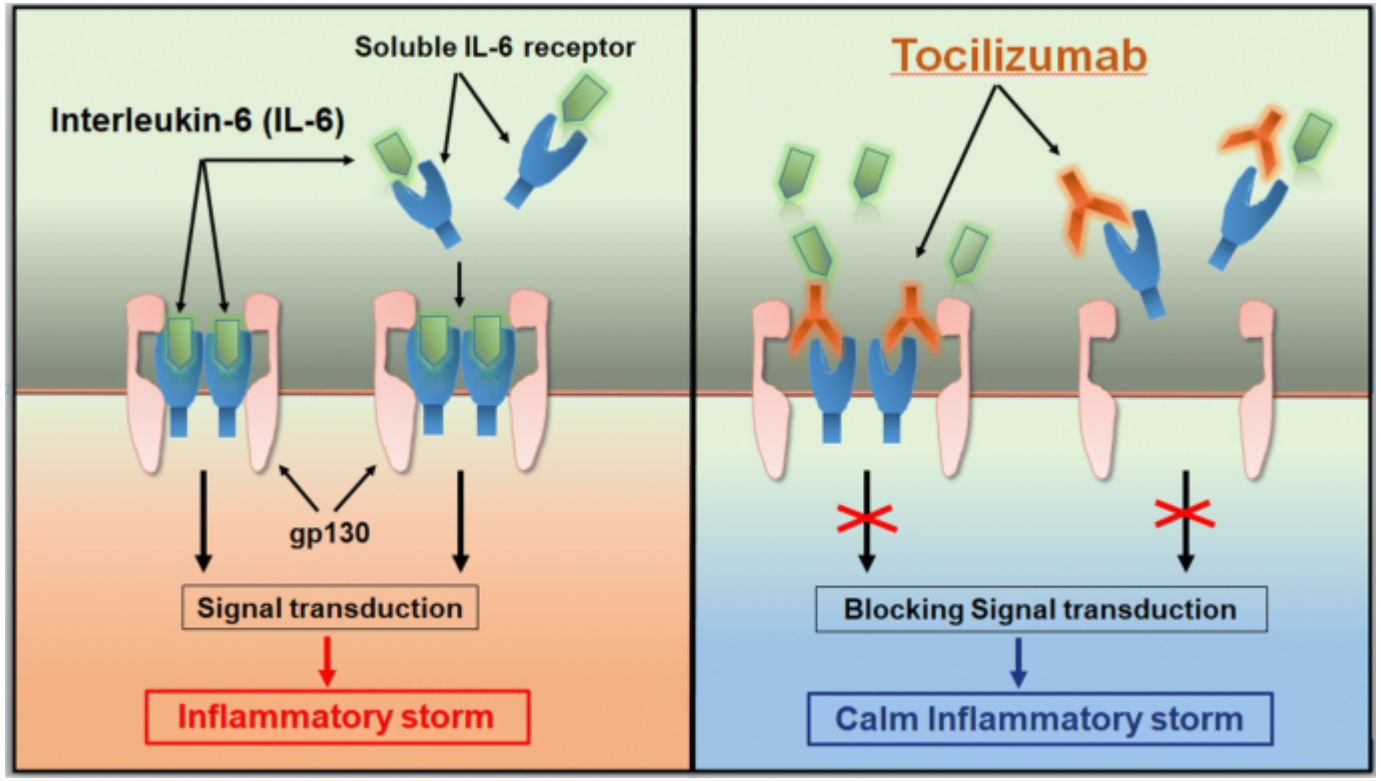
‡ Low-flow nasal cannula is defined as oxygen delivered at ≤6 L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at >6 L/minute.

CRS TREATMENT

GRADE 1 : fever	• Supportive care • Exclude infection • Anti-seizure medication
GRADE 2 : fever + hypotension (no vasopressor) + low dose O2 requirement	• Supportive care • IVF, Oxygen • Tocilizumab 8 mg/kg IV q8h x 4 • +/- Dexamethasone 10 mg q6-12h
GRADE 3 : fever + hypotension (vasopressor) +/- high flow/face mask O2	• Supportive care/transfer to ICU • IVF, Oxygen, pressor support • Tocilizumab +/- Dexamethasone
GRADE 4 : fever + hypotension (>1 vasopressor) + CPAP/BIPAP/intubation/ventilation	• Transfer to ICU • Tocilizumab • Methylprednisolone 1,000 mg IV

TOCILIZUMAB

- IL6 receptor inhibitor
- FDA approved treatment for CRS
- REMS program of commercial products require at minimal TWO doses of Tocilizumab on site prior to CAR T cell infusion
- No effect on anti-tumor response



https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-020-02339-3

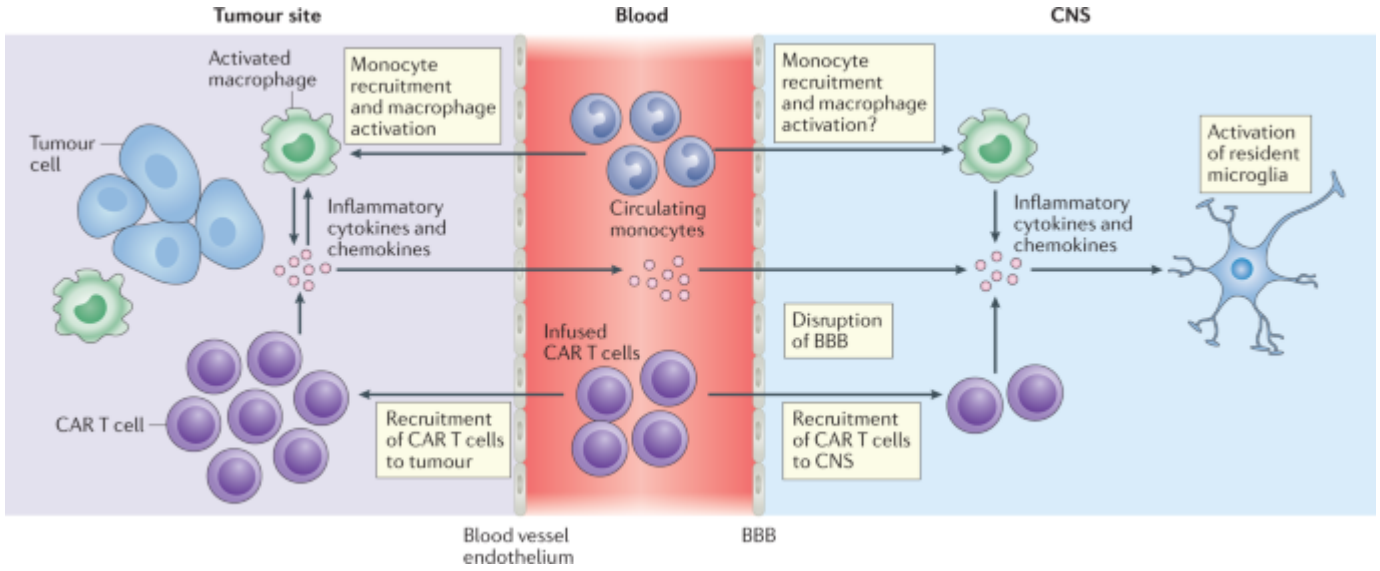
CORTICOSTEROIDS

- Used if no clinical response within 24 hours of Tocilizumab or for severe CRS
- In conjunction with Tocilizumab as first line
- Some evidence to suggest steroids may inhibit CAR T cell persistence and anti-tumor effect but concern not well founded.

IMMUNE EFFECTOR CELL-ASSOCIATED NEUROTOXCITY SYNDROME (ICANS)

PATHOPHYSIOLOGY

- Not well understood
- Endothelial cell dysfunction >> vascular instability, capillary leak, blood brain barrier disruption >> allowing cytokines/ chemokines to cross into the CNS, recruiting CAR T cells into the blood brain barrier.



SIGNS/SYMPTOMS

- Dysgraphia
- Confusion
- Agitation
- Expressive aphasia
- Somnolence
- Encephalopathy
- Focal deficits
- Seizures

**May occur concurrently with CRS or following resolution of CRS

SCREENING TOOL

Encephalopathy Assessment Tools for Grading of ICANS

CARTOX-10 [12]	ICE
• **Orientation:** orientation to year, month, city, hospital, president/prime minister of country of residence: 5 points • **Naming:** ability to name 3 objects (eg, point to clock, pen, button): 3 points • **Writing:** ability to write a standard sentence (eg, "Our national bird is the bald eagle"): 1 point • **Attention:** ability to count backwards from 100 by 10: 1 point	• **Orientation:** orientation to year, month, city, hospital: 4 points • **Naming:** ability to name 3 objects (eg, point to clock, pen, button): 3 points • **Following commands:** ability to follow simple commands (eg, "Show me 2 fingers" or "Close your eyes and stick out your tongue"): 1 point • **Writing:** ability to write a standard sentence (eg, "Our national bird is the bald eagle"): 1 point • **Attention:** ability to count backwards from 100 by 10: 1 point

CARTOX-10 (left column) has been updated to the ICE tool (right column). ICE adds a command-following assessment in place of 1 of the CARTOX-10 orientation questions. The scoring system remains the same.
Scoring: 10, no impairment;
7-9, grade 1 ICANS;
3-6, grade 2 ICANS;
0-2, grade 3 ICANS;
0 due to patient unarousable and unable to perform ICE assessment, grade 4 ICANS.

GRADING SYSTEM

Table 4
Published Neurotoxicity Grading Systems

Grading System	Adverse Event Term/Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
CTCAE v5.0 [13].*	Encephalopathy	Mild symptoms	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated
	Seizure	Brief partial seizure and no loss of consciousness	Brief generalized seizure	New-onset seizures (partial or generalized); multiple seizures despite medical intervention	Life-threatening consequences
	Dysphasia	Awareness of receptive or expressive characteristics; not impairing ability to communicate	Moderate receptive or expressive characteristics; impairing ability to communicate spontaneously	Severe receptive or expressive characteristics; impairing ability to read, write, communicate intelligibly	
	Tremor	Mild symptoms	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care ADL	
	Headache	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL	
	Confusion	Mild disorientation	Moderate disorientation; limiting instrumental ADL	Severe disorientation; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated
	Depressed level of consciousness	Decreased level of alertness	Sedation; slow response to stimuli; limiting instrumental ADL	Difficult to arouse	Life-threatening consequences; coma; urgent intervention indicated
	Cerebral edema			New onset; worsening from baseline	Life-threatening consequences; urgent intervention indicated
CARTOX criteria [12]	Neurologic Assessment Score (CARTOX-10)	7–9 (mild impairment)	3–6 (moderate impairment)	0–2 (severe impairment)	Patient in critical condition, and/or obtunded and cannot perform assessment of tasks
	Elevated ICP	N/A	N/A	Stage 1–2 papilledema† or CSF opening pressure <20 mmHg	Stage 3–5 papilledema†, or CSF opening pressure ≥20 mmHg, or cerebral edema
	Seizures or motor weakness	N/A	N/A	Partial seizure or nonconvulsive seizures on EEG with response to benzodiazepine	Generalized seizures or convulsive or nonconvulsive status epilepticus, or new motor weakness

ADL indicates activities of daily living; CSF, cerebrospinal fluid; EEG: electroencephalography.
* CTCAE: Under CRS listing: "Also consider neurologic toxicities such as psychiatric disorders: hallucinations or confusion; nervous system disorders: seizure, dysphasia, tremor, headache."
† Papilledema grading is performed according to the Modified Frisén scale [35].

https://www.sciencedirect.com/science/article/pii/S1083879118316914

TREATMENT

GRADE 1: CARTOX (7-9)	• If not on Keppra, start Keppra 500 mg BID
GRADE 2 : CARTOX (3-6)	• Dexamethasone IV 10 mg q6-12h until symptoms resolve followed by rapid taper
GRADE 3 : CARTOX (0-2)	• Dexamethasone IV 10 mg q6-12h until symptoms resolve followed by rapid taper
GRADE 4 : Critical condition/obtunded	• Methylprednisolone 1,000 mg IV x 3 days or until resolution of Grade 4 symptoms

TREATMENT

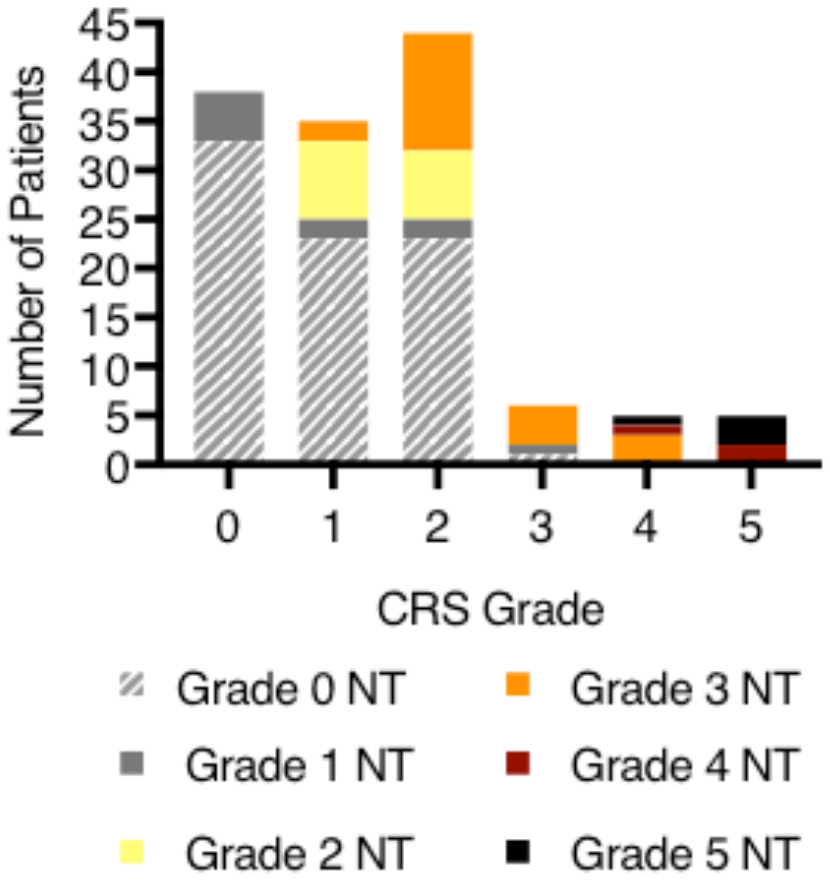
- Dexamethasone as first line because excellent CNS penetration
- Unclear if Tocilizumab has beneficial effect
  - Saturation of IL6 receptors may increase serum IL6, which could lead to increase in CSF IL6 levels that could worsen neurologic toxicities
- Research undergoing for other treatments: Anakinra (IL1 receptor antagonist) and intra-thecal hydrocortisone

RISK FOR CRS & ICANS

- Larger disease burden = higher risk of CRS/ICANS because more cytokines/chemokines release
- Dependent on CAR T product
  - Yescarta and Tecartus higher risk of CRS/ICANS
- Reversible in most cases

CRS AND ICANS CAN OVERLAP

- CRS typically precedes ICANS but temporal overlap is common
- Rare to observe severe CRS in the absence of severe ICANS
- Patients with severe CRS often found with vascular dysfunction with capillary leak + consumptive coagulopathy >> Increased blood brain permeability



Hay et al. Blood, 2017;
Gust, Hay, Hanafi et al, Cancer Discovery, 2017

OTHER TOXICITIES

HEMATOLOGIC TOXICITIES

- Associated with severe CRS and higher marrow burden
- More anemia, thrombocytopenia, neutropenia
- Delayed count recovery
- ~30% at day 60 with cytopenias, requiring transfusions and GCSF support

INFECTION RISK

- B-cell aplasia from effects of CD19 targeting & lymphodepletion
- Prolonged neutropenia
- Underlying immunosuppression
- Will require re-vaccination of childhood vaccines
- May need intravenous immunoglobulin repletion (IVIG)
- At risk for CMV reactivation and fungal infection especially with prolonged steroid treatment

CHEMOTHERAPY RELATED TOXICITIES

- Cytopenia
- Nausea
- Vomiting
- Diarrhea
- Fatigue

CAR T CELL PROPHYLAXIS

- Neutropenic ppx: Levofloxacin 750 mg QHS, Fluconazole 200 mg QD for prolonged neutropenia
- Anti-viral ppx: Acyclovir 800 mg BID or Valacyclovir 500 mg BID
- PJP ppx: Bactrim 800 mg/160 mg BID qMTue
- Anti-fungal ppx: Fluconazole 200 mg QD when neutropenic or Posaconazole 300 mg QD for prolonged steroid treatment
- Seizure ppx: Keppra 500 mg BID, high disease burden, H/O CNS lymphoma, fever, and Yescarta/Tecartus patients
- TLS ppx: Allopurinol 300 mg QD
- GCSF for when ANC <500

FOLLOW UP POST-CAR T

- Evaluate for treatment response at day 28 and follow up imaging depending on initial response
- Weekly blood draw to monitor for cytopenias +/- transfusions/ GCSF
- Monitor IgG levels and replete as needed, IgG >400
- Revaccinate childhood vaccinations at 6 months post
- Continue antiviral x 1 year and PJP ppx x 6 months

SUMMARY

- CAR T cells are the newest and most promising treatment for B-cell malignancies and multiple myeloma
- Main CAR T cell related toxicities
  - CRS, ICANS, Infection, Cytopenia
- CRS and ICANS are easily treated and reversible in most cases
- Future of T cell therapy to include solid tumors
- Research for steroid sparing agents in treatment of CRS/ICANS

THANK YOU!