CAR T cell neurotoxicity: translational insights

Juliane Gust, MD PhD

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Topics for today:

• Neurotoxicity/ICANS: one or many disorders?
• Animal models of neurotoxicity
• In vitro models
• The way forward
**T cell redirecting therapy**

**Chimeric antigen receptor (CAR) T cells:**
- Nonclassical immune synapse
- Signaling through CAR similar but not the same as T cell receptor signaling

**Bispecific T cell engagers:**
- Engages T cell receptor by binding CD3
- As far as we know, induces physiological T cell signaling

Goebeler et al., 2020; Larson and Maus, 2021
ICANS A.k.a immune effector cell associated neurotoxicity

Frequency:
- ~40% with CD19-CAR
- ~25% with CD22 and BCMA-CAR
- Bispecifics: blinatumomab 40-50%, less with CD20/CD3
- NONE with solid tumor CARs
- Brain tumor CARs: toxicity usually related to focal edema

Manifestations:
- encephalopathy/language dysfunction (most patients)
- seizures, coma (5-10%)
- cerebral edema (<1%)

Gust et al., 2018 CNS Drugs; Lee et al., 2019 Biol Blood Marrow Transp
A typical case of ICANS

6 year old girl with refractory CD19+ ALL

**Day 1:** CAR T cells

**Day 4:** first fever, bilateral hand tremor

**Day 5:** persistent fever, becomes less responsive, receives tocilizumab

**Day 6:** seizure, intubated. Ongoing seizures on EEG. Receives high dose steroids and anakinra.

**Day 10:** extubated, rapidly returns to normal mental status
6 year old girl with CAR T neurotoxicity

Characteristic MRI findings on day 7:

- Bilateral thalamic edema
- White matter symmetric T2 lesions
Feared complication: cerebral edema

8yo boy with B-ALL, treated with CD19 CAR T cells

Severe CRS day 4-6, improving

Becomes sleepy and vomits, then unresponsive

Brain herniation within hours after symptom onset

➢ only seen with CD19 CAR
➢ different from “regular ICANS”?
What do we know about neurotoxicity?

**Time course:** closely follows CAR T cell proliferation and cytokine release syndrome

**Treatment:** corticosteroids, IL-1 blockade, intracranial-pressure directed and neuroprotective interventions

Gust et al., 2018 CNS Drugs; Lee et al., 2019 Biol Blood Marrow Transp
Risk factors for neurotoxicity

- Systemic cytokine release syndrome (CRS) - affected by tumor burden, CAR T proliferation, CAR construct
- Some CAR products have more neurotoxicity than would be expected by incidence of CRS
- Prior neurologic comorbidities (weak signal)

Gust et al., Frontiers Immunology 2021
Specific cytokines in ICANS

IFNγ  IL-10  IL-6  IL-15

Cohen 2019 (37)  0 vs ≥1
Gofshteyn 2018 (5)  0 vs ≥1
Gust 2017 (6)  0-2 vs ≥3
Gust 2019 (7)  0 vs ≥1
Kochenderfer 2017 (38)  0-2 vs ≥3
Neelapu 2017 (9)  0-2 vs ≥3
Santomasso 2018 (11)  0-2 vs ≥3
Shalabi 2018 (39)  0 vs ≥1

CSF
Gust 2017 (6)  pre vs peak
Gust 2019 (7)  pre vs peak
Santomasso 2018 (11)  pre vs peak

P<0.05  P≥0.05  not reported or not done

Gust et al., Frontiers Immunology 2021
BBB model based on clinical findings

Blood

Endothelium

Pericytes

Astrocytes

Brain parenchyma

Gust et al., 2020
Could it have been predicted?

Xenograft: no toxicity (Brentjens 2003, Brentjens 2007, Cheadle 2009)

Syngeneic: mouse CD19 CAR T cells, +/- murine CD19+ tumors

no toxicity: Cheadle 2009, 2010 (1\textsuperscript{st} gen CAR, BALB/c), Kochenderfer 2010; Davila 2013 (2\textsuperscript{nd} gen CD28 CAR, C3H or B6 mice)

CRS: Cheadle et al., 2014 (2\textsuperscript{nd} gen CAR, higher dose of lymphodepletion and CAR T cells)

2010: CRS recognized in human CD19 CAR trial (Brentjens 2010)

2013-2016: neurotoxicity recognized as separate from CRS. ROCKET CD19 CAR T trial suspended for excessive deaths from cerebral edema
Modeling approaches

Syngeneic animal models: capture full spectrum of immune-brain interactions, but can’t use clinical CAR constructs

Humanized mouse models: can use clinical constructs but may not accurately model interaction of immune system with neurovascular unit

In vitro models: can use patient samples, difficult to capture complexity of the neurovascular unit

In silico: predict on-target toxicity by expression profiling, identify rare targets, cannot predict off-target toxicity
Animal models

SGM3 humanized mice treated with human CD19 CAR T cells (Norelli et al., 2018). Neurotoxicity develops late (day 28-30), macrophage infiltration. Prevented by IL-1 blockade.

Xenograft, human CD19 CAR T cells: reduced contrast extravasation on brain MRI after GM-CSF blockade. No histology shown (Sterner et al., 2019)

Nonhuman primate treated with CD20-CAR T cells: feasible model of neurotoxicity (Taraseviciute et al., 2018)
Blinatumomab (CD3/CD19 bispecific) increases T cell adhesion to endothelial cells in a flow chamber (Klinger et al., 2020). No cancer target in model.

CD19-CAR T cell patient serum applied to endothelial cells alters VWF string unit formation, indicating coagulopathy (Gust et al., 2017)
Is CD19-CAR different?

Persistent concern for on-target, off-tumor toxicity

**Parker et al., 2020 Cell:**
- CD19 may be expressed in FETAL human brain vascular mural cells
- Data not convincing for adult human, or mouse

**Adult brain scRNAseq (Allen Institute):**
- very few mural cells/pericytes in dataset
- no CD19 expressed
Syngeneic mouse model of ICANS

BALB/c, no tumor, 250mg/kg cy, 10 x 10^6 murine CD19 CAR T cells

Neurophenotype score

Open field test (day 4)

Faulhaber et al., 2021
Widespread cerebral microhemorrhages

loss of pericyte processes

CAR T mock

string capillaries

Faulhaber et al., 2021
Two-photon in vivo: brain capillaries

Day 4 + 6

no dye leakage

>10% capillaries blocked

Faulhaber et al., 2021
Leukocytes adhering to microvessels

All plugs are CD45.2+ leukocytes, only some are CAR T cells
Leukocytes adhering to microvessels

Next questions:
- does this occur in humans?
- what causes increased adhesion of leukocytes?
- is capillary plugging pathogenic?
- can reversal of capillary plugging ameliorate neurotoxicity?
How can in vitro models be useful?

Scalable vs naturalistic

What questions do we want to answer?
- Why do different CAR products have different toxicity profiles?
- Is there something patient specific, i.e., cytokine profiles or secreted factors?
- How do immune cells (CAR T and other) and secreted factors interact with the blood-brain barrier?
In vitro microvascular modeling

In vitro modeling approach with Ying Zheng @ UW:
- microvessels molded in collagen matrix
- seed with primary human brain microvascular endothelial cells
- perfuse with plasma/serum

healthy donor serum
CAR T day 7 serum
baseline, perfuse x 24h
Changes in microvascular structure

24h culture with serum perfusion

Caitlin Howard, unpublished data
Tight junctions seem functional

Measure dextran leakage after 6h perfusion with plasma

Yu-Jung Shin
Microchannels created by multiphoton ablation of hydrogel

Human umbilical vein endothelial cells cover the channels (min 5μm diameter)

Arakawa et al., Science Advances 2020
Human 3D capillary model

Perfuse RBCs, can also do citrated whole blood, serum, PBMCs
Measure flow parameters, adhesion, deformation etc

Arakawa et al., Science Advances 2020
Take home points

• Neurotoxicity was not predictable with current methods
• Mechanism of ICANS remains elusive
  - CAR T interaction with host immune system
  - brain-immune interaction
• Way forward: iterative loop of
  • careful clinical phenotyping + data sharing,
  • on-target expression analysis,
  • syngeneic/humanized mouse models,
  • organ modeling to test specific hypotheses
Thank you!

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