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Cytokine Release Syndrome

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Conflicts of Interest

- Research Contract with Intellia Therapeutics
- Member of Advisory Boards/Consultant/Speaker:
  Molmed, Intellia, TxCell, Novartis, GSK, Allogene,
  Kite/Gilead, Miltenyi, Kiadis
- Patents (Adoptive T cell therapy field)

CAR-T cells from bench to bedside

FDA News Release

FDA approval brings first gene therapy to the United States

CAR T-cell therapy approved to treat certain children and young adults with B-cell acute lymphoblastic leukemia

August 30, 2017

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Approved: August 22, 2018

FDA News Release

FDA approves CAR-T cell therapy to treat adults with certain types of large B-cell lymphoma

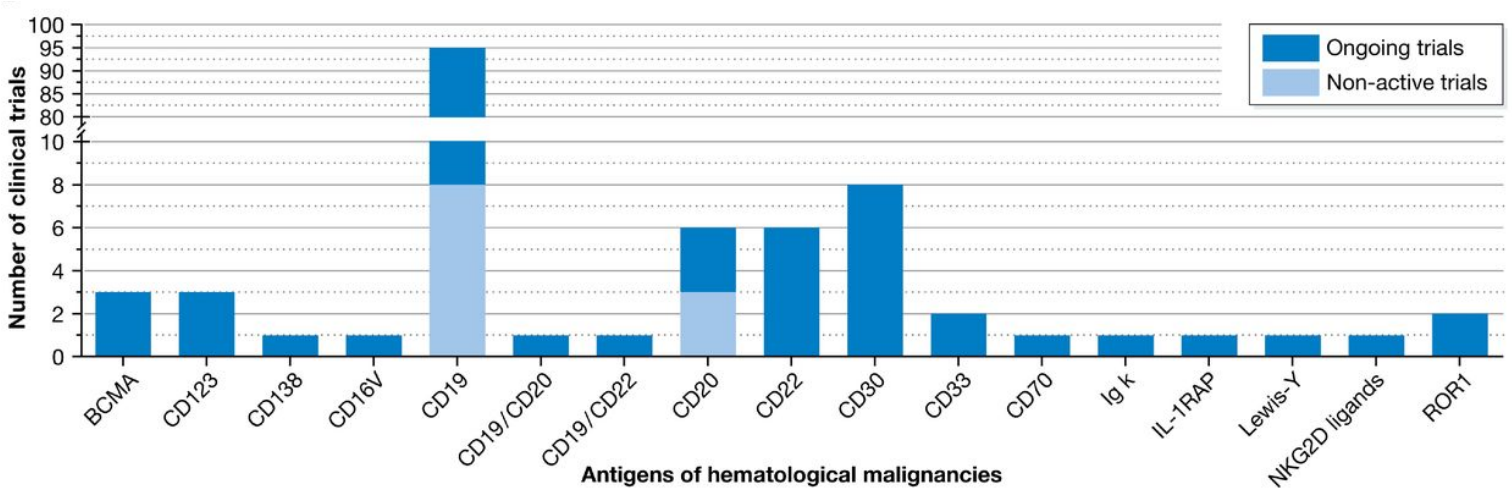
Yescarta is the second gene therapy product approved in the U.S.

October 18, 2017

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Approved: August 23, 2018

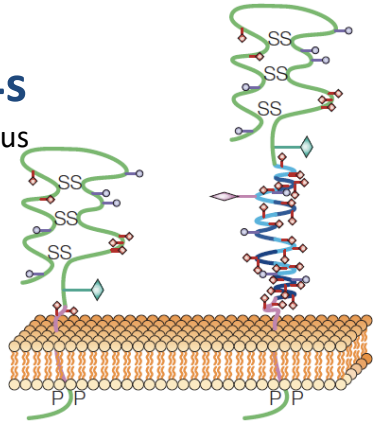
CAR-T cells for heme tumors



Adapted from Hartmann J., EMBO Mol Med, 2017

CAR-T cells against CD44v6

Hyaluronic acid, osteopontin, a_vb_3, homotypic?



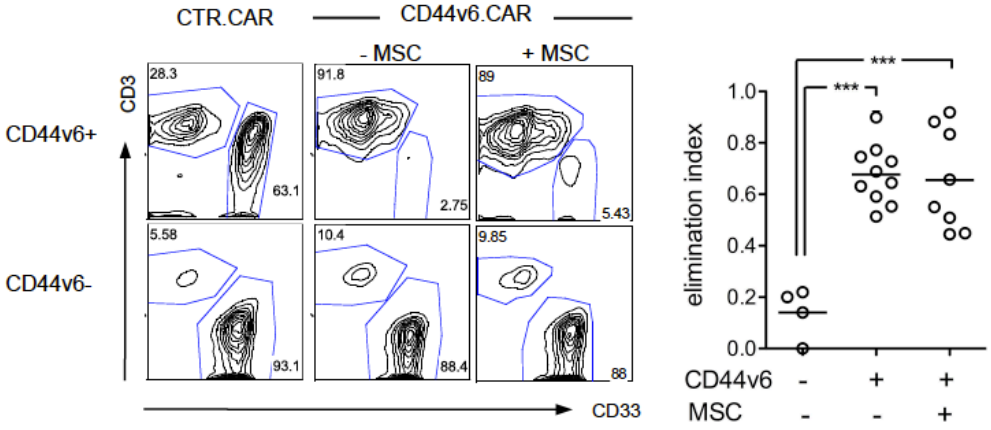
CD44s

Ubiquitous

CD44v6

Keratinocytes
Monocytes
Epithelial cancers
Blood cancers

Cytotoxicity mediated by CD44v6-CART cells

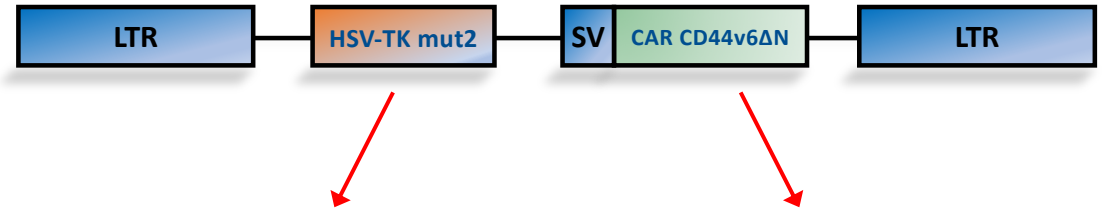


Cancer-stem cell antigen?

CD44v6 correlates with **AML prognosis** (Legras, *Blood* 1998)
CD44v6 correlates with **MM prognosis** (Liebisch, *Blood* 2005)
CD44 mAb eradicates **LSCs** (Jin et al, *Nat Med* 2006)
CD44 $^{-/-}$ HSC resist **leukemogenesis** (Krause, Nat Med 2006)

Casucci, *Blood* 2013
Casucci, *Frontiers in Imm.* 2017
Norelli, *Nat. Med.* 2018

A phase I/IIa clinical trial of anti-CD44v6 CAR-TK cells (EURE-CART)



TK suicide gene
Reversal of skin toxicity

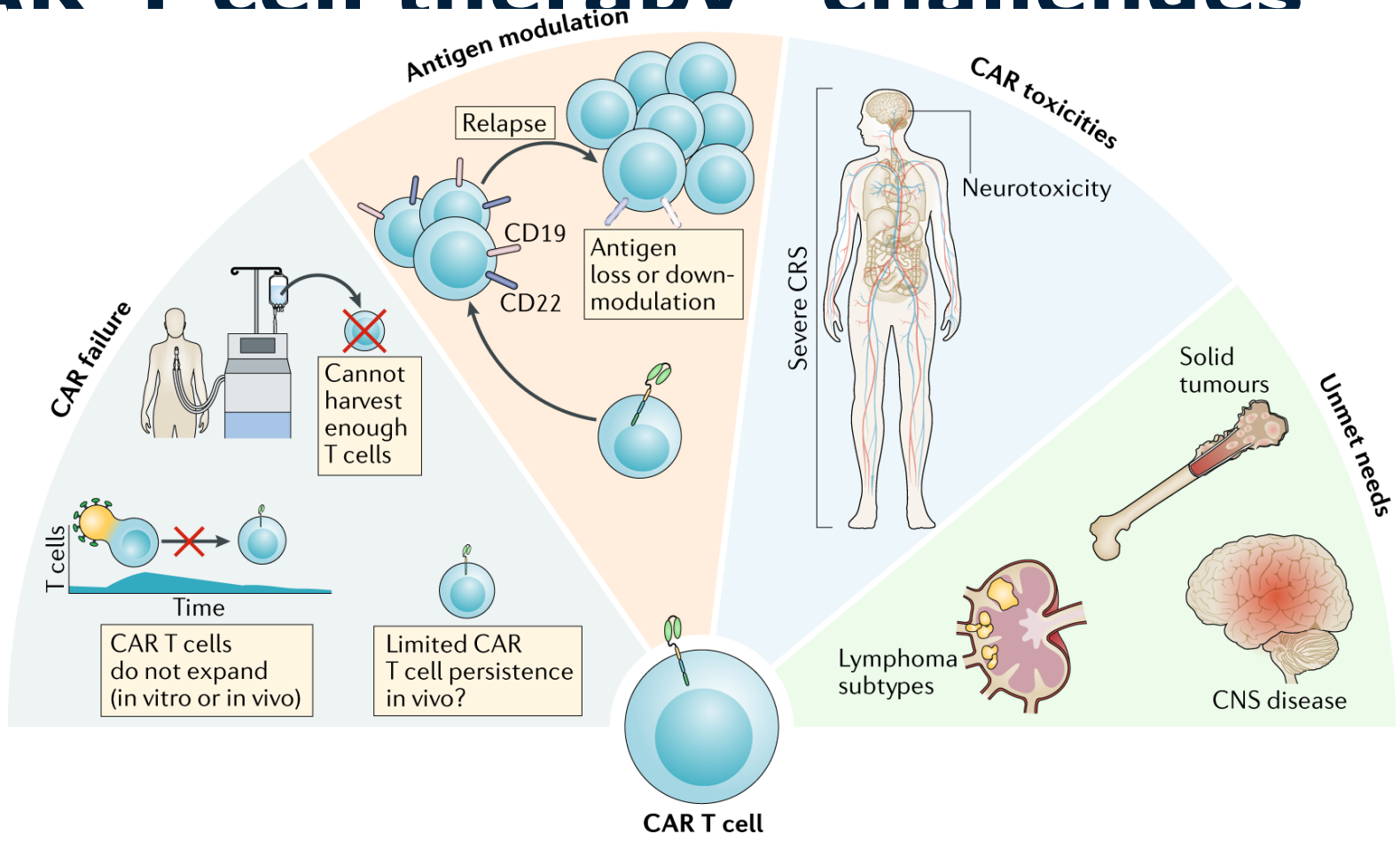
CD44v6 CAR
Antitumor effects in AML and MM
Selection and in vivo tracking
of CAR T cells by anti-ΔLNGFR mAb

Participating centers:
San Raffaele, Milano (A Bondanza, C Bonini F Ciceri)
Wurzburg University (H Einsele)
Ospedale Pediatrico Bambino Gesù, Roma (F Locatelli)
Sant Pau Hospital, Barcelona (J Sierra)
University Hospital Ostrava (R Hajek)

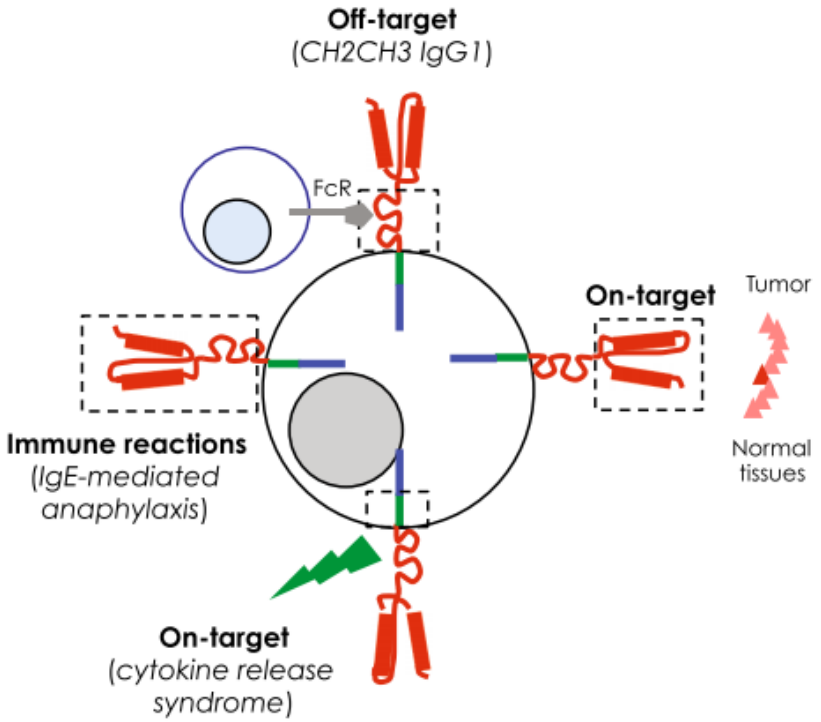
CAR T manufacturing: Molmed Spa
Clinical trial management: [redacted] Frechen
Project management: ARTTIC, Paris
Funding H2020 EURE-CART

Clinical trial started in 2019

CAR-T cell therapy "challenges"

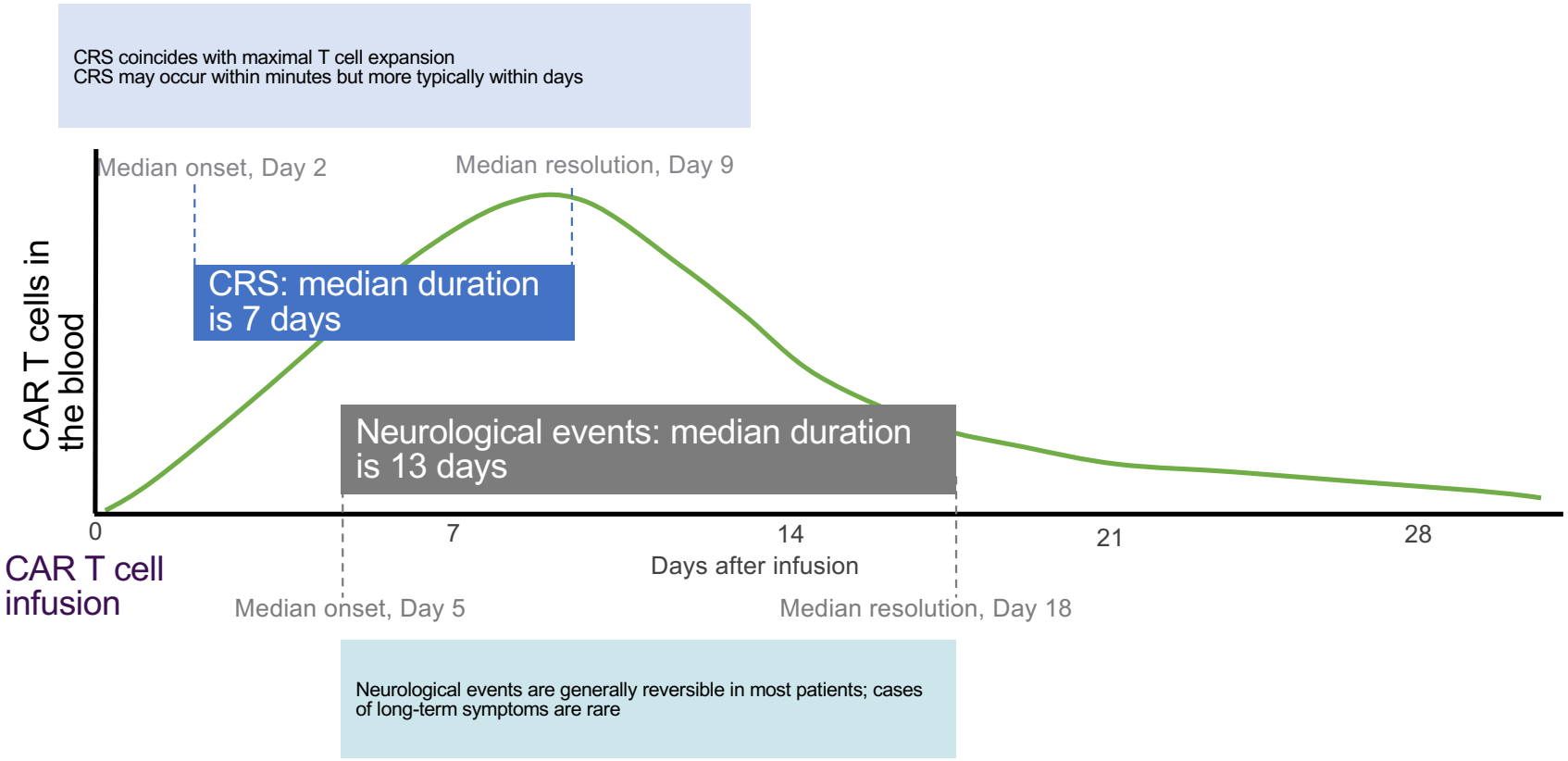


CAR-T cell safety concerns



Casucci M. et al, *Cancer Immunol Immunother*, 2015

Kinetics of AEs associated with CAR T cell therapy



1. Lee DW, *et al*. *Blood* 2014; 124:188–195. 2. Yescarta SmPC (May 2019; available at www.ema.europa.eu).

Cytokine Release Syndrome

Clinical syndrome resulting from generalized immune activation

Median onset: 2 days after CART cells infusion.

CRS incidence and severity varies according to:

- CAR construct
  → earlier onset with CD28 than 41BB costimulation
- CAR-T cell manufacturing
- Diagnosis
- Eligibility criteria

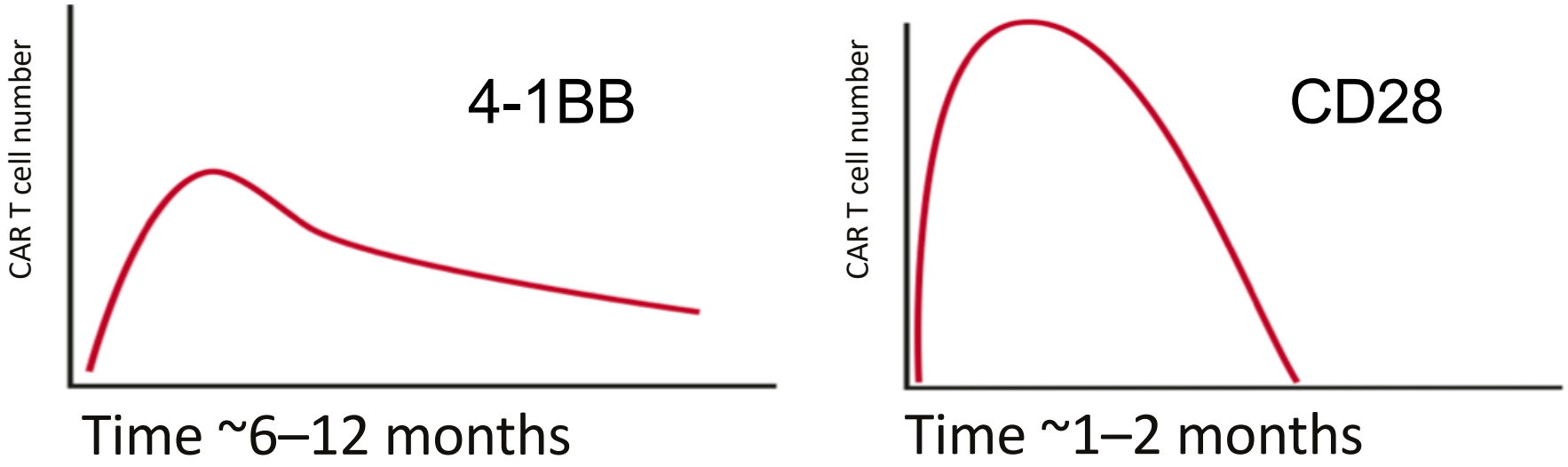
Observed with:

- CD19
- CD22
- BCMA CARs

Efficacy and toxicity of CAR-CD19 for DLBCL

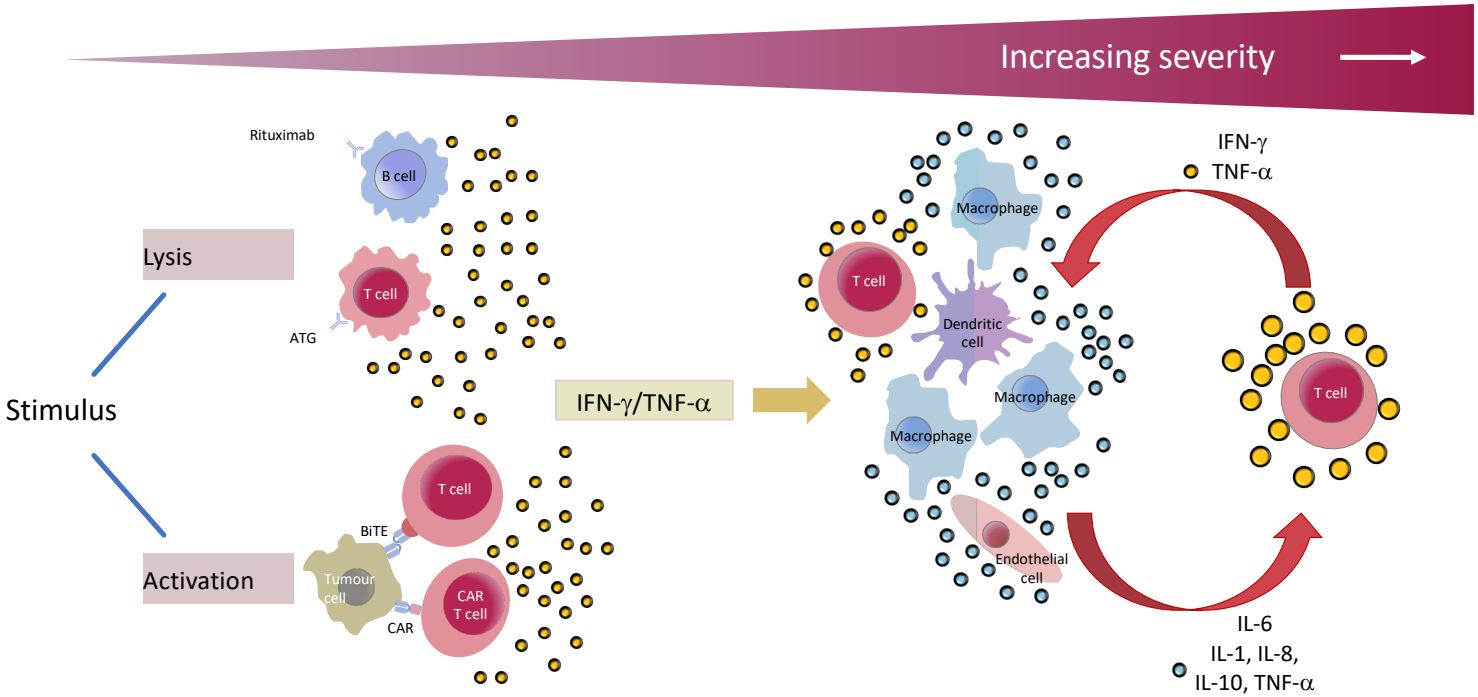
TRIAL	Zuma-1	Juliet	Transcend full	bb2121 in MM
Product	Axi-cel	CTL19	JCAR017	b2121
OR%	82	52	80	85
CR%	54	40	59	45
CRS%	93	58	39	76
Grade 3+ CRS%	13	22	1	6
NT%	64	21	25	42
Grade 3+ NT%	28	12	15	3

Kinetic of CAR T cell expansion and persistence



Davis KL & Mackall CL. *Blood Adv* 2016; 1:265–269.

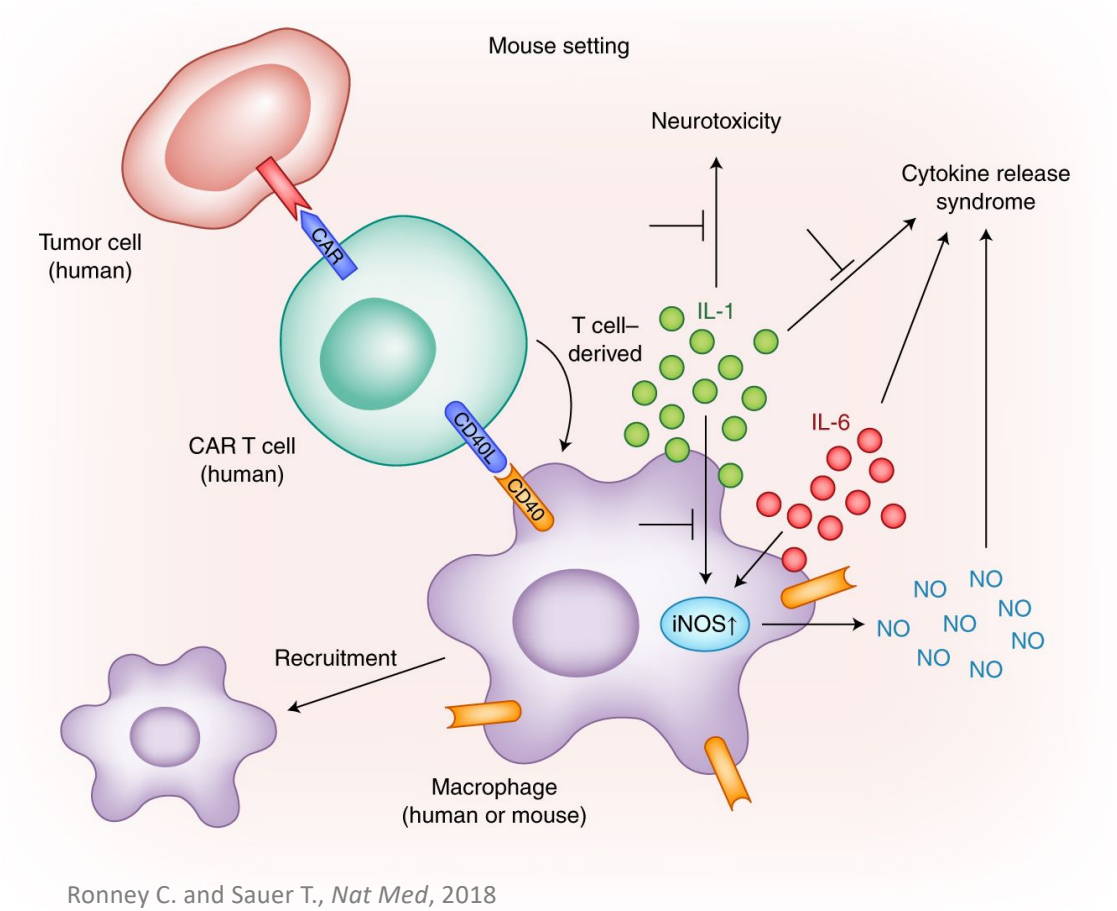
CRS is an inflammatory response caused by overactivation of immune-effector cells



ATG: anti-thymocyte globulin; BiTE: Bi-specific T cell engager; IFN: interferon; TNF: tumour necrosis factor

Shimabukuro-Vornhagen A, *et al*. *J Immunother Cancer* 2018; 6:56.

CRS initiating cascade



Ronney C. and Sauer T., *Nat Med*, 2018

CRS pathophysiology

Massive systemic inflammatory cytokine release by both infused T cells and bystander immune cells (Monocytes/macrophages)

CRS is associated with factors inducing increased CAR-T cell activation and expansion

→ High disease burden
→ High CAR-T cell dose
→ Addition of fludarabine to cyclophosphamide lymphodepletion

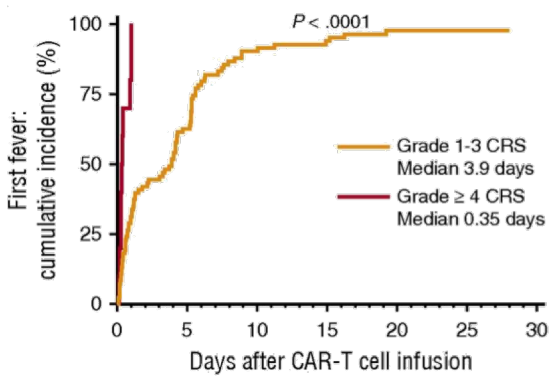
CRS pathophysiology

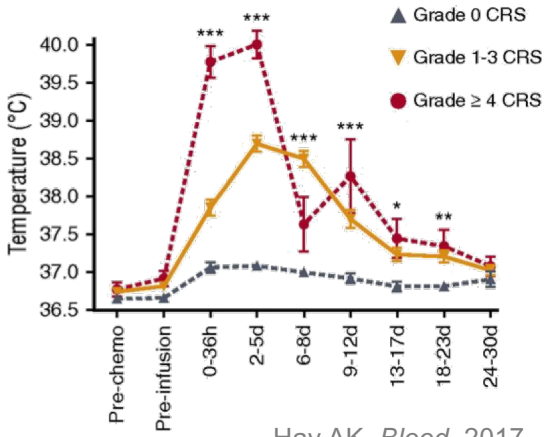
→ IL-1 preceeding IL-6 and NO production
→ Vasodilatation and hypotension
→ Endothelial activation → release of VWF and Angiopoietin 2

→ Hemodynamic instability
Capillary leak
Consumptive coagulopathy

The cytokine release syndrome

Signs and symptoms





Hay AK, *Blood*, 2017

- Fever (first hallmark)
  - → earlier in CD28 rather than 41-BB CARs (1-2 weeks delay)
  - → 1-2 days when most severe CRS occurs
- Myalgias
- Fatigue

→ CRS resolution typically by 2 to 3 weeks

The cytokine release syndrome

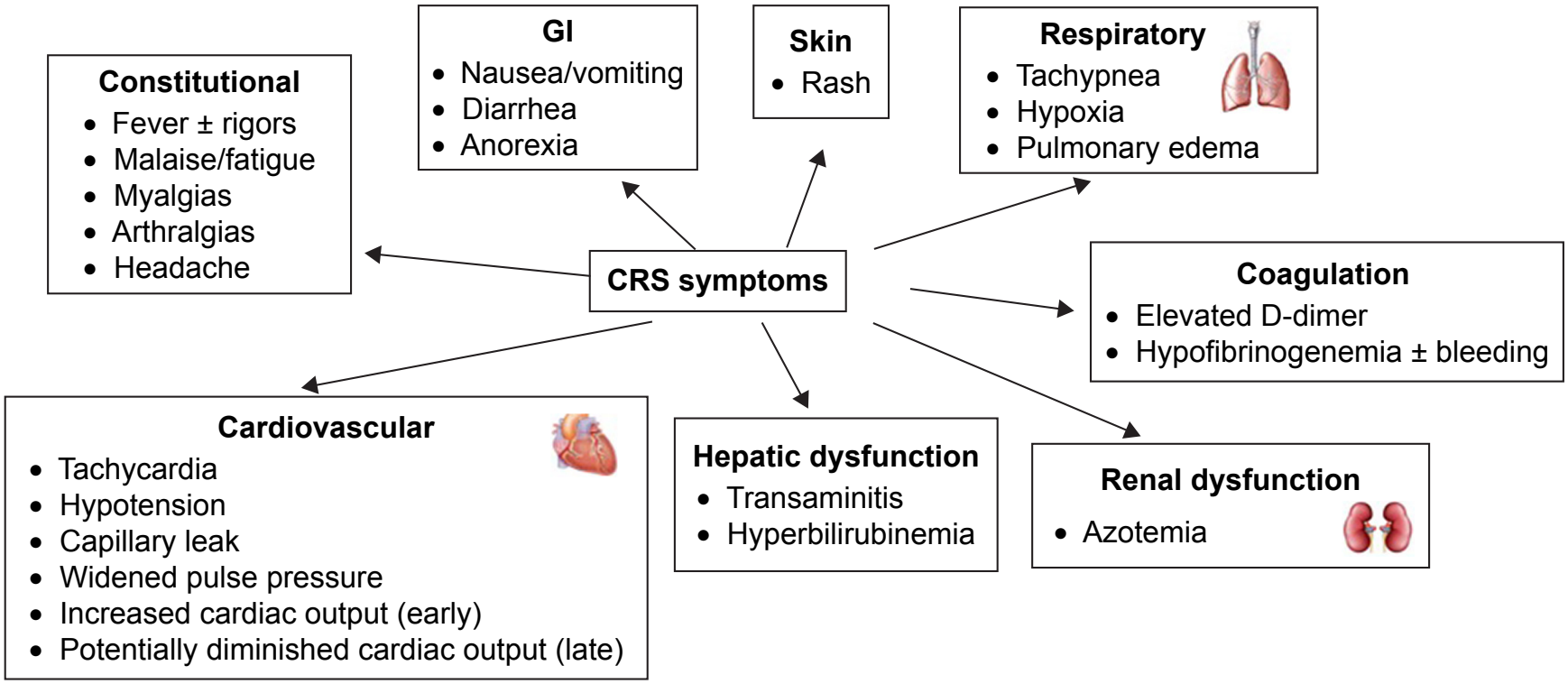


Figure 1 Symptoms of CRS.
Notes: CRS affects a number of organ systems. It requires fever at a minimum but is frequently associated with any of the symptoms shown. Additional manifestations may also rarely occur.
Abbreviations: GI, gastrointestinal; CRS, cytokine release syndrome.

Riegler et al, Therapeutics and Clinical Risk Management 2019

Cytokine Release Syndrome

Clinical markers include:

- Cytopenia
- High ferritin
- High C-reactive protein
- High IFNg, soluble IL-2R
- High IL-6, IL-10, associated with macrophage activation

→CRS can progress to life-threatening vasodilatatory shock, capillary leak, hypoxia and end-organ dysfunction

CRS consensus grading 2018

Table 5 2018 CRS consensus grading by Lee et al[30]

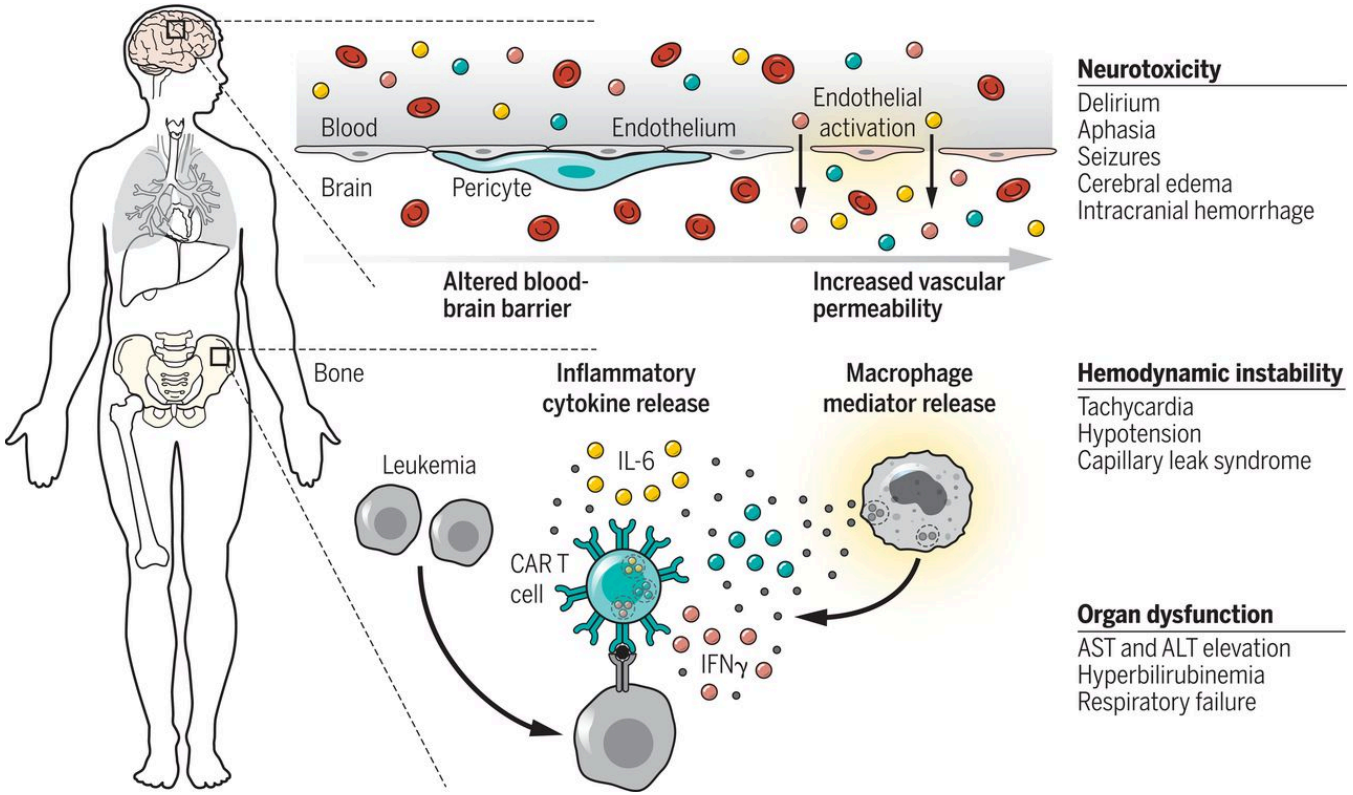
Grade 1	Fever[a] ≥38°C
Grade 2	Fever[a] ≥38°C with hypotension not requiring vasopressors and/or hypoxia requiring low-flow nasal cannula or blow-by oxygen
Grade 3	Fever[a] ≥38°C with hypotension requiring one vasopressor with or without vasopressin and/or hypoxia requiring high-flow nasal cannula, facemask, non-rebreather mask, or Venturi mask not attributable to any other cause
Grade 4	Fever[a] ≥38°C with hypotension requiring multiple vasopressors (excluding vasopressin) and/or hypoxia requiring positive pressure (eg, CPAP, BiPAP, intubation, and mechanical ventilation) not attributable to any other cause
Grade 5	Death

Notes: [a]Fever is defined as temperature ≥38°C. In patients who have CRS then receive 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.

Abbreviations: CRS, cytokine release syndrome; CPAP, continuous positive airway pressure; BiPAP, bilevel positive airway pressure.

Lee et al, BBMT 2018

CRS and NT pathogenesis

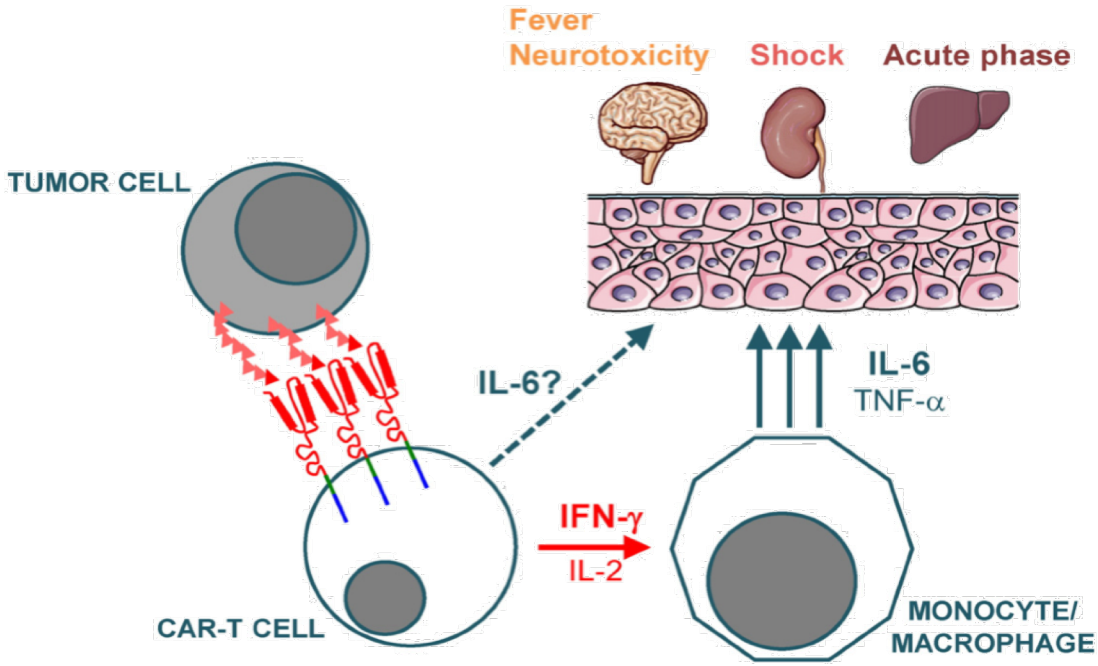


June CH., *Science*, 2018

Neurotoxicity: pathogenesis

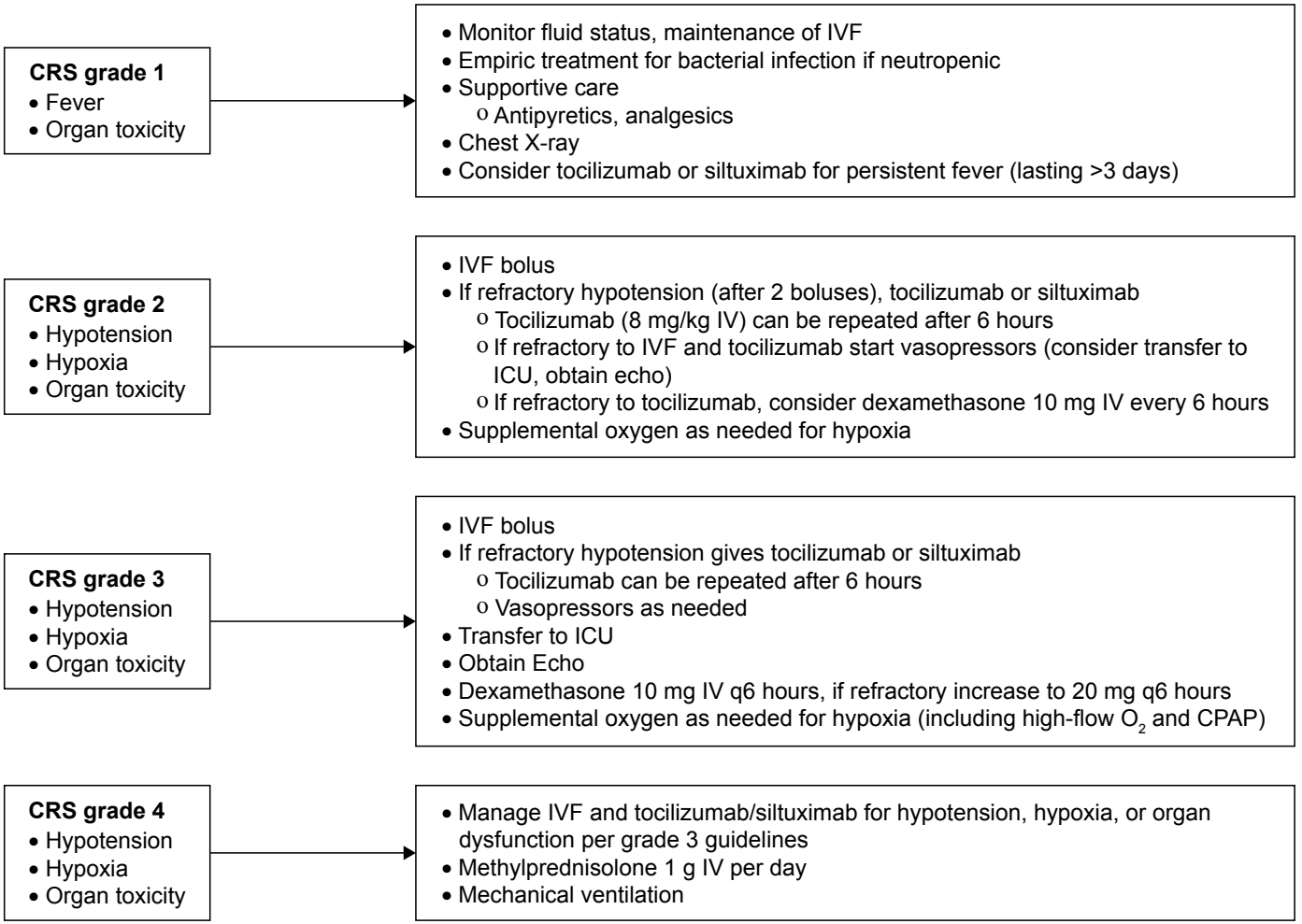
Pathogenesis less known:

- Endothelial activation and BBB breakdown
- CART and non CART in CSF
- Higher levels of CTK in CSF than in blood



Modified from Norelli et al., BBA on Cancer, 2016

CRS management flow-chart



Neelapu et al,
Nat Rev Clin Oncol 2018

Management of CRS and neurotox

Refractory CRS:

Corticosteriods → Together with Toci as first line therapy

In second-line therapy if CRS is refractory to toci or in case of neurotoxicity

→ Can be detrimental for CAR-T cells at high doses

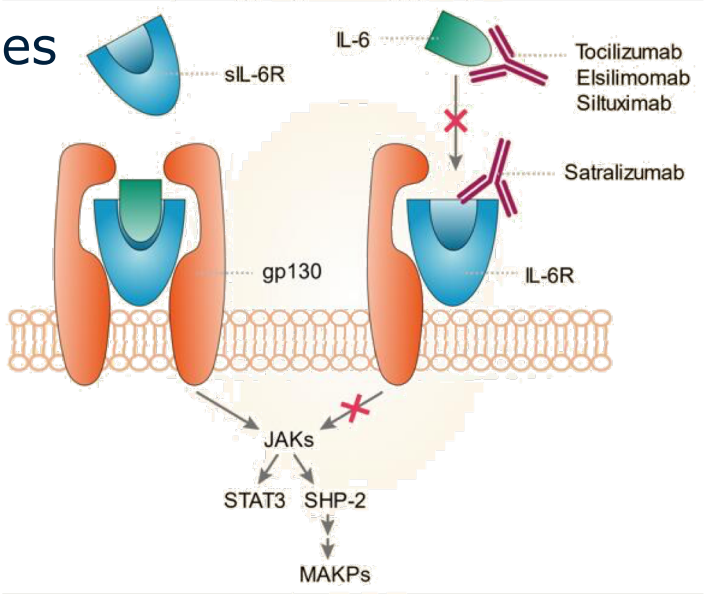
Other compounds:

Anti-IL-6 mAb Siltuximab

Anti-IL-1R antagonist Anakinra

Anti-Janus kinase Ruxolitinib

→ Still not conclusive available data



New Perspectives

1. Modelling CART cells toxicity
(ie: Norelli et al., Nat Med 2018; Giavridis et al., Nat Med 2019)

2. Modify CAR properties
(ie: CAR affinity modulation, Inhibitory CARs, Dual/Tandem CARs)

3. Introduce safety switch sytem in the CAR vector

Modeling CART cell toxicity

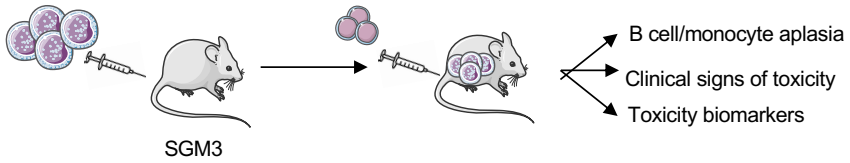
nature medicine

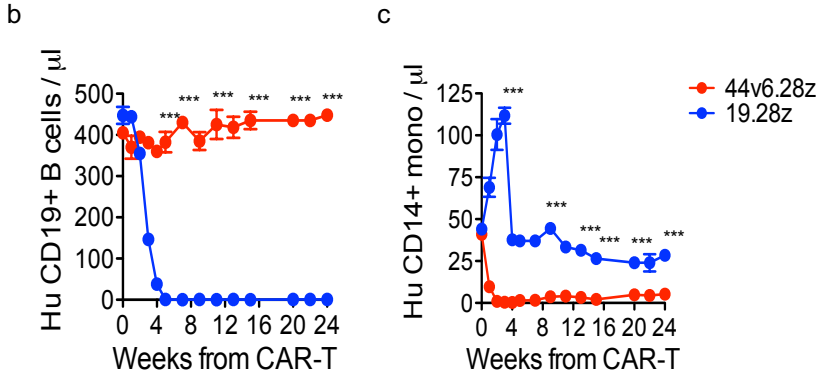
ARTICLES

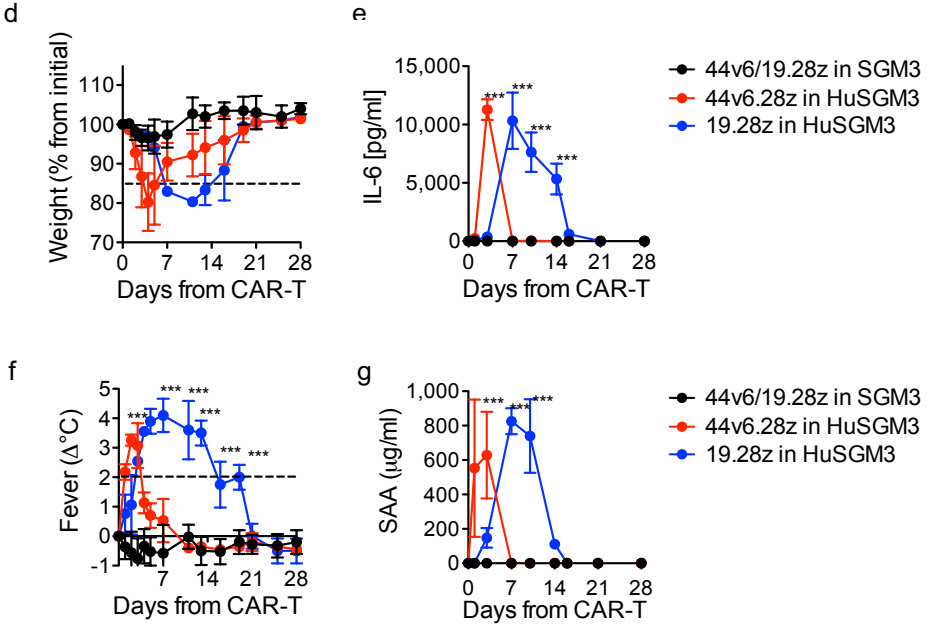
https://doi.org/10.1038/s41591-018-0036-4

Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells

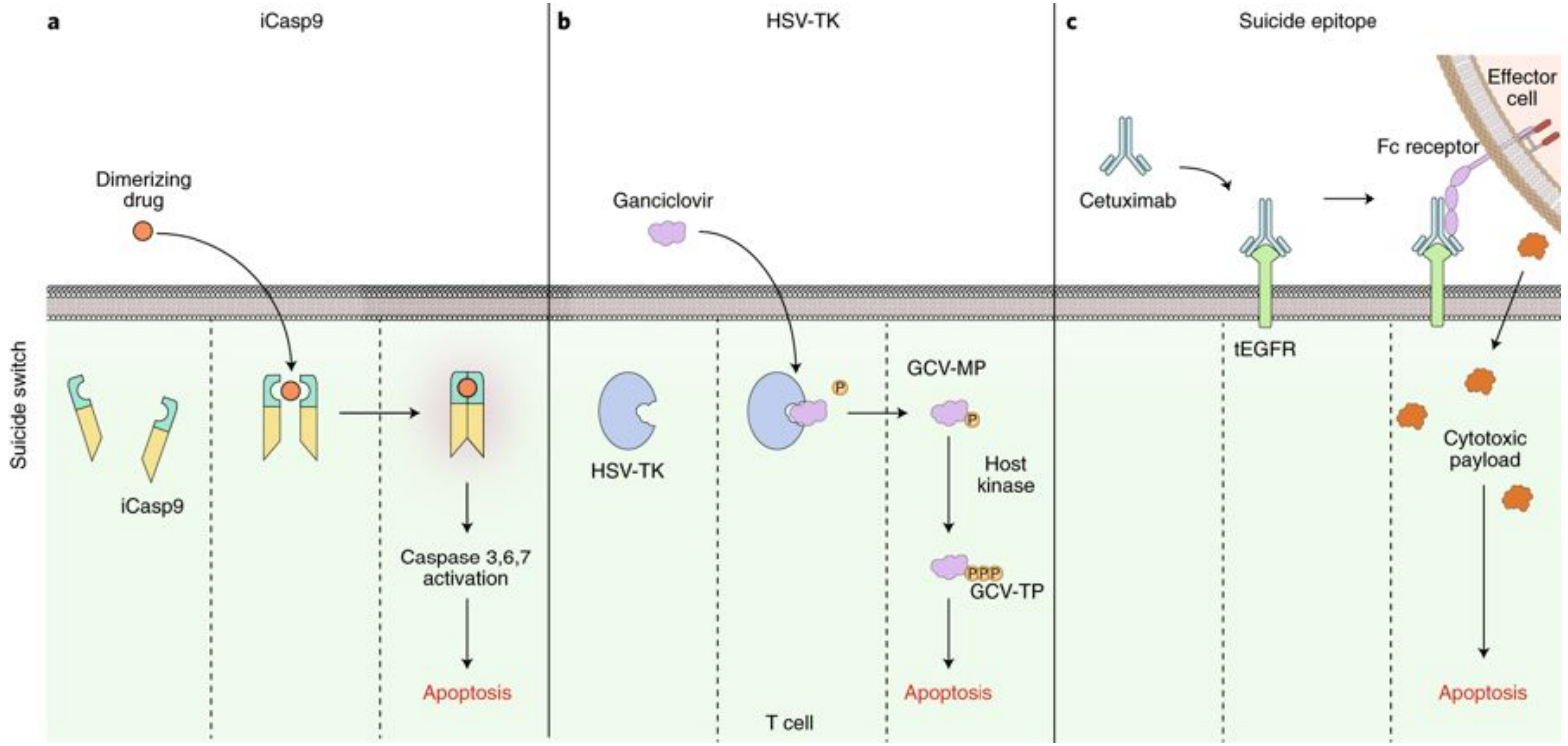
Margherita Norelli[1,2], Barbara Camisa[1], Giulia Barbiera[3], Laura Falcone[1], Ayurzana Purevdorj[1], Marco Genua[3], Francesca Sanvito[4], Maurilio Ponzoni[4], Claudio Doglioni[4], Patrizia Cristofori[5], Catia Traversari[6], Claudio Bordignon[2,6], Fabio Ciceri[2,7], Renato Ostuni[3], Chiara Bonini[2,8], Monica Casucci[1] and Attilio Bondanza[1,2]*



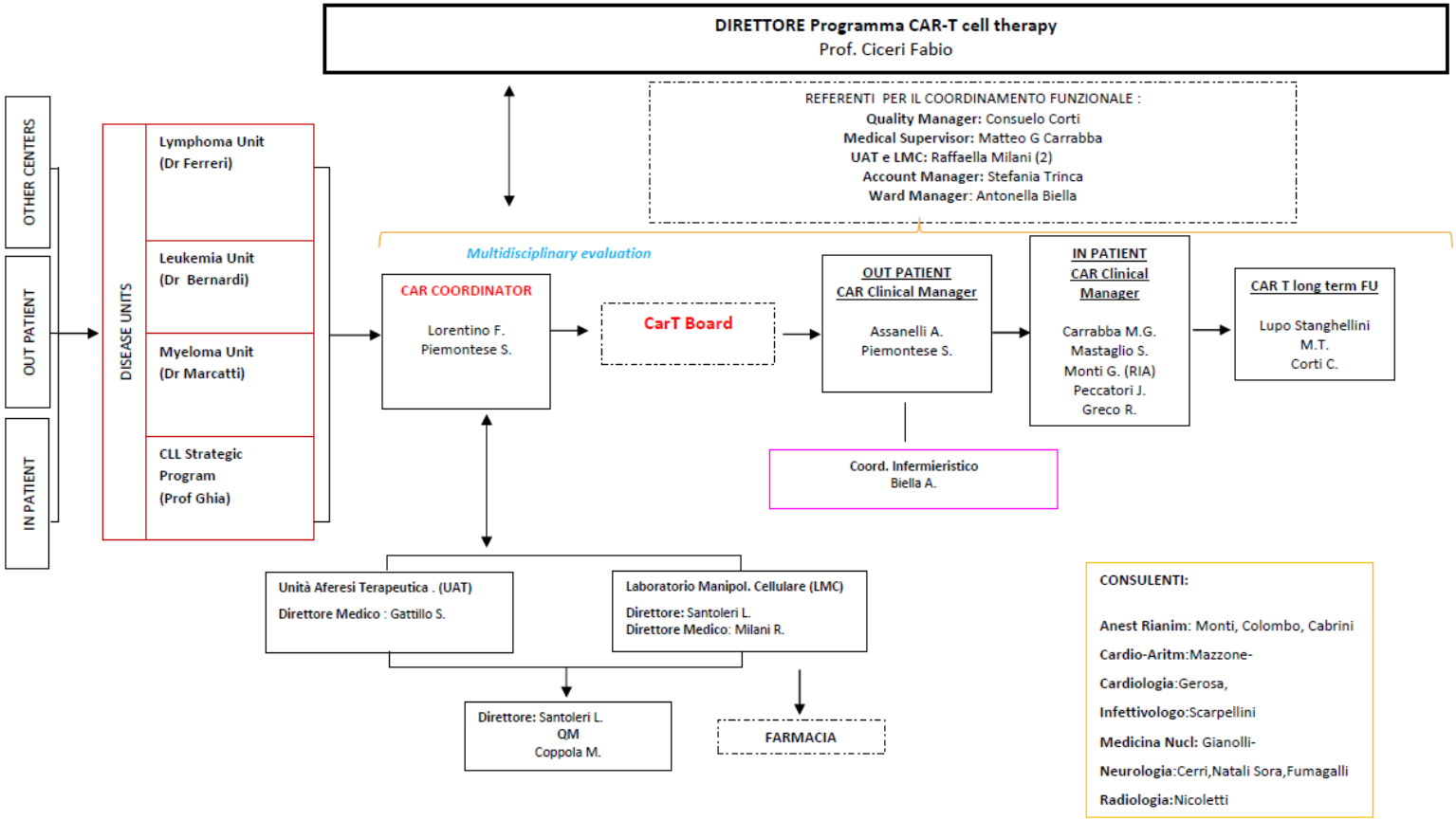




CAR-T cell safety switches



Labanieh L., *Nat Biol Engineer*, 2018


DIRETTORE Programma CAR-T cell therapy
Prof. Ciceri Fabio
REFERENTI PER IL COORDINAMENTO FUNZIONALE :
Quality Manager: Consuelo Corti
Medical Supervisor: Matteo G Carrabba
UAT e LMC: Raffaella Milani (2)
Account Manager: Stefania Trinca
Ward Manager: Antonella Biella
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IN PATIENT
DISEASE UNITS
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Leukemia Unit (Dr Bernardi)
Myeloma Unit (Dr Marcatti)
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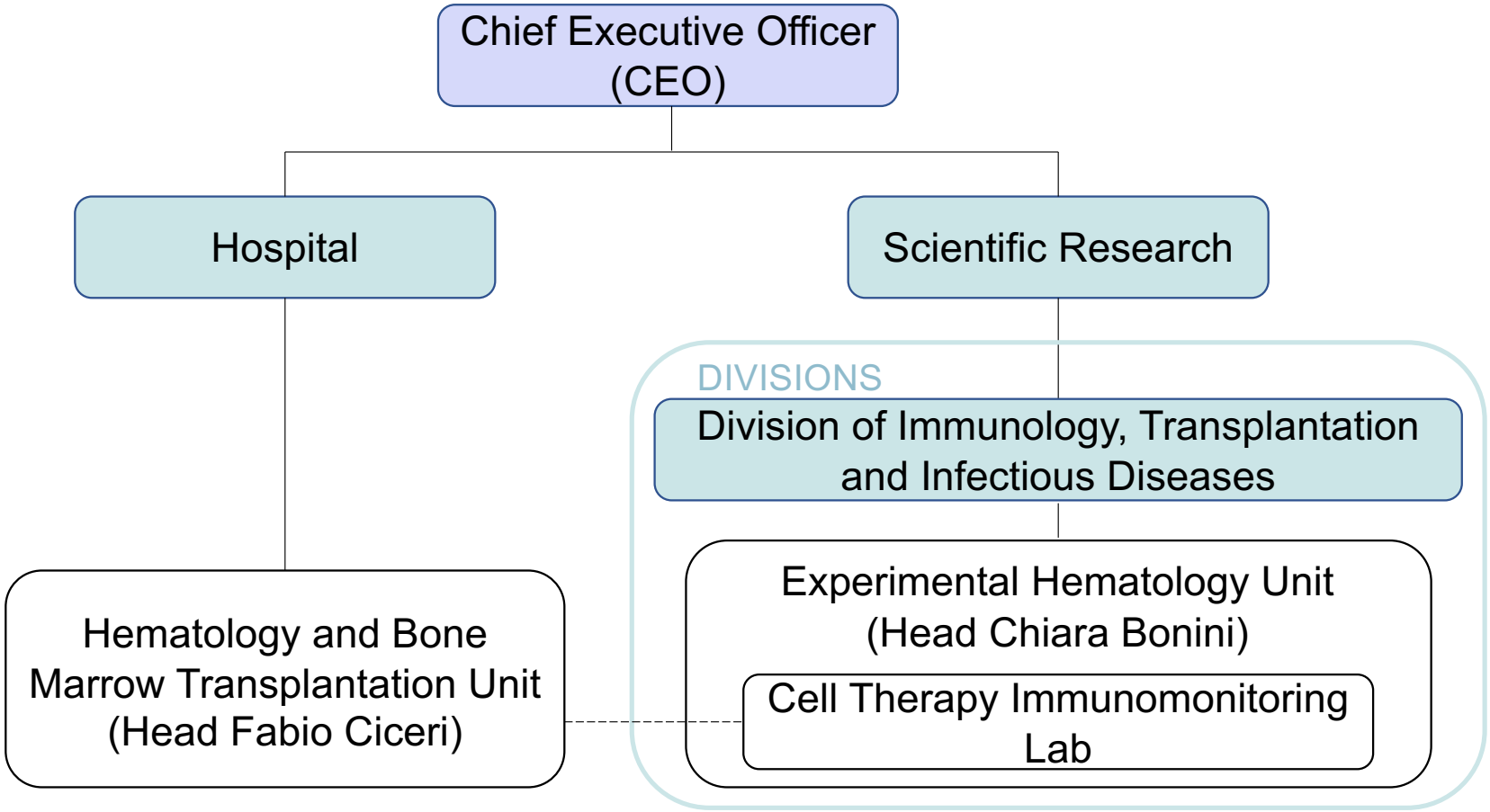
Cardiologia:Gerosa,
Infettivologo:Scarpellini
Medicina Nucl: Gianolli-
Neurologia:Cerri,Natali Sora,Fumagalli
Radiologia:Nicoletti

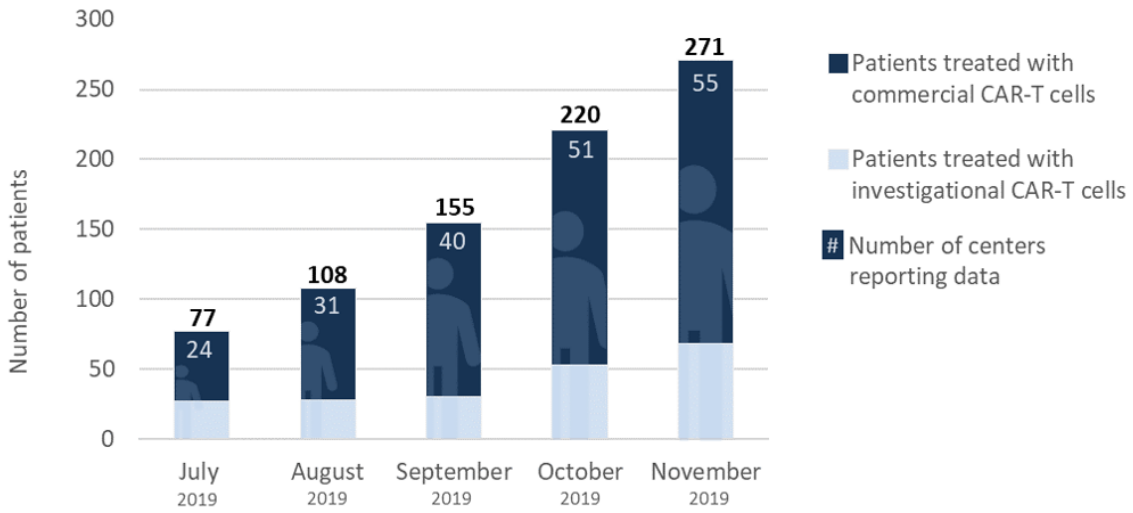
Cell Therapy Immunomonitoring Lab organizational chart



MISSION: To monitor immune responses in clinical trials with the final aim of providing additional information to tailor patient management and treatment

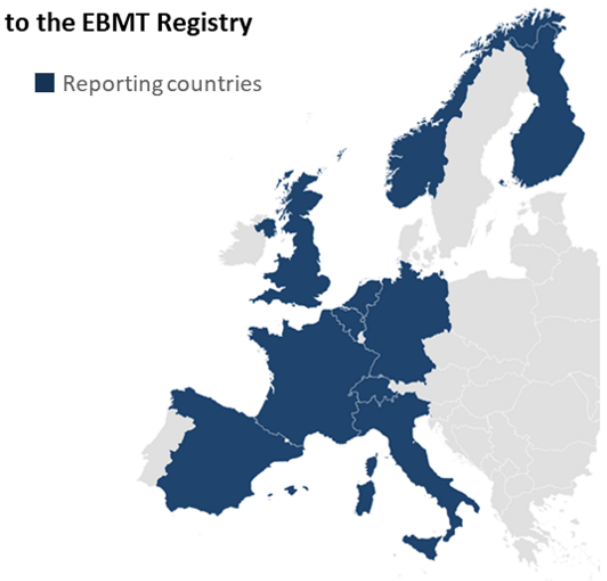
CART cells in Europe: the EBMT registry

Number of CAR-T cell treated patients registered in the EBMT Registry



Source: EBMT Registry, November 2019

Countries reporting CAR-T cell treated patients to the EBMT Registry



Source: EBMT Registry, November 2019

Aknowledgments

Monica Casucci (Innovative Immunotherapies Unit)

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