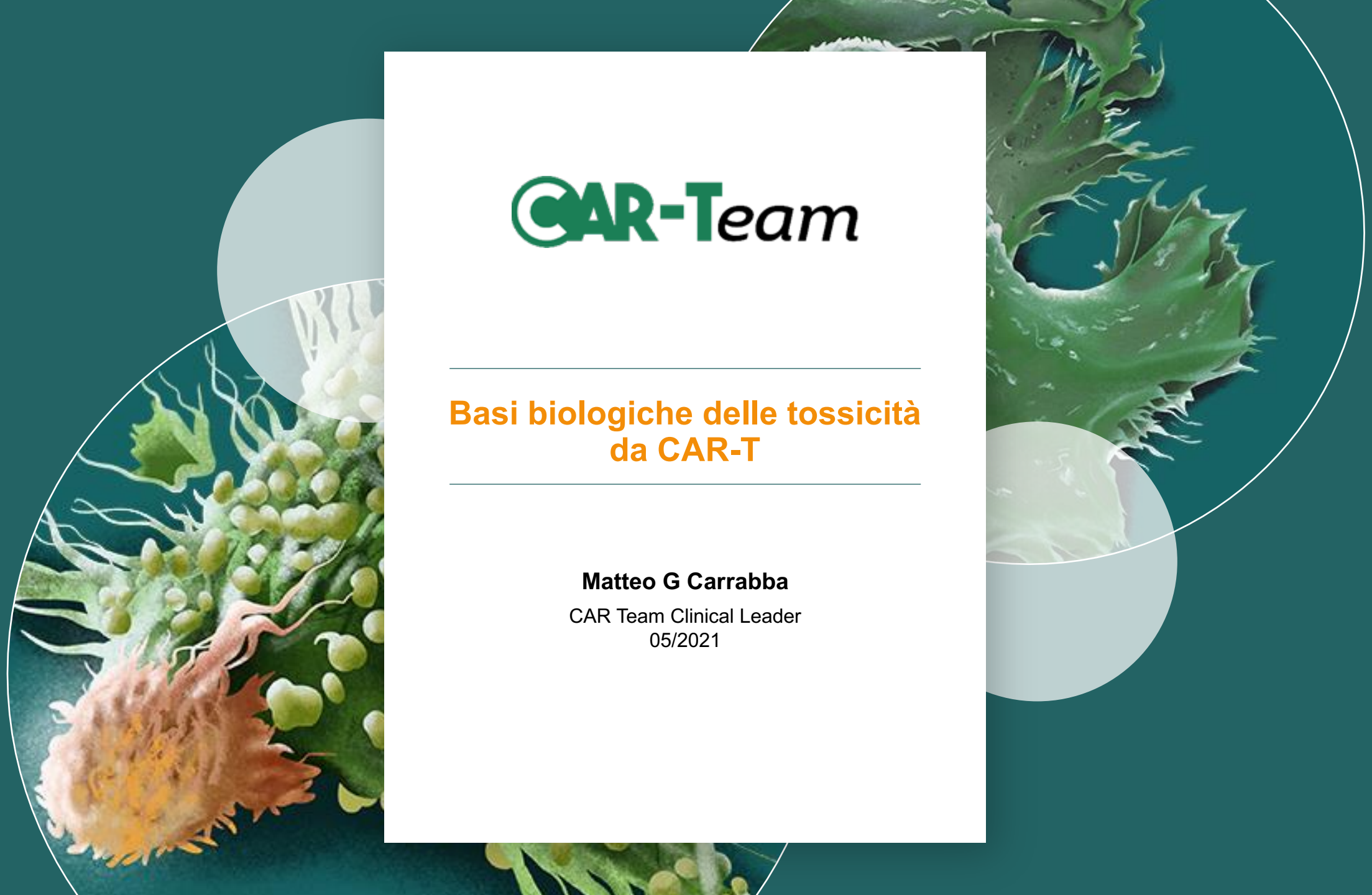


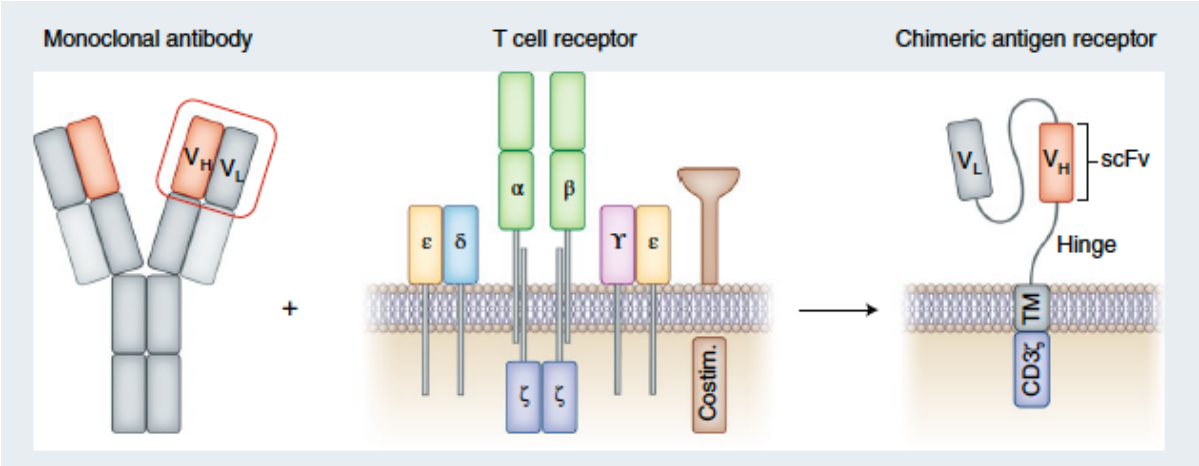
# Basi biologiche delle tossicità da CAR-T

**Matteo G Carrabba**

CAR Team Clinical Leader

05/2021

# Chimeric antigen receptors



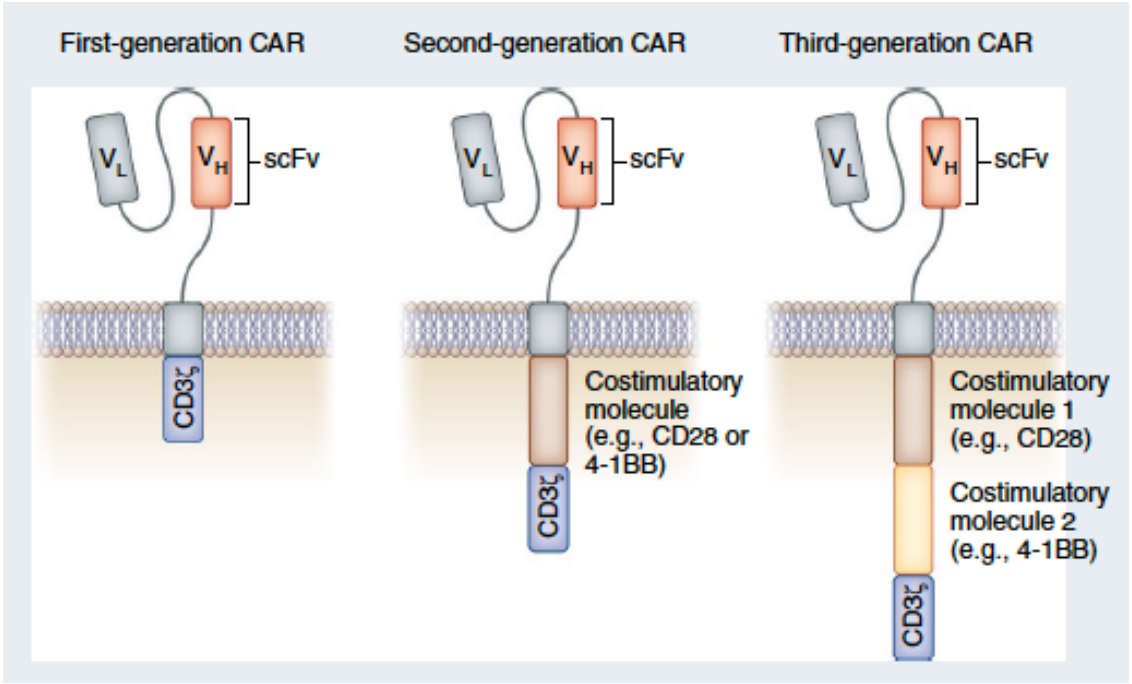

## Key advantages

- Independency from MHC restriction
- Targeting of proteins, sugars, lipids
- Multiple effector mechanisms
- Living drugs (expansion, memory)

*Mod. da Majzner RG, et al. Nat Med 2019; 25: 1341-1355*

# CAR generations

## Natural T cells require costimulation to fully activate




*Mod. da Majzner RG, et al Nat Med 2019; 25: 1341-1355*

# CD19 CAR-T in B-cell tumors

| Cancer | Site | n | CAR | CR (%) | Reference |
|---|---|---|---|---|---|
| B-ALL | NCI | 21 | 28z | 60 | Lee, Lancet 2015 |
| B-ALL | MSKCC | 53 | 28z | 67 | Park, NEJM 2018 |
| B-ALL | FHCC | 30 | BBz | 93 | Turtle, JCI 2016 |
| B-ALL | Multiple ELIANA | 75 | BBz | 81 | Maude, NEJM 2018 |
| NHL | Multiple ZUMA-1 | 101 | 28z | 54 | Neelapu, NEJM 2018 |
| NHL | Multiple JULIET | 93 | BBz | 40 | Schuster, NEJM 2019 |
| NHL | FHCC | 32 | BBz | 33 | Turtle, STM 2016 |
| CLL | UPenn | 14 | BBz | 29 | Porter, STM 2015 |
| CLL | FHCC | 24 | BBz | 17 | Turtle, JCO 2017 |

# CD19 CAR-T products for lymphoma

| | Axi-cel | Tisa-cel | Liso-cel | Brexu-cel |
|---|---|---|---|---|
| Institution | NCI/MDACC | UPenn | SCH/FHCRC | MDACC |
| Sponsor | Kite-Gilead | Novartis | Juno-BMS | Kite-Gilead |
| Trial | ZUMA-1 | JULIET | TRANSCEND | ZUMA-2 |
| FDA approval | 2017 (Yescarta®) | 2018 (Kymriah®) | 2021 (Breyanzi®) | 2020 (Tecartus®) |
| Tumor | DLBCL, t-FL, PMBCL | DLBCL, t-FL | DLBCL, t-FL | MCL |
| CAR design | CD28-CD3z | 41BB-CD3z | 41BB-CD3z | CD28-CD3z |
| Reference | Locke, Lancet 2019 | Schuster, NEJM 2019 | Abramson, Lancet 2020 | Wang, NEJM 2020 |

**Strong interaction between academia and industries**

# Main CAR-related toxicities

## On-target off-tumor toxicity

- Damage of healthy tissues expressing the target antigen
- Quite relevant: tumor-specific antigens are rare
- Severity depends on how vital, accessible and widespread the targeted tissue is (CD19 → B-cell aplasia → Immunoglobulin Replacement Therapy)
- Particularly dangerous for solid tumors

# Main CAR-related toxicities

## Cytokine release syndrome

| Cancer | Site | CAR | Severe CRS (%) | Reference |
|---|---|---|---|---|
| B-ALL | NCI | 28z | 29 | Lee, Lancet 2015 |
| B-ALL | MSKCC | 28z | 26 | Park, NEJM 2018 |
| B-ALL | FHCC | BBz | 23 | Turtle, JCI 2016 |
| B-ALL | Multiple ELIANA | BBz | 47 | Maude, NEJM 2018 |
| NHL | Multiple ZUMA-1 | 28z | 13 | Neelapu, NEJM 2018 |
| NHL | Multiple JULIET | BBz | 22 | Schuster, NEJM 2019 |
| NHL | FHCC | BBz | 13 | Turtle, STM 2016 |
| CLL | UPenn | BBz | 43 | Porter, STM 2015 |
| CLL | FHCC | BBz | 8 | Turtle, JCO 2017 |

# CRS

- Systemic **inflammatory** reaction
- Rapid onset **within a few days** after CAR-T cell infusion
- Fever, hypotension, hypoxia, capillary leak, coagulopathy
- Potentially **life-threatening**

- Severe CRS associated with:
  - Higher **tumor burden**
  - Higher **T-cell dose**
  - Cy/Flu **lymphodepletion**

    → More robust CAR-T cell expansion in vivo

*Teachey DT, et al. Cancer Discov 2016; 6: 664-679*
*Hill JA, et al. Blood 2018; 131:121-130*

# CRS biomarkers

- Laboratory markers of inflammation and organ failure
  - Including **C-reactive protein** (CRP) and ferritin
- Inflammatory cytokines
  - Including **IL-6**, IL-8, IFN-$\gamma$, MCP1, MIP1$\alpha$, GM-CSF
- Laboratory markers of **coagulopathy**
- Markers on **endothelial activation**
  - Including VWF, increased angiopoietin-2/angiopoitin-1 ratio

*Teachey DT, et al. Cancer Discov 2016; 6: 664-679*
*Hill JA, et al. Blood 2018; 131:121-130*

# CRS pathophysiology

- Initiated by **CAR-T cells activation upon antigen engagement**
- Which other cellular compartments are involved?

→ Development of **animal models** recapitulating CRS development

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]*

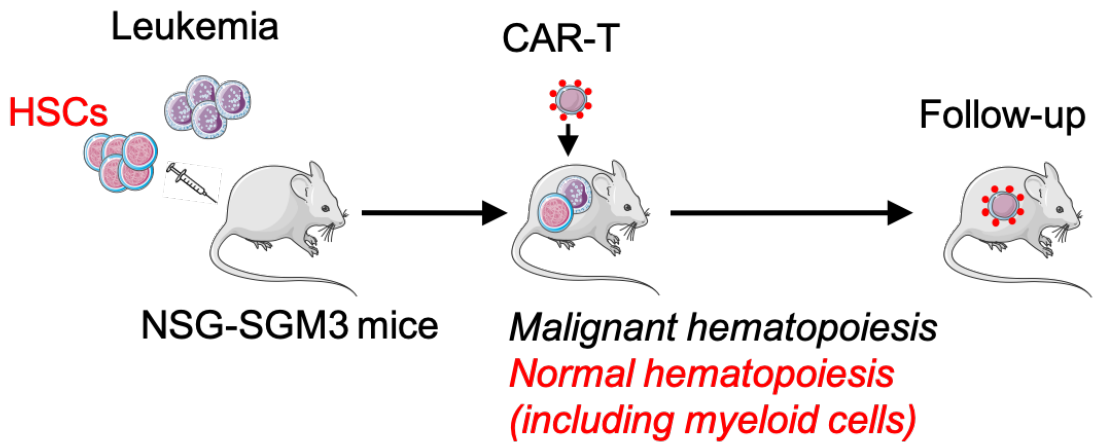
nature medicine
LETTERS
https://doi.org/10.1038/s41591-018-0041-7

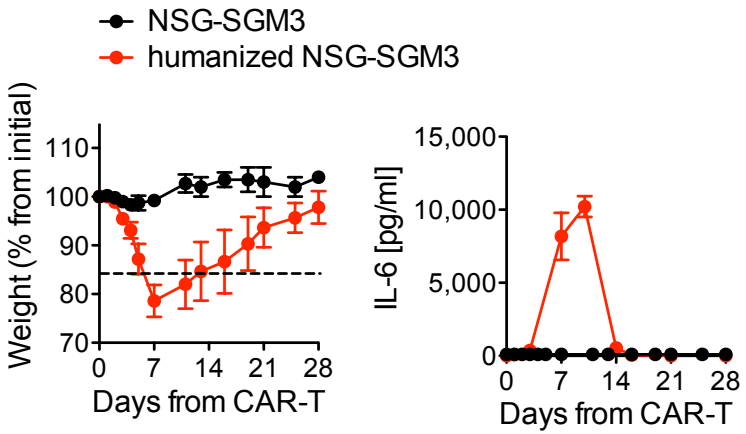
## CAR T cell–induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade

Theodoros Giavridis[1], Sjoukje J. C. van der Stegen[1], Justin Eyquem[1], Mohamad Hamieh[1], Alessandra Piersigilli[2] and Michel Sadelain[1]*

# Humanized model for CAR-T

## Efficacy and CAR-related toxicities

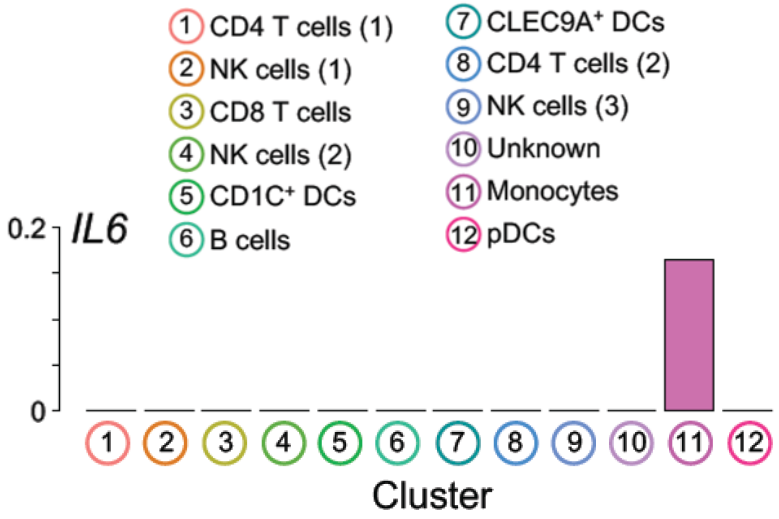


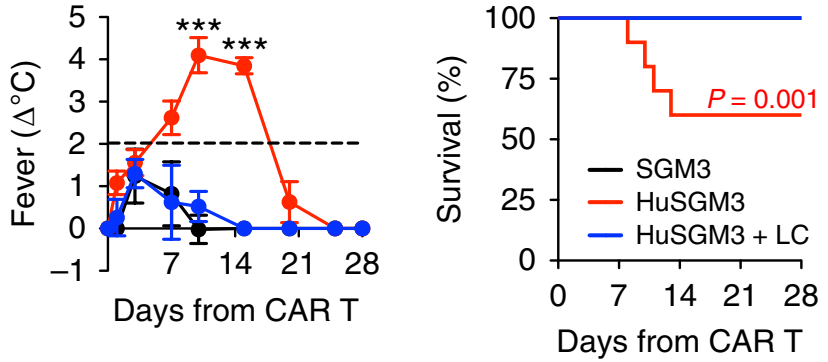

*Mod. da Norelli M, et al. Nat Med 2018; 24: 739-748*

# Role of monocytes in CRS

## IL-6 sources



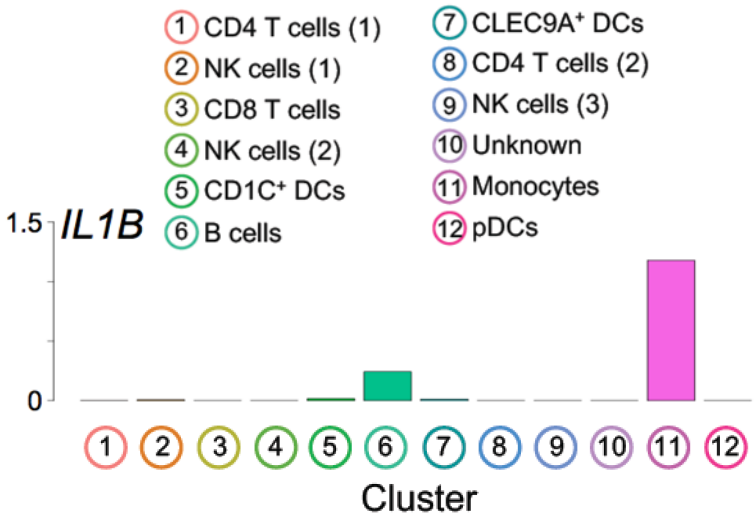

## Monocyte's ablation




*Mod. da Norelli M, et al. Nat Med 2018; 24: 739-748*

# IL-1 precedes IL-6 release

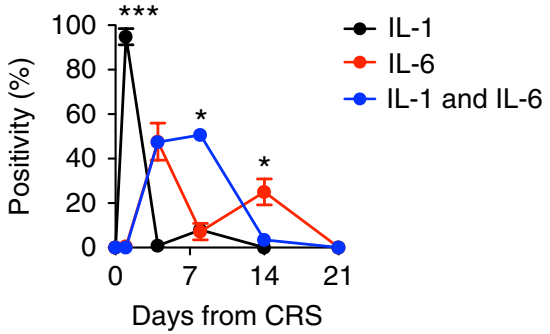
## IL-1 source




## IL-1 kinetics

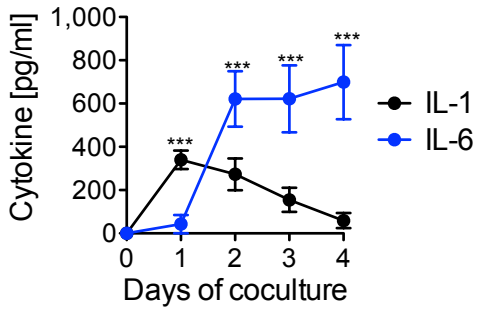
*In vivo*

*IC staining monocytes*



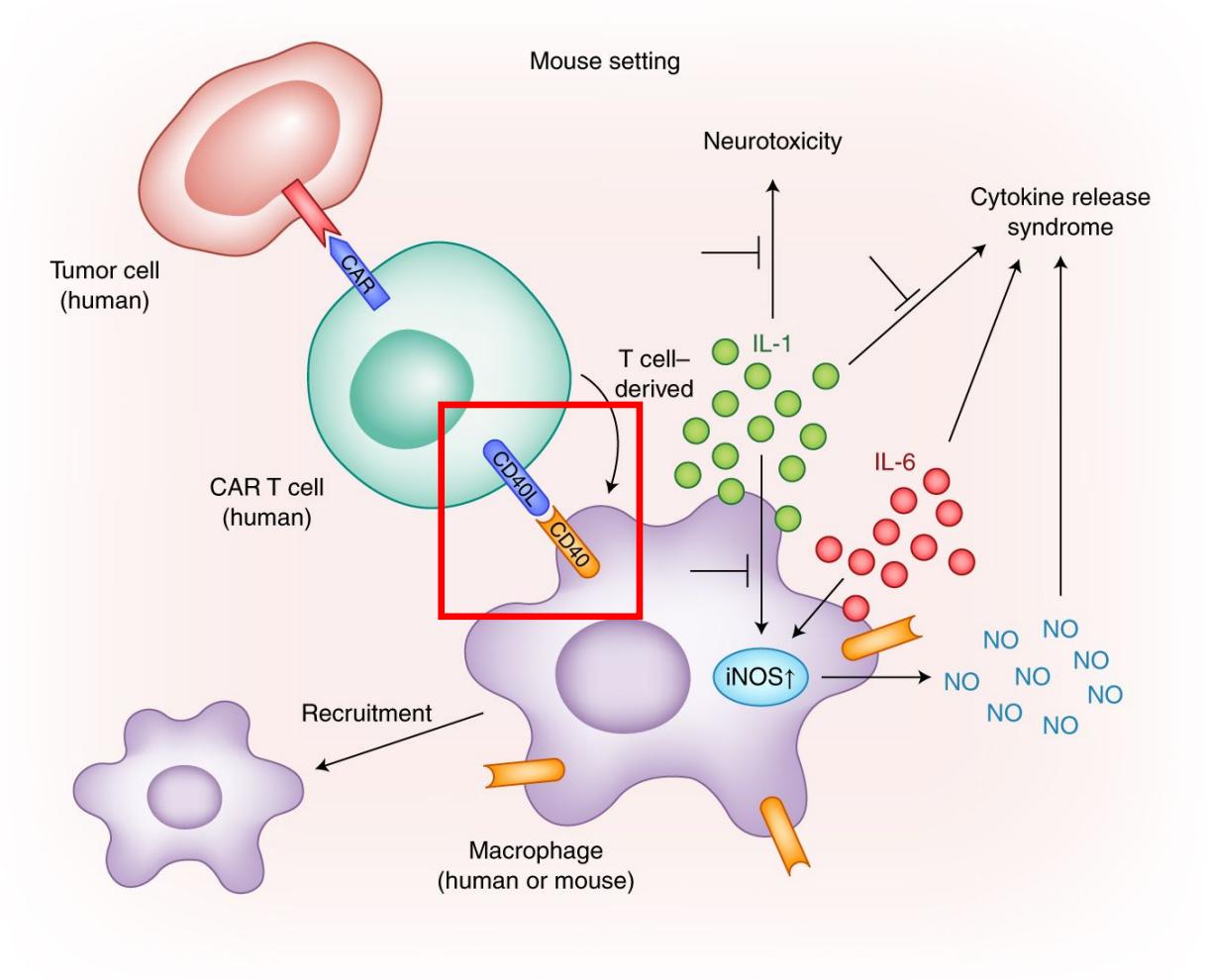

*In vitro co-cultures*

*19.CAR28z + target + mTHP-1*




*Mod. da Norelli M, et al. Nat Med 2018; 24: 739-748*

# CRS initiating cascade




*Mod. da Rooney C, Sauer T. Nat Med 2018; 24: 705-706*

CAR-T cells release **perforin** to form pores, leading to the entry of **granzyme B** into target tumor cells, which causes the subsequent activation of **GSDME** and **pyroptosis** (programmed necrotic cell death)

Proptosis supernatants contain **ATP** and **HMGB1** that induce macrophages to release **IL-1b** and **IL-6**, respectively

*Liu YL, et al. Science Immunol 2020; 5: eaax7969*

# Main CAR-related toxicities

## Neurotoxicity

| Cancer | Site | CAR | Severe NTX (%) | Reference |
|---|---|---|---|---|
| B-ALL | NCI | 28z | 5 | Lee, Lancet 2015 |
| B-ALL | MSKCC | 28z | 42 | Park, NEJM 2018 |
| B-ALL | FHCC | BBz | 50 | Turtle, JCI 2016 |
| B-ALL | Multiple ELIANA | BBz | 13 | Maude, NEJM 2018 |
| NHL | Multiple ZUMA-1 | 28z | 28 | Neelapu, NEJM 2018 |
| NHL | Multiple JULIET | BBz | 12 | Schuster, NEJM 2019 |
| NHL | FHCC | BBz | 28 | Turtle, STM 2016 |
| CLL | UPenn | BBz | 7 | Porter, STM 2015 |
| CLL | FHCC | BBz | 25 | Turtle, JCO 2017 |

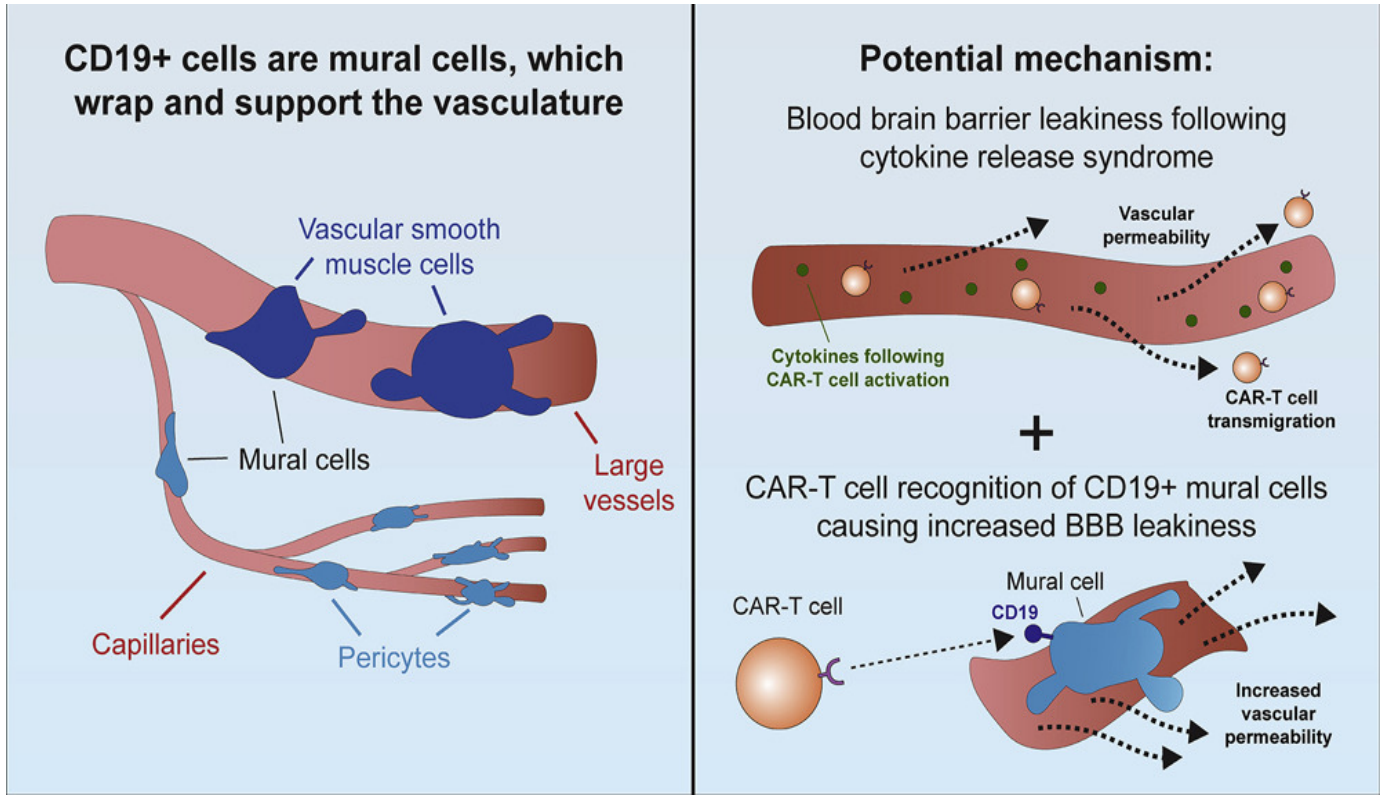
# Neurotoxicity

- Characterized by aphasia, delirium, headache, seizures and edema
- Potentially **life-threatening** (cerebral hemorrhage and edema)
- Characterized by **endothelial activation**
- Characterized by **increased permeability of the BBB**
- Typically occurs **after CRS**

- Severe neurotoxicity is frequently associated with:
  - Earlier and more severe **CRS** (fever and cytokines)
  - Higher **tumor burden**
  - Cy/Flu **lymphodepletion**
  - More robust **CAR-T cell expansion** in vivo
  - Higher **CAR T-cell dose**
  - Neurologic **comorbidities**

*Gust J, et al. Cancer Discov 2017; 7: 1404-1419*
*Santomasso BD, et al. Cancer Discov 2018; 8: 958-971*

# Neurotoxicity pathophysiology




1

2

# CRS and neurotoxicity management

- **Tocilizumab**
  - Anti-IL-6R antibody
  - Active against CRS
  - Unable to control neurotoxicity in most of patients
- **Corticosteroids**
  - At high-doses can be detrimental for efficacy

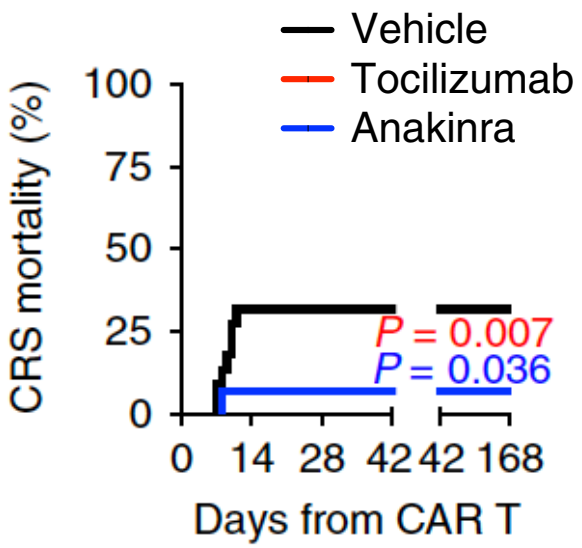
**The search for strategies to mitigate these toxicities is extremely active**

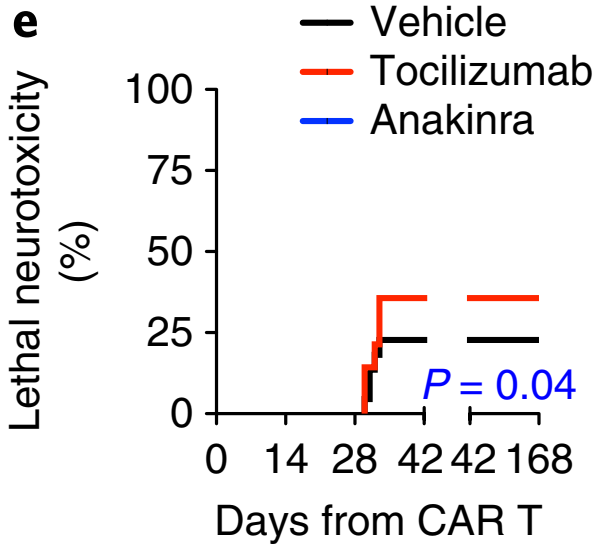
# Mitigating CRS and neurotoxicity

1. **Early intervention in patients at risk of developing severe toxicities**
   - Identification of predictive biomarkers
2. **Cytokine inhibitors**
   - IL-6, IL-1, GM-CSF, catecholamine

# Anakinra abolished neurotoxicity in mice

**CRS**



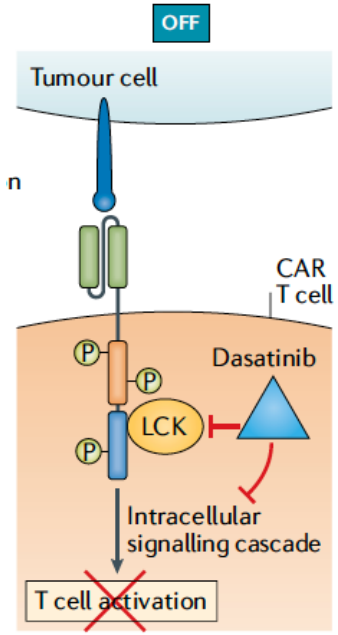

*Mod. da Norelli M, et al. Nat Med 2018; 24: 739-748*

# Mitigating CRS and neurotoxicity

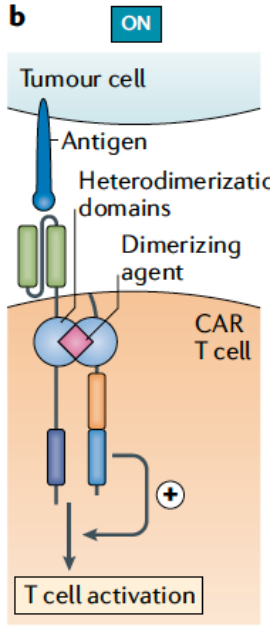
1. **Early intervention in patients at risk of developing severe toxicities**
   - Identification of predictive biomarkers
2. **Cytokine inhibitors**
   - IL-6, IL-1, GM-CSF, catecholamine
3. **On/off switches**
   - Pharmacological control over CAR T-cell activity (drugs or CAR designs)

# On/off switches to mitigate toxicity

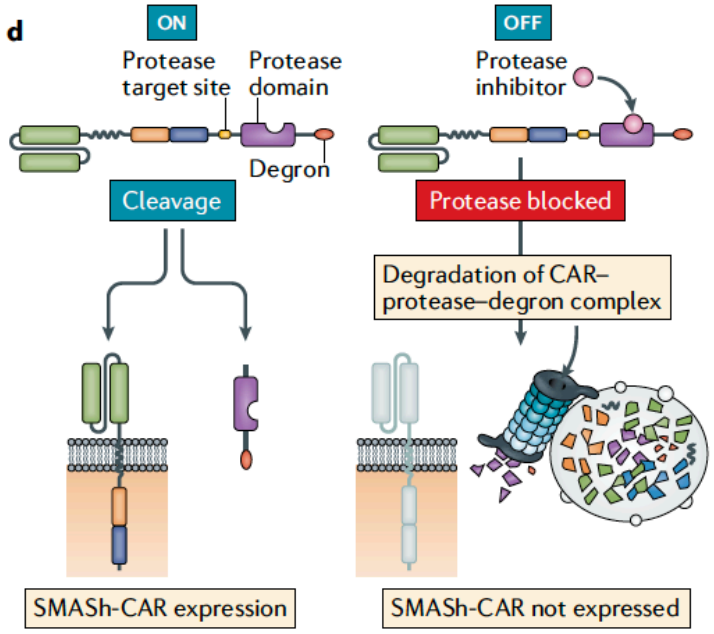
Short treatment with **dasatinib** can rapidly and temporary switch-off CAR T-cell function




CAR constructs able to induce full T-cell activation only upon administration of **a dimerizing agent**




CAR constructs including a domain that enable **drug-dependent degradation** of the CAR protein




*Mod. da Rafiq S, et al. Nat Rev Clinical Oncol 2020; 17: 147-167*