Synergism between human apoptotic cell infusion (Allocetra-OTS) and human chimeric antigen receptor (CAR)-T therapy in fighting solid human tumor in SCID Bg mice

September 23, 2019

Joint IIS-ISCR Special Conference - The Cutting Edge of Immunology, Cancer and Immunoncology Research

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Disclosures:

Dror Mevorach

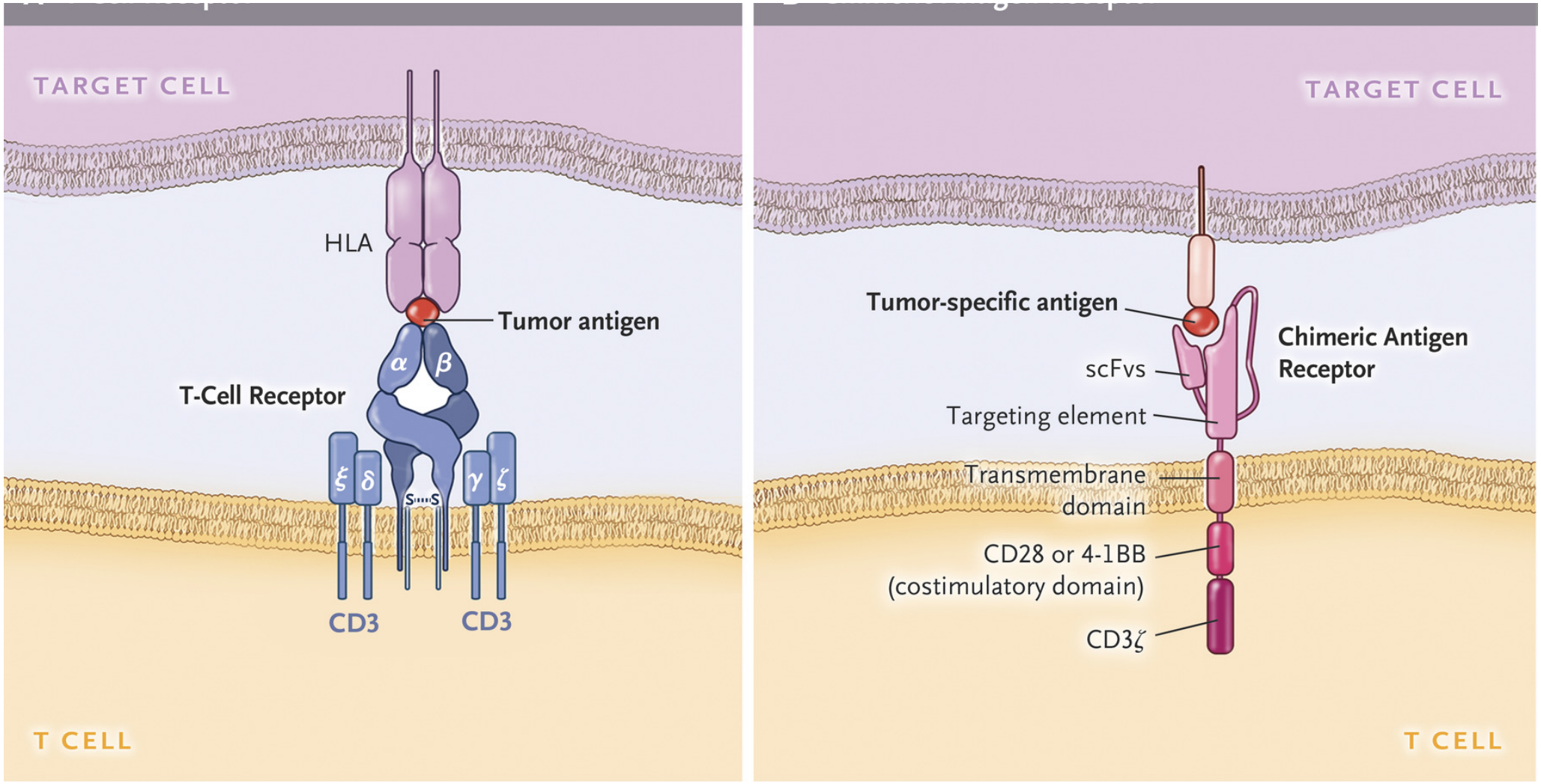
-The Founder, CSO, CMO of Enlivex Ltd.

-A member of the Scientific Advisory Board for anti C5 monoclonal antibody, Regeneron, US

Chimeric antigen receptor (CAR) T cells

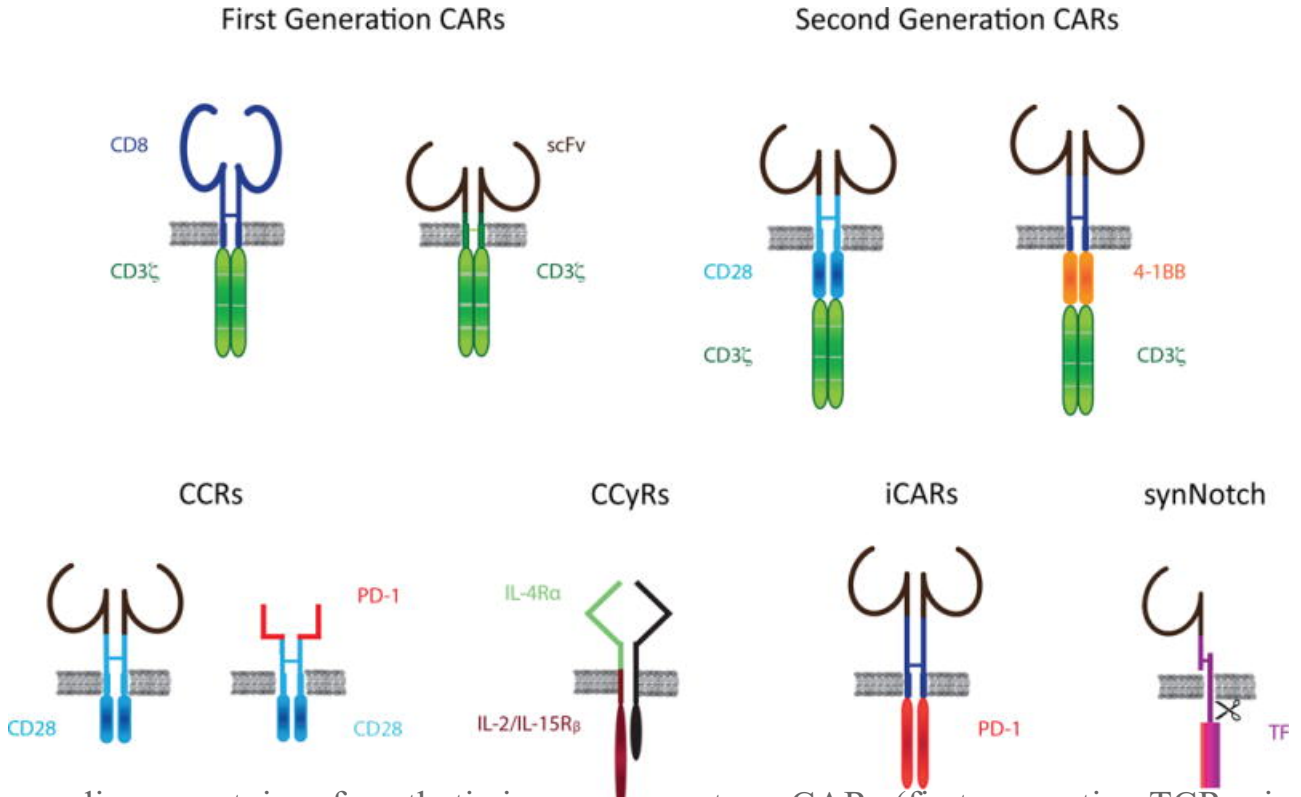
- Chimeric antigen receptor (CAR) T cells are genetically modified T cells that express a CAR directed against specific tumor antigens. CAR T cells are able to kill target tumor cells and may result in long-lasting immune responses *in vivo.* Carl H. June and Michel Sadelain. Chimeric Antigen Receptor Therapy. N Engl J Med 2018.

- The rapid development of CAR technologies has led to clinical trials in hematological cancers and CAR T cells might evolve into a standard treatment in the next few years. Maude, 2014 ; Davila et al, Sci Tr Med, 2014; Lee et al, Lancet 2015, Kochenderfer, JCO 2015.

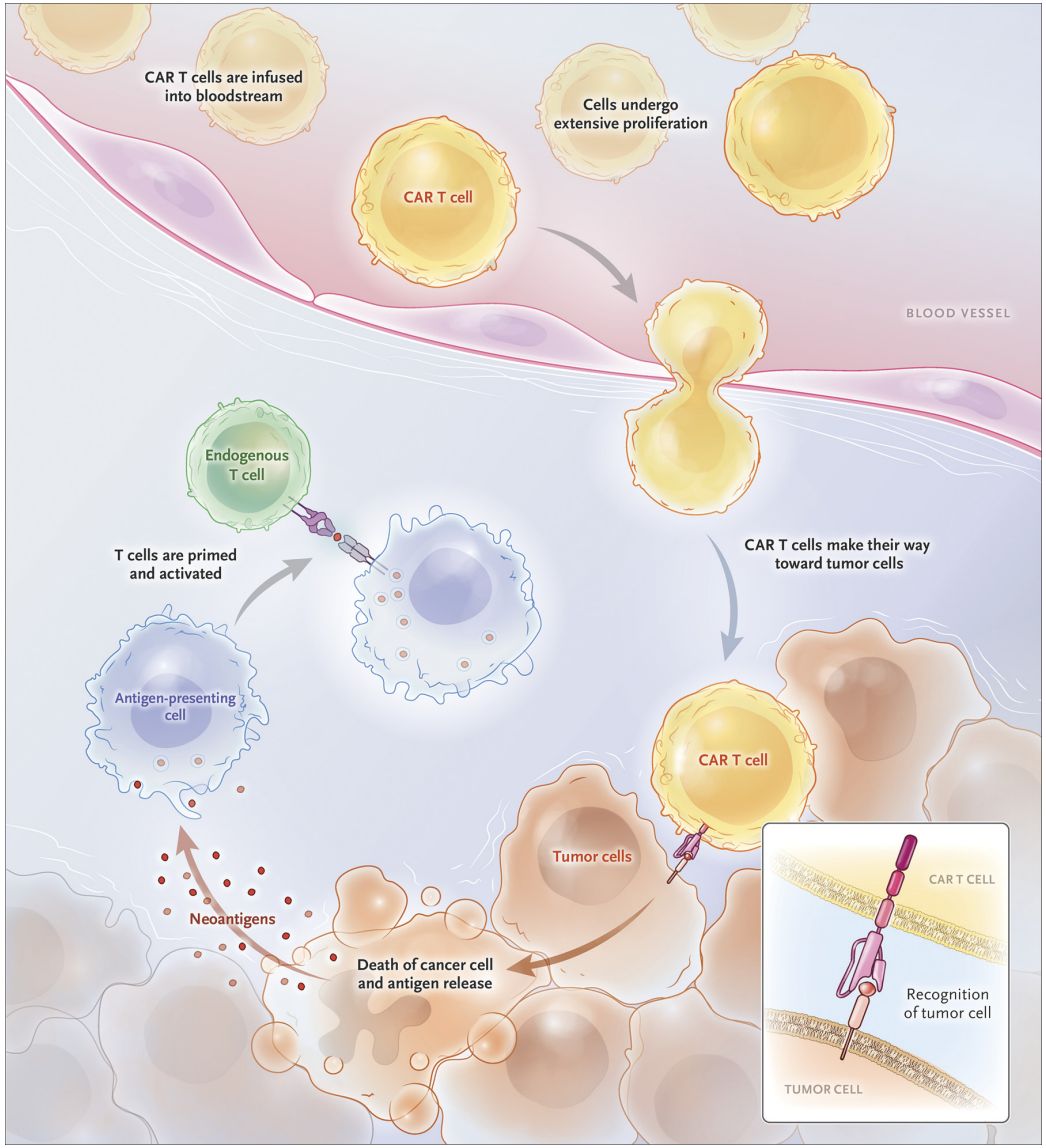


June & Sadelain. Chimeric Antigen Receptor Therapy. N Engl J Med 2018.

CAR T CELL THERAPY



The expanding repertoire of synthetic immunoreceptors: CARs (first generation TCR mimetics, **second generation providing integrated activating and costimulatory signals**; CCRs, chimeric costimulatory receptor; CCyRs, chimeric cytokine receptors; iCARs, inhibitors of T cell activation; synNotch, synthetic Notch receptors. **Third generation CARs are conceptually similar to second generation CARs, except for their use of multiple costimulatory components.**



- Chimeric Antigen Receptor (CAR) T Cells Engrafting, Trafficking to Tumor, and Proliferating Extensively after Infusion.

- After infusion, CAR T cells leave the blood and travel to sites of tumor, where they identify and kill tumor cells.
- This can trigger extensive proliferation of CAR T cells and the release of tumor antigens, which activates the immune system to recruit non–CAR T cells, thus eliciting further antitumor responses in a process known as cross priming. June & Sadelain. Chimeric Antigen Receptor Therapy. N Engl J Med 2018.

Table 1. Responses to CAR T-Cell Therapy.*

Disease	Response Rate	Comments	Reference
	percent		
Leukemia			
B-cell acute lymphoblastic leukemia (in adults)	83–93	High initial remission rates; unresolved issue is whether CAR T-cell therapy is definitive therapy or should be followed by allogeneic hematopoietic stem-cell therapy	Park et al.,[35] Davila et al.,[36] Turtle et al.[37]
B-cell acute lymphoblastic leukemia (in children)	68–90	Approximately 25% of patients reported to have a relapse with CD19-negative or CD19-low leukemia; CD22 CAR T cells may improve survival among some patients with CD19 relapses	Maude et al.,[34] Maude et al.,[38] Fry et al.,[39] Lee et al.[40]
Chronic lymphocytic leukemia	57–71	Relapse is rare in patients who have a complete response; ibrutinib appears to increase response rates	Porter et al.,[41] Turtle et al.[42]
Lymphoma			
Diffuse large B-cell lymphoma	64–86	Approximately 40–50% of patients reported to have a durable complete response	Turtle et al.,[43] Kochenderfer et al.,[44] Schuster et al.,[45] Neelapu et al.[46]
Follicular lymphoma	71	At a median follow-up of 28.6 mo, the response was maintained in 89% of patients who had a response	Schuster et al.[45]
Transformed follicular lymphoma	70–83	A total of 3 of 3 patients with transformed follicular lymphoma had a complete response	Turtle et al.,[43] Schuster et al.,[45] Neelapu et al.[46]
Refractory multiple myeloma	25–100	B-cell maturation antigen CAR T cells; stringent complete response in approximately 25% of patients	Ali et al.,[47] Fan et al.,[48] Berdeja et al.[49]
Solid tumors			
Glioblastoma	ND	In case report from phase 2 study, complete response on magnetic resonance imaging after intravenous and cerebrospinal fluid administration of CAR T cells; response lasted 7.5 mo	Brown et al.[50]
Pancreatic ductal adenocarcinoma	17	In one patient with liver metastasis, CAR T-cell treatment produced a complete metabolic response in the liver but was ineffective against the primary pancreatic tumor	Beatty et al.[51]

* ND denotes not determined.

June & Sadelain. Chimeric Antigen Receptor Therapy. N Engl J Med 2018.

Table 2. Reported Toxic Effects of CAR T Cells.

CAR Specificity and Adverse Effect	Reference
CD19 CAR	
B-cell aplasia and hypogammaglobulinemia	Kochenderfer et al.,[52] Kalos et al.[53]
Cytokine release syndrome	Davila et al.,[36] Lee et al.,[54] Teachey et al.[55]
Dermatitis	Rubin et al.[56]
Hematophagocytic lymphohistiocytosis and macrophage activation syndrome	Grupp et al.,[32] Porter et al.,[41] Teachey et al.[55]
Neurologic effects such as ataxia and aphasia	Brudno and Kochenderfer[57]
Cerebral edema	Gust et al.[58]
B-cell maturation antigen CAR: the cytokine release syndrome	Riches et al.[59]
Mesothelin CAR: anaphylaxis (antibody to murine single-chain variable fragments)	Maus et al.[60]
Carbonic anhydrase IX CAR: cholangitis (on-target)	Lamers et al.[61]
HER2/neu CAR: lethal cytokine release syndrome	Morgan et al.[62]
Carcinoembryonic antigen–related cell-adhesion molecule 5 (CEACAM5) CAR: hemorrhagic colitis (on-target)	Thistlethwaite et al.[63]

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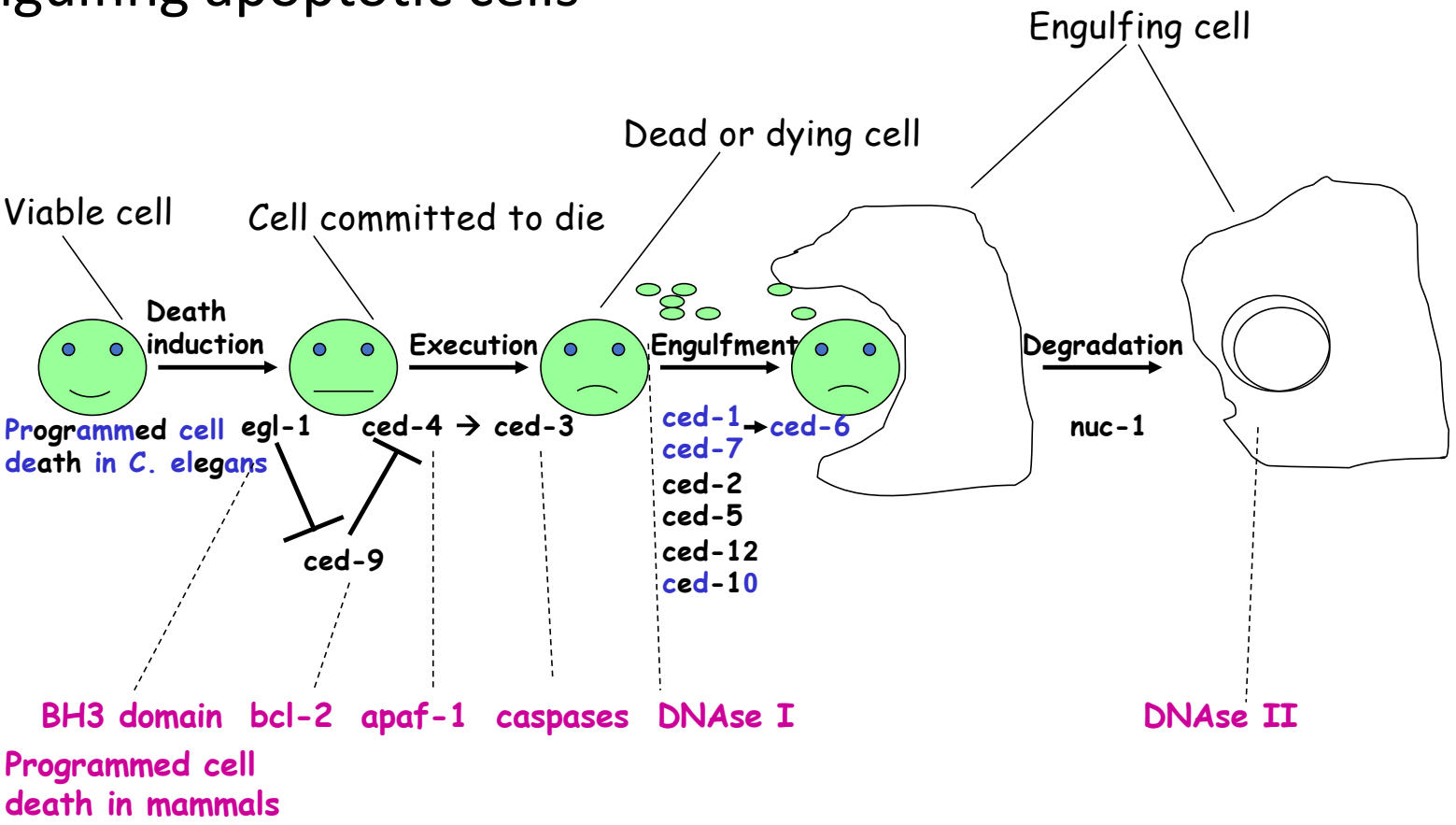
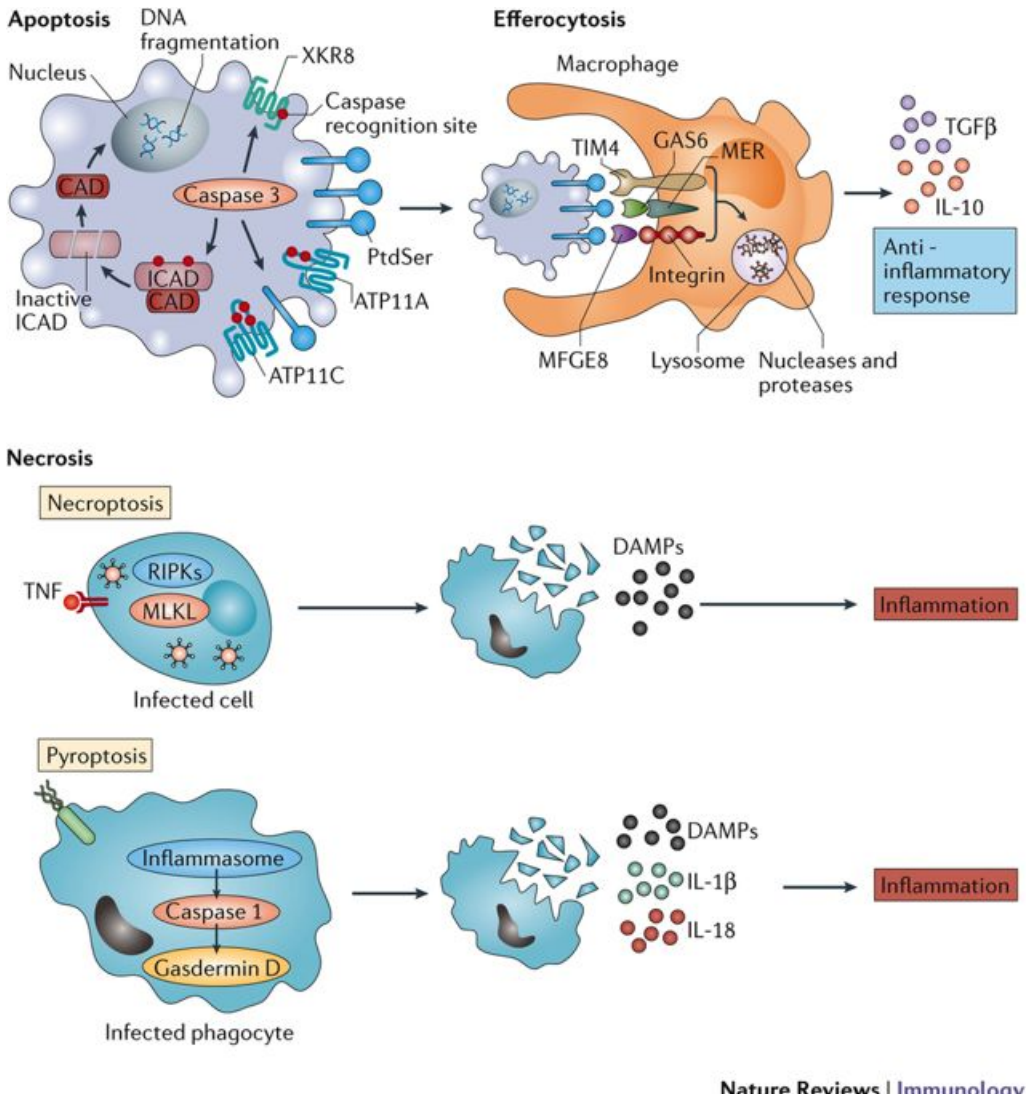
Engulfing apoptotic cells





S Nagata et al.

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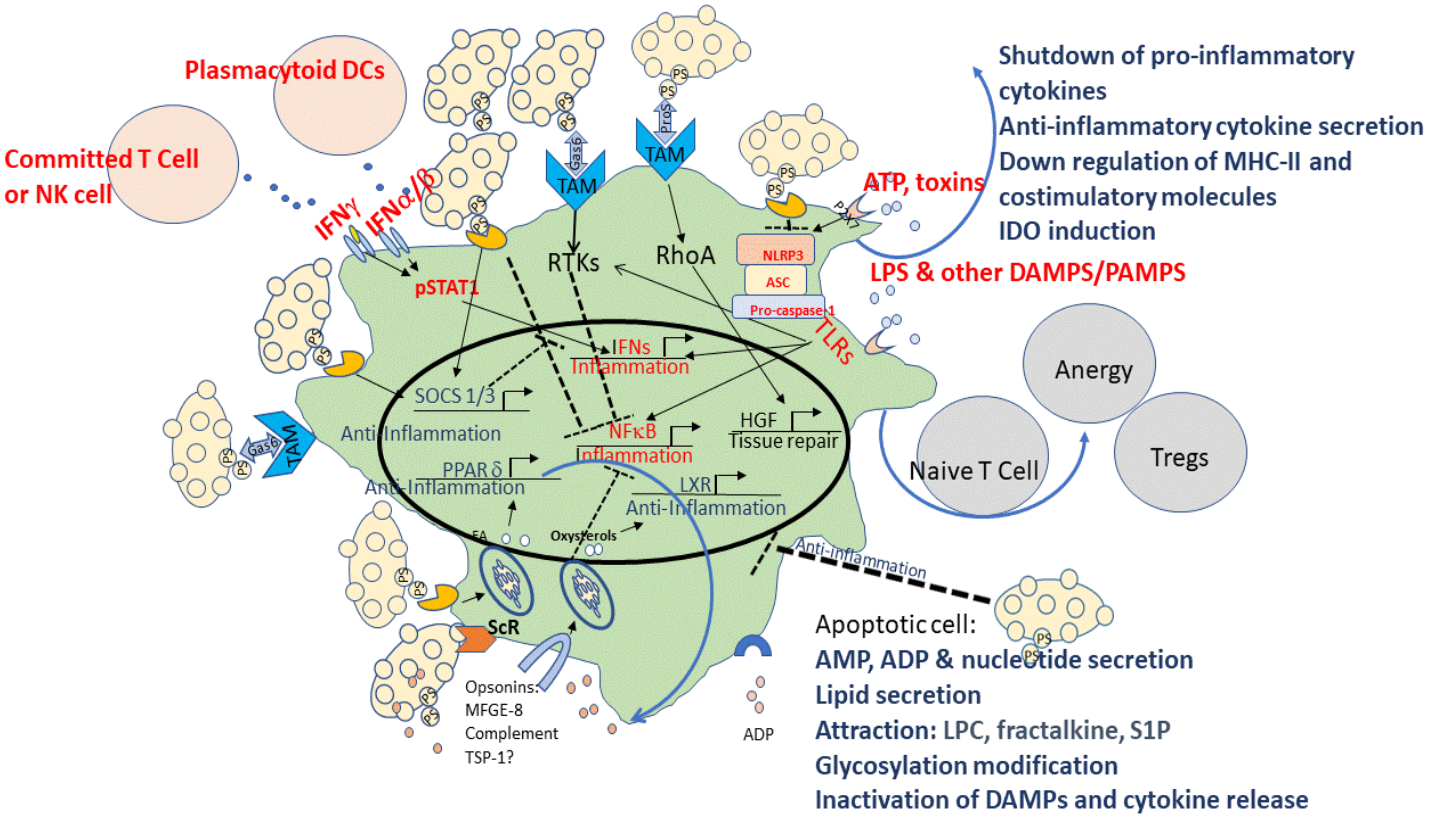
Apoptotic cells: Mechanisms of Immune modulation

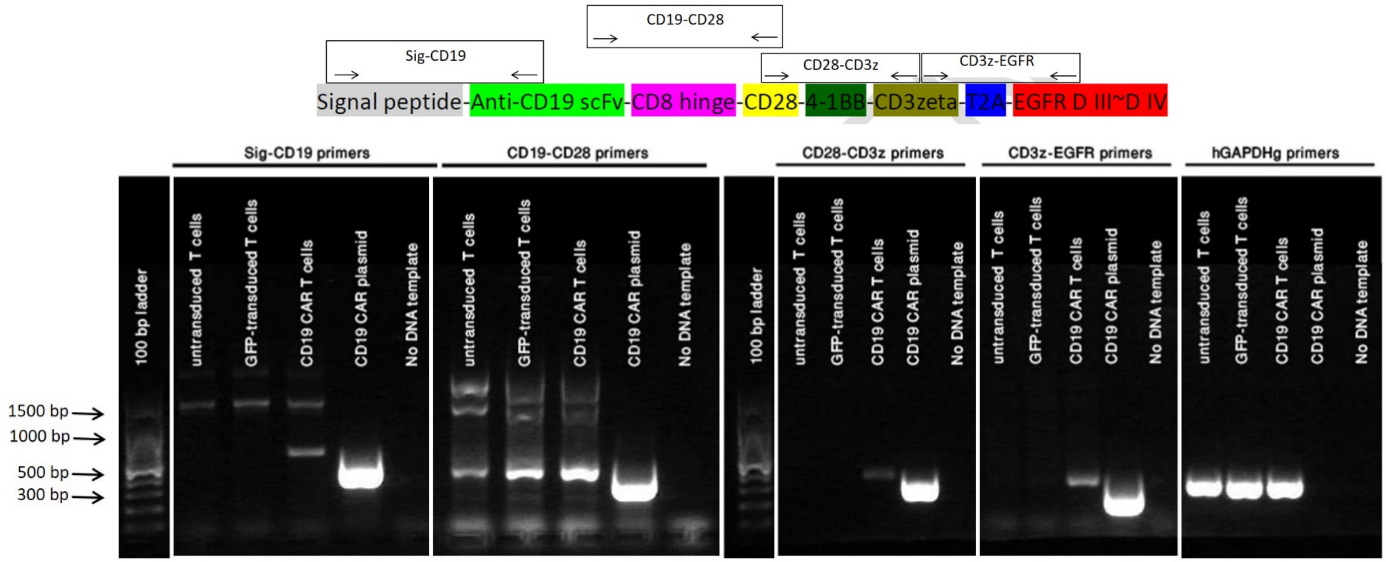


From Trahtemberg and Mevorach, Frontiers in Immunology 2017

METHODS

- SCID-Bg mice were injected intra-peritoneally with human HeLa-CD19-luciferase cells, apoptotic cells or vehicle, and CD19-CAR T cells or mock T cells.
- HeLa-CD19 was stably transduced with pLenti-PGK-V5-Luc-Neo and CAR was prepared using 3rd generation CD19-CAR plasmids.
- Luminex was used for measuring cytokine/chemokines levels
- Flow-cytometry and single cell analysis were used to characterize the macrophages.

Methods: Third generation CAR T anti CD19



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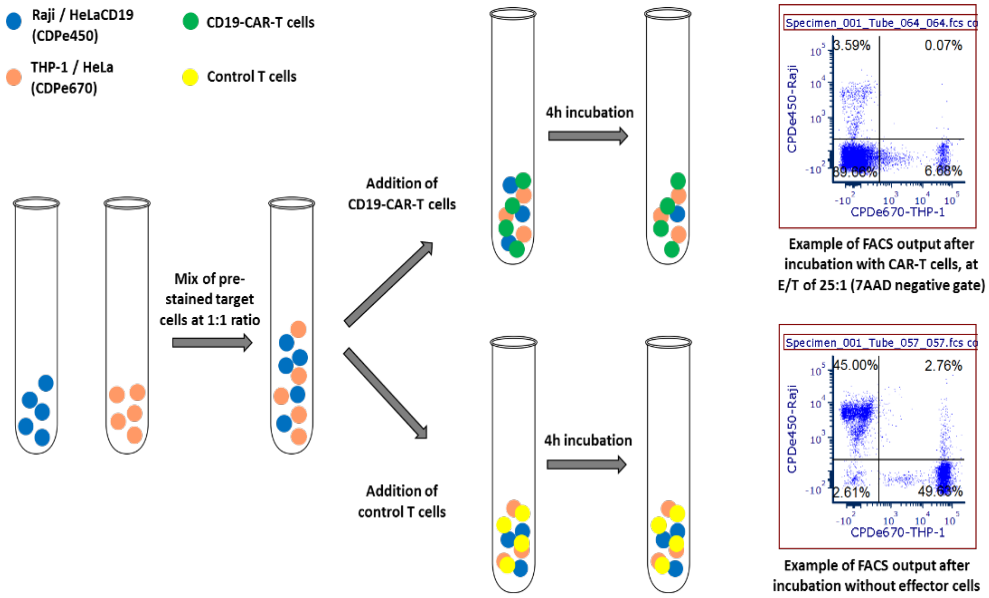


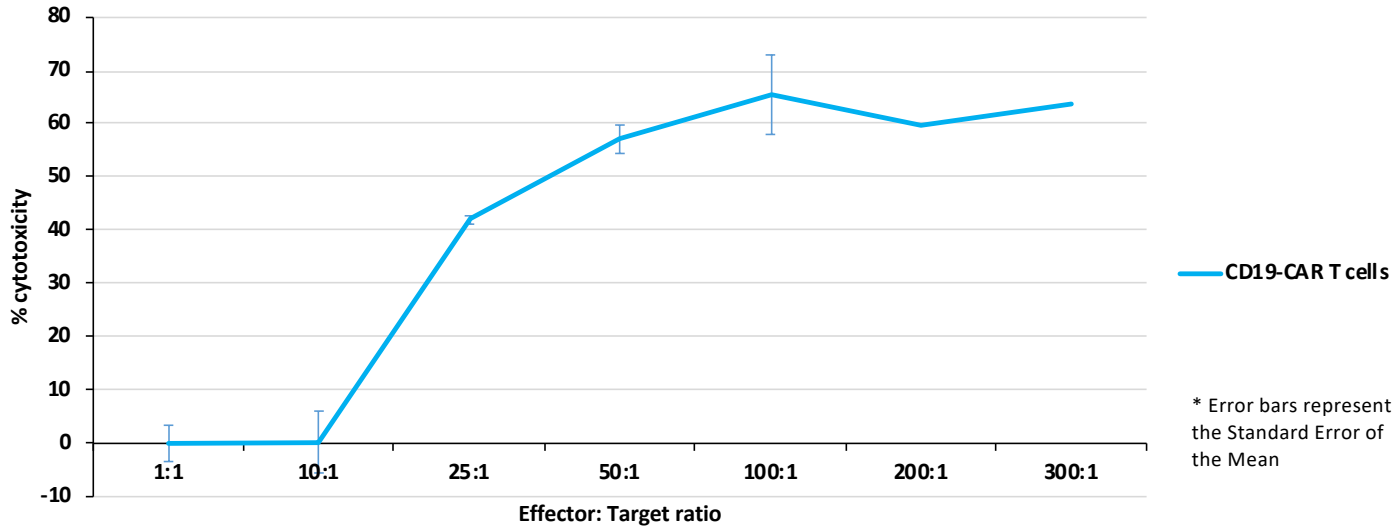
Example of FACS output after incubation with CAR-T cells, at E/T of 25:1 (7AAD negative gate)

Example of FACS output after incubation without effector cells (7AAD negative gate)

Kochenderfer J. et al. J Immunother 2009, 32 (7):689-702

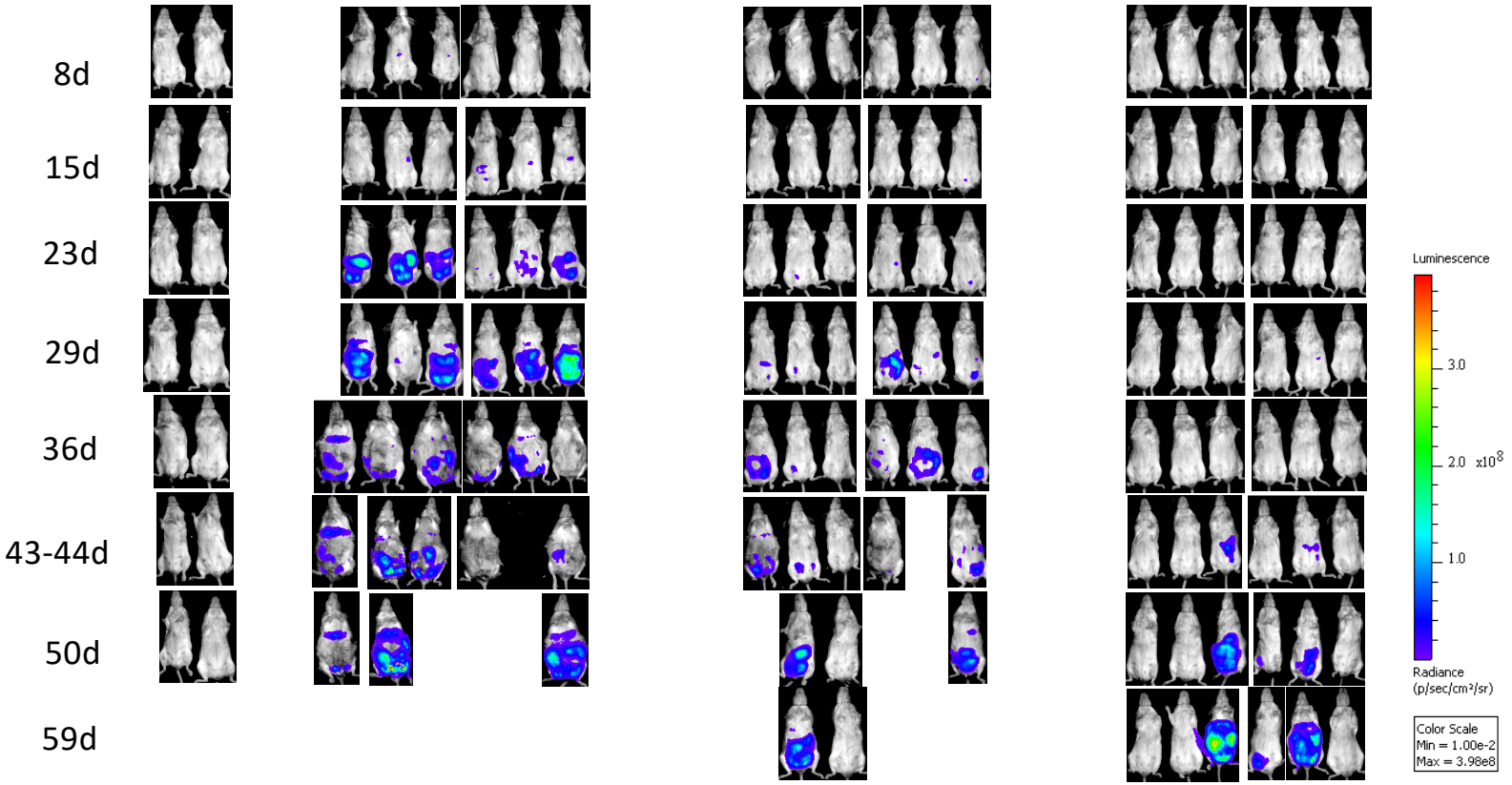
% cytotoxicity of CAR (day 14 post-infection)

according to Kochenderfer J. et al. J Immunother 2009, 32 (7):689-702

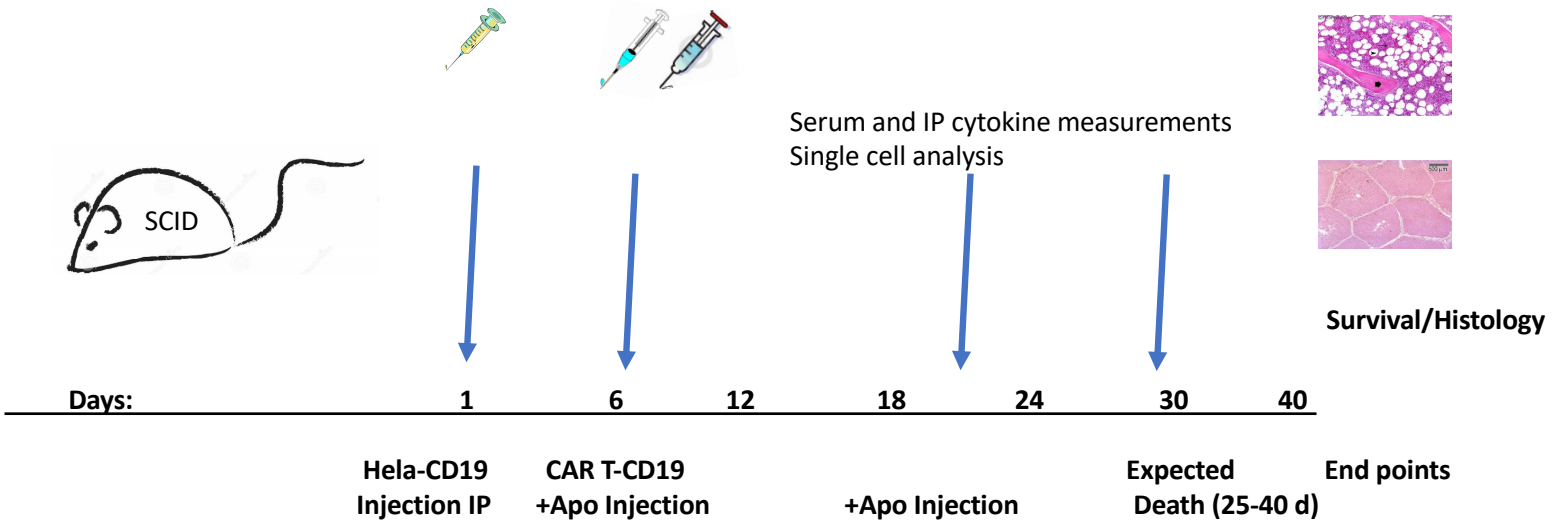


* Error bars represent the Standard Error of the Mean

METHODS: SCID WITH HUMAN ABDOMINAL HELA-LUCIFERASE-CD19

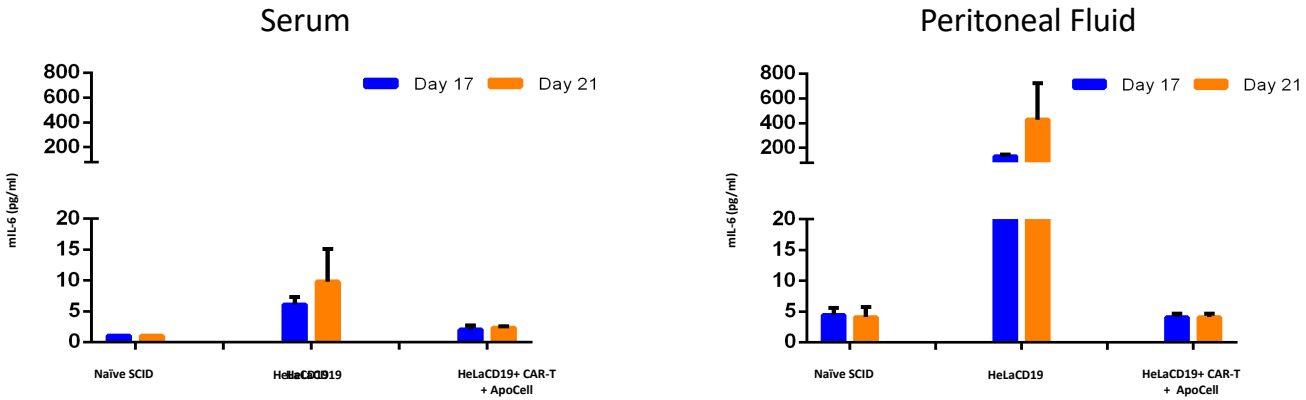


Experiment Scheme



RESULTS

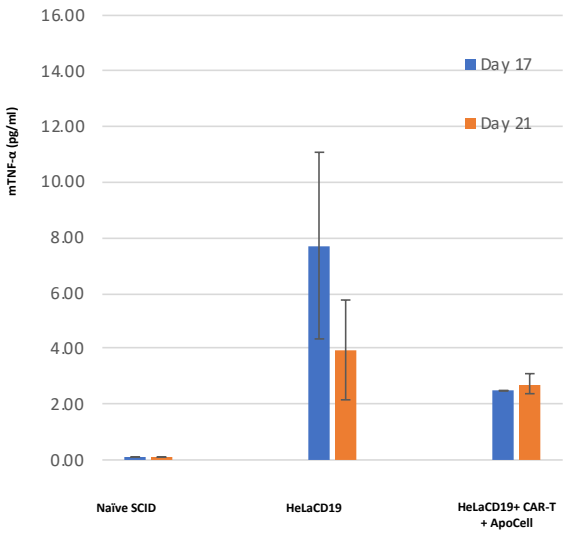
Mouse IL-6



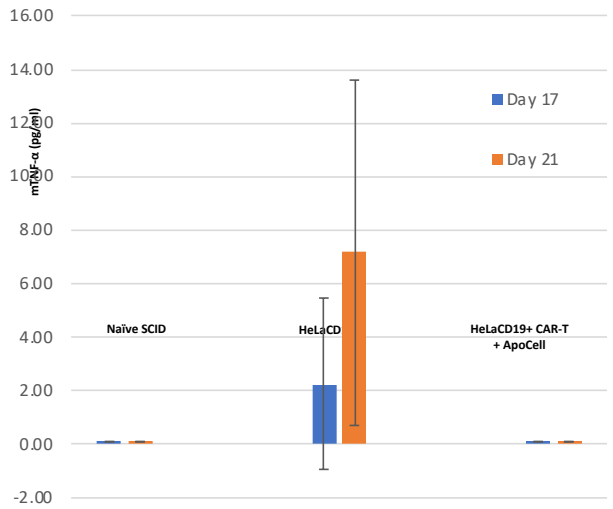
RESULTS

Mouse TNF-α

Serum

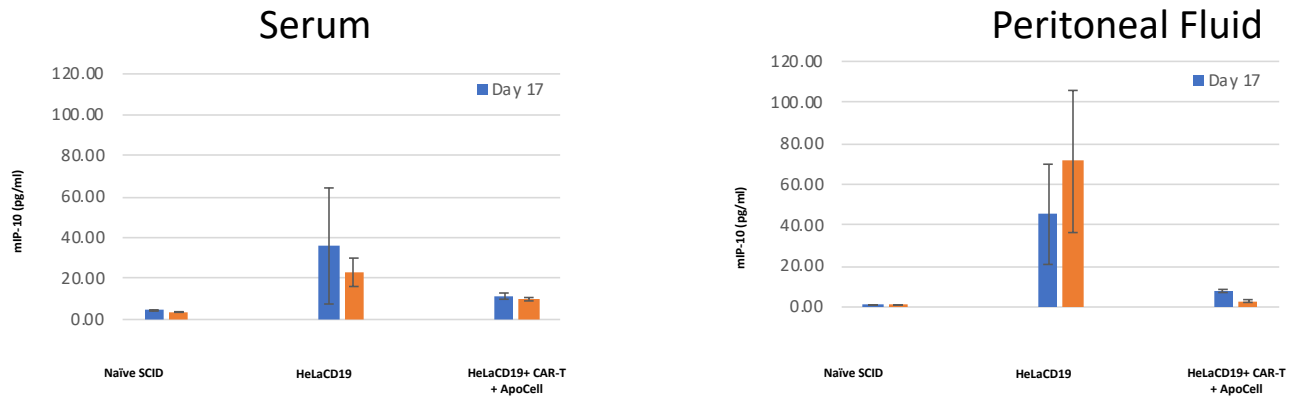


Peritoneal Fluid



RESULTS

Mouse IP-10



RESULTS: CAR-T SIGNIFICANTLY AMELEIORATES SURVIVAL OF SCID WITH HUMAN ABDOMINAL HELA

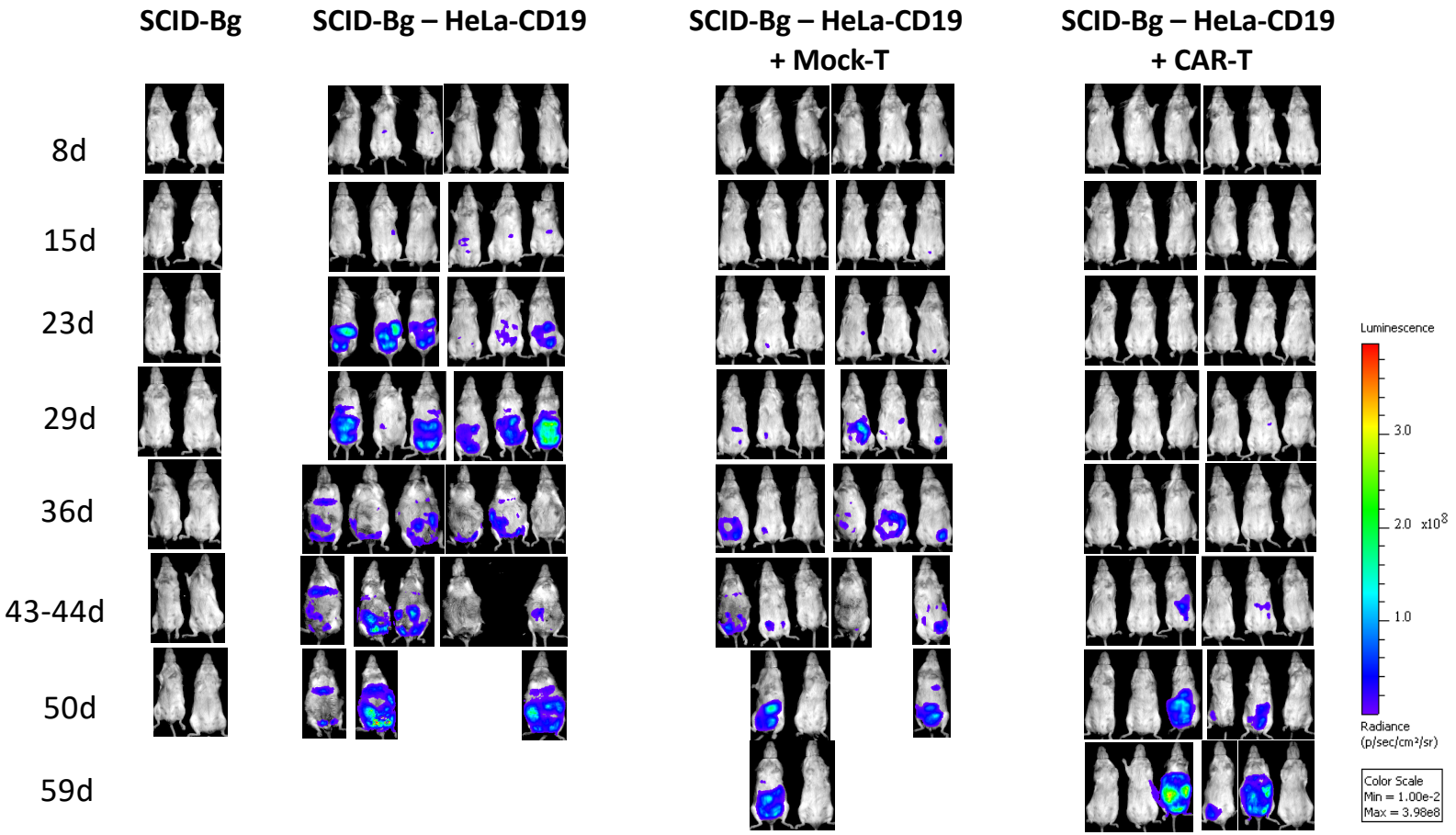


Figure 1B.

RESULTS: CAR-T SIGNIFICANTLY AMELEIORATES SURVIVAL OF SCID WITH HUMAN ABDOMINAL HELA

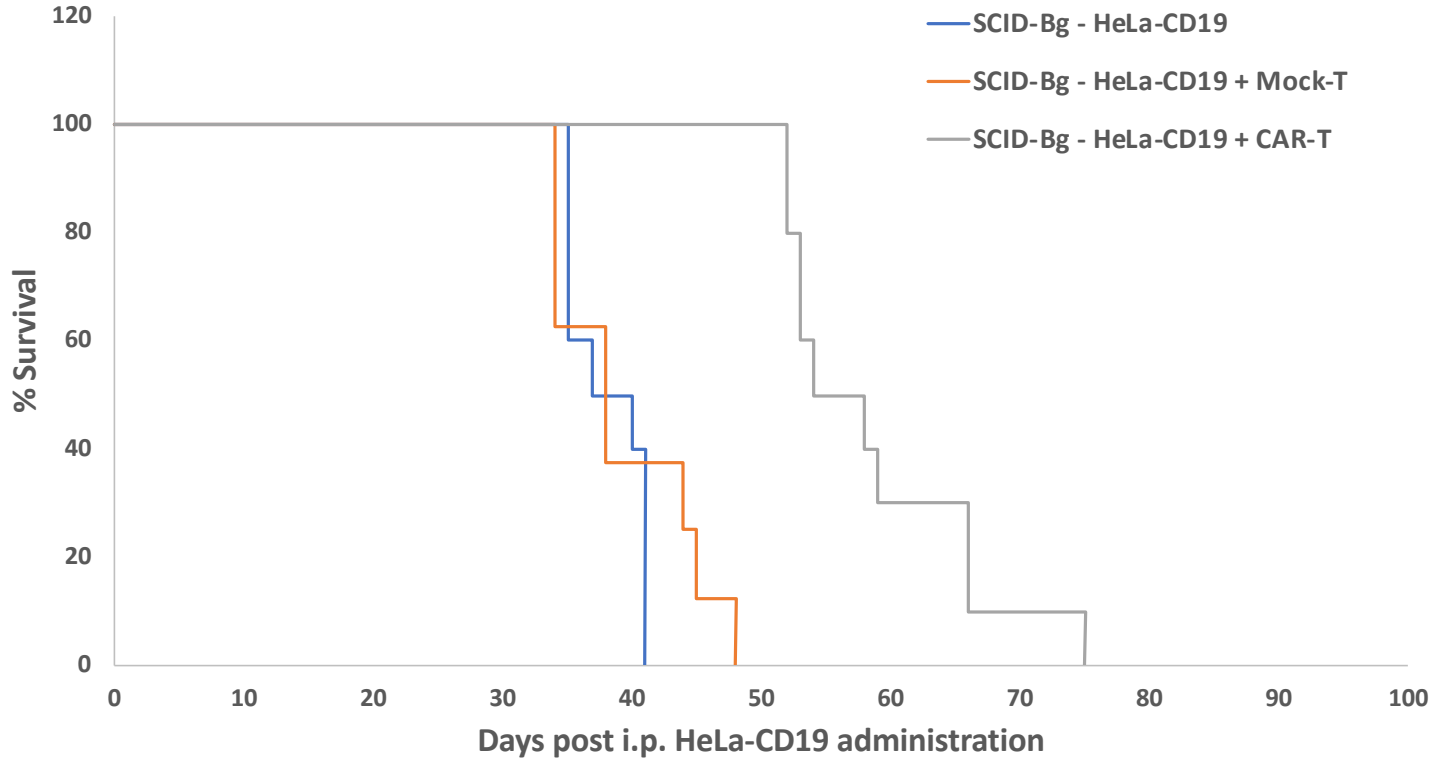


Figure 1A.

Apoptotic cells (Allocetra-OTS) dramatically ameliorate CAR T anti cancer function

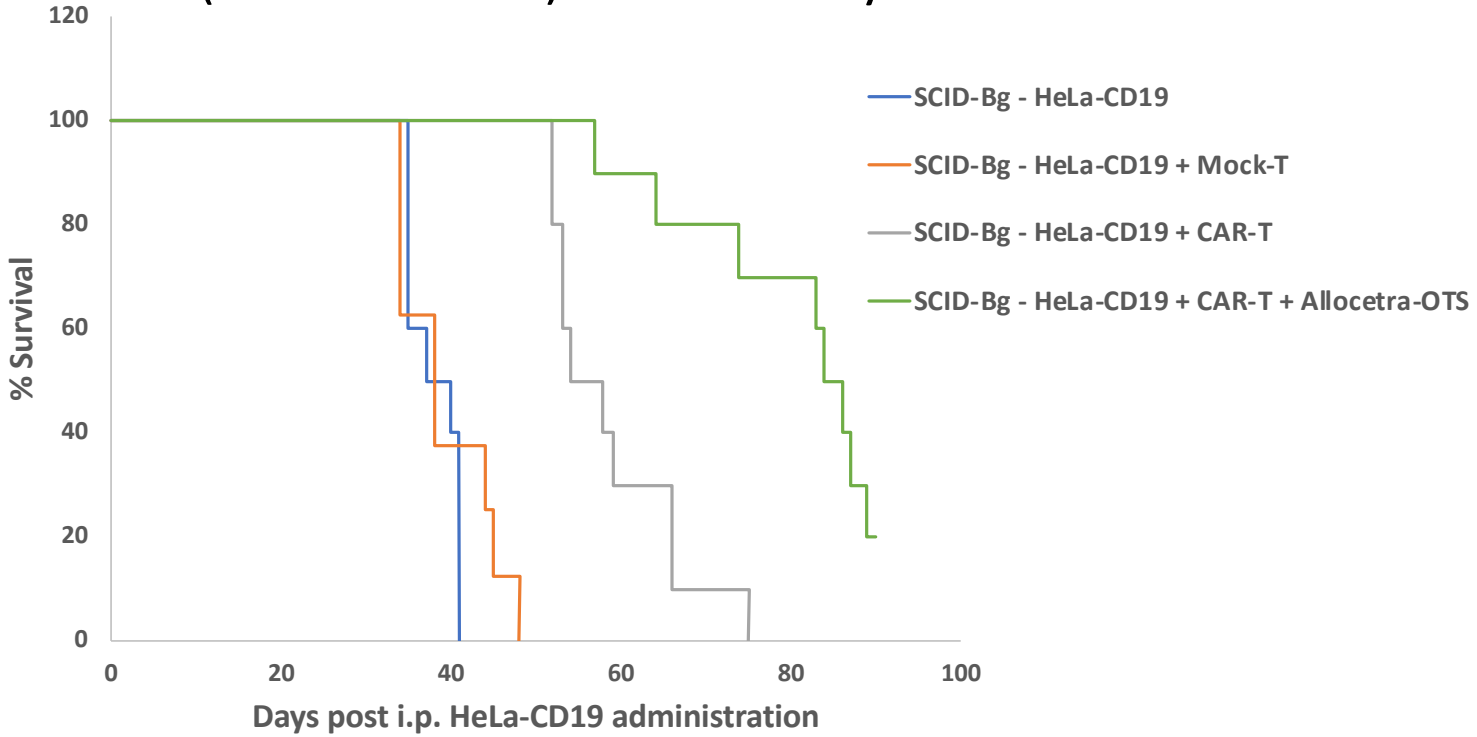
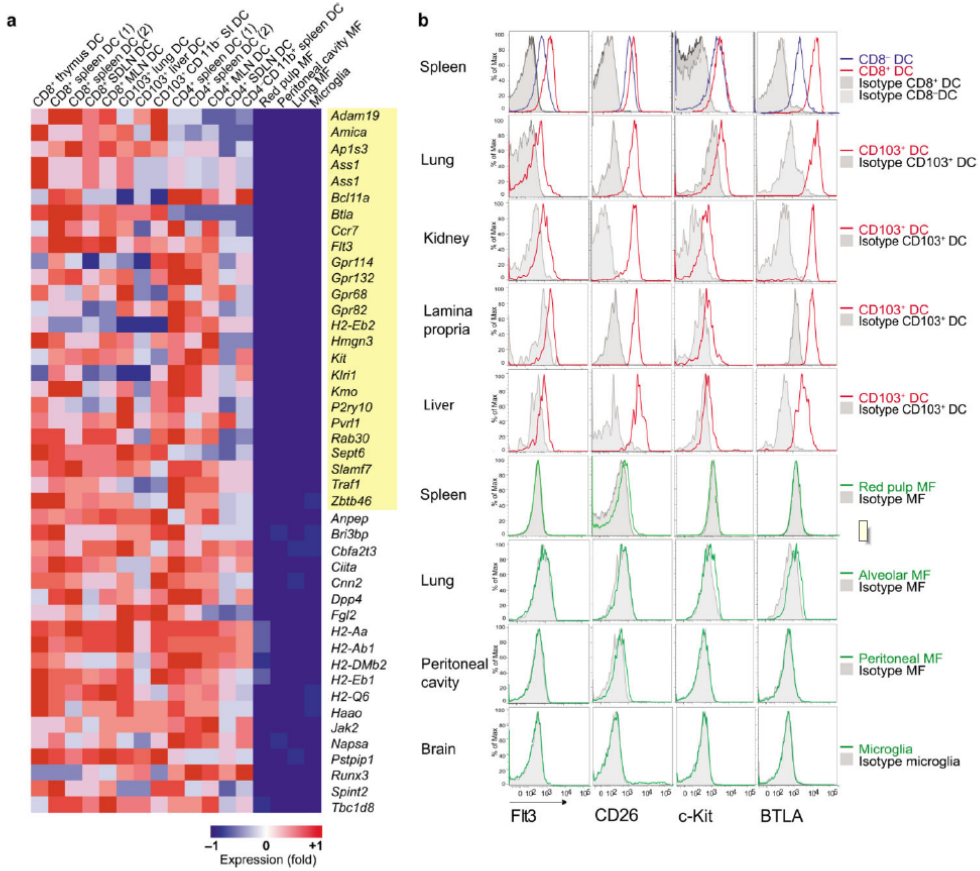


Figure 4A

Robust results from treatment of hematological malignancies with CAR-T were not replicated to date in solid tumors

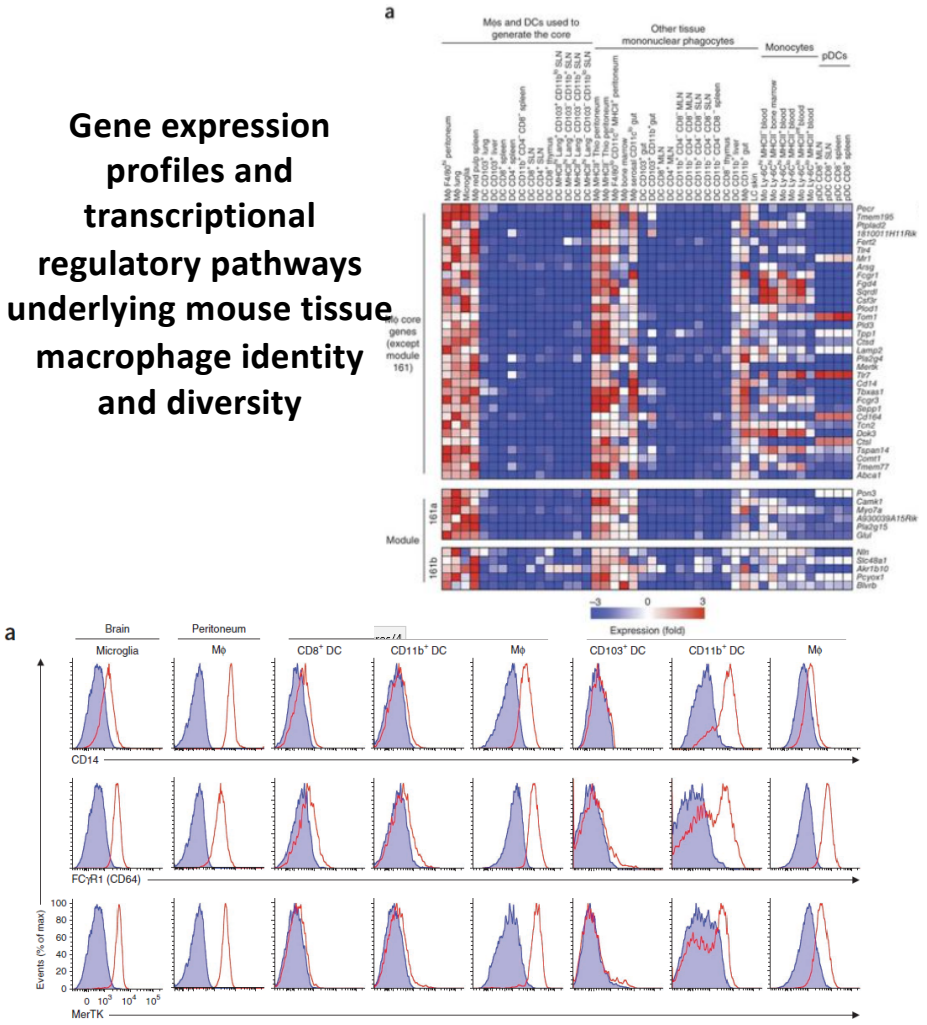
- However, despite their great results in hematological malignancies, no similar efficacy was shown in solid tumors Carl H. June and Michel Sadelain. Chimeric Antigen Receptor Therapy. N Engl J Med 2018.
- The likely reasons for their failure include lack of adequate antigens, poor trafficking, CAR-T exhaustion, and a hostile tumor microenvironment. Martinez et al. 2019.
- As a consequence, the major methods for immunotherapy in solid tumors involve T cell checkpoint blocking and stimulating antibodies Rotte et al. 2018.

Deciphering the transcriptional network of the DC/Macrophages/Monocyte lineage



Jennifer C Miller et al., *Nature Immunology* (2012)

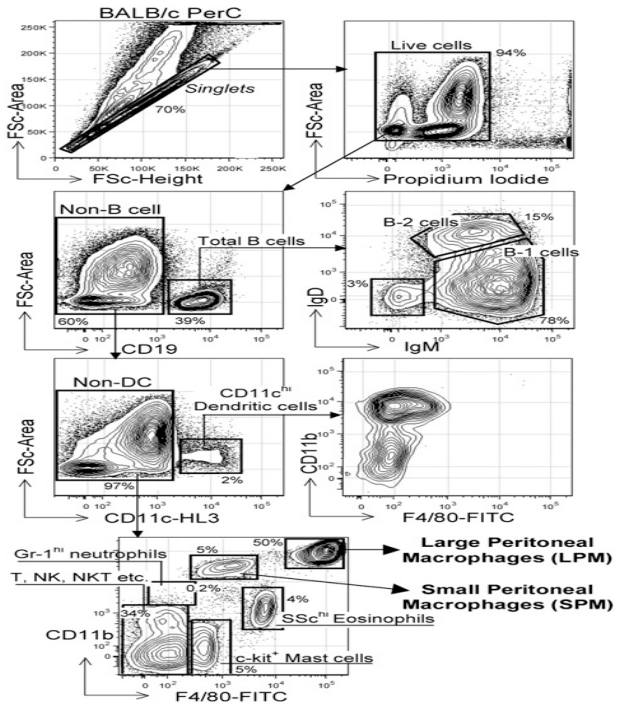
Gene expression profiles and transcriptional regulatory pathways underlying mouse tissue macrophage identity and diversity



Emmanuel L. Gautier et al., *Nature Immunology* (2012)

Characterization of Peritoneal Macrophages

Ghosn et al. (2010) Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. PNAS



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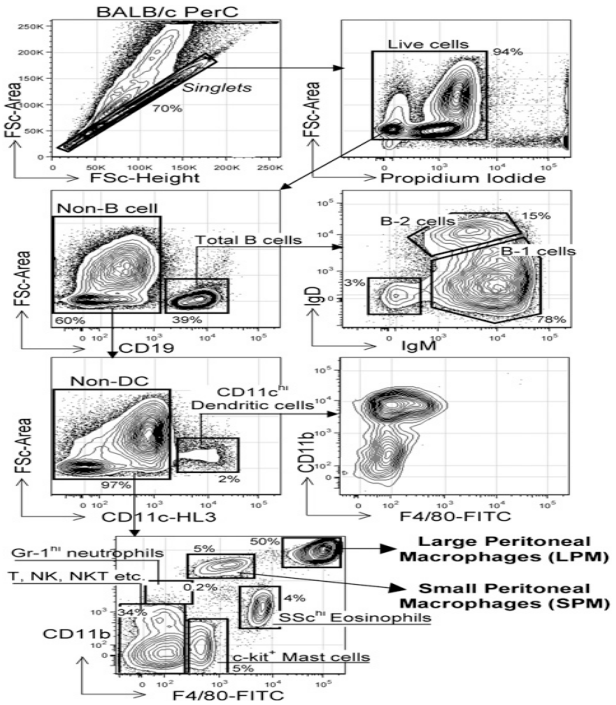
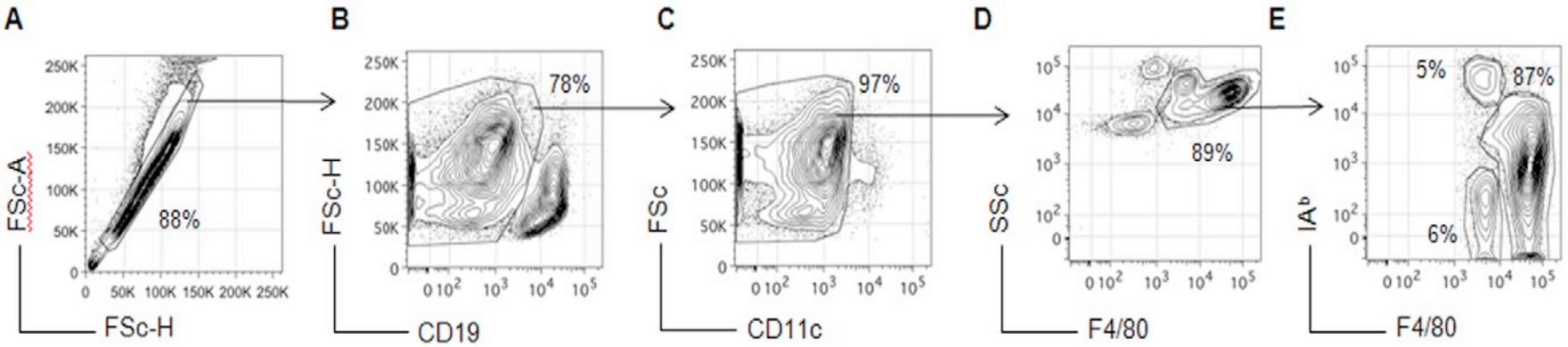
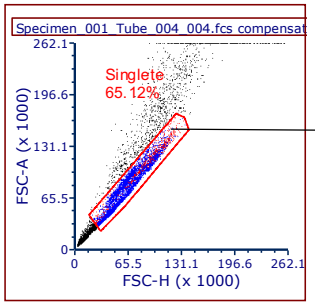


Large peritoneal macrophages (LPM)
Small peritoneal macrophages (SPM)
Granulocytes
Dendritic cells
Lymphocytes

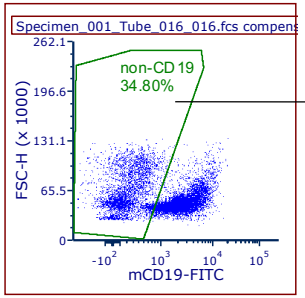


Cassado et al. (2011) PLoS One.

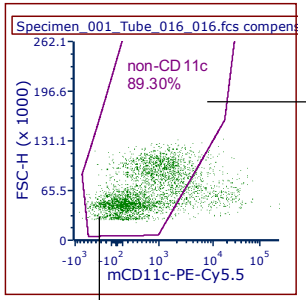
Peritoneal Macrophages Characterization – Analysis Hierarchy



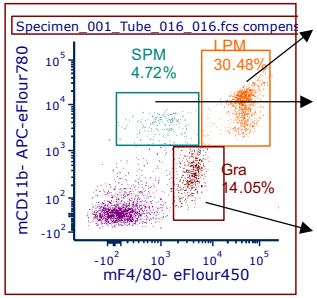
Doublet discrimination



Non B cells gating



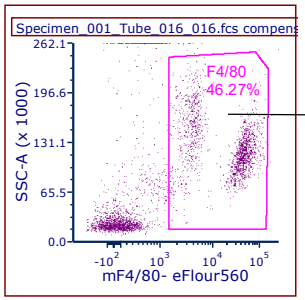
Non Dendritic cells gating



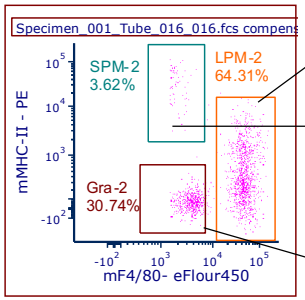
Large peritoneal macrophages (LPM). F4/80^{high}CD11b^{pos}

Small peritoneal macrophages (SPM). F4/80^{low}CD11b^{pos}

Granulocytes (Gra). F4/80^{low}CD11b^{neg}



Macrophages gating



Large peritoneal macrophages (LPM). F4/80^{high}MHC-II^{neg}

Small peritoneal macrophages (SPM). F4/80^{low}MHC-II^{pos}

Granulocytes (Gra). F4/80^{low}MHC-II^{neg}

Single cell analysis: Macrophages changes during tumor progression

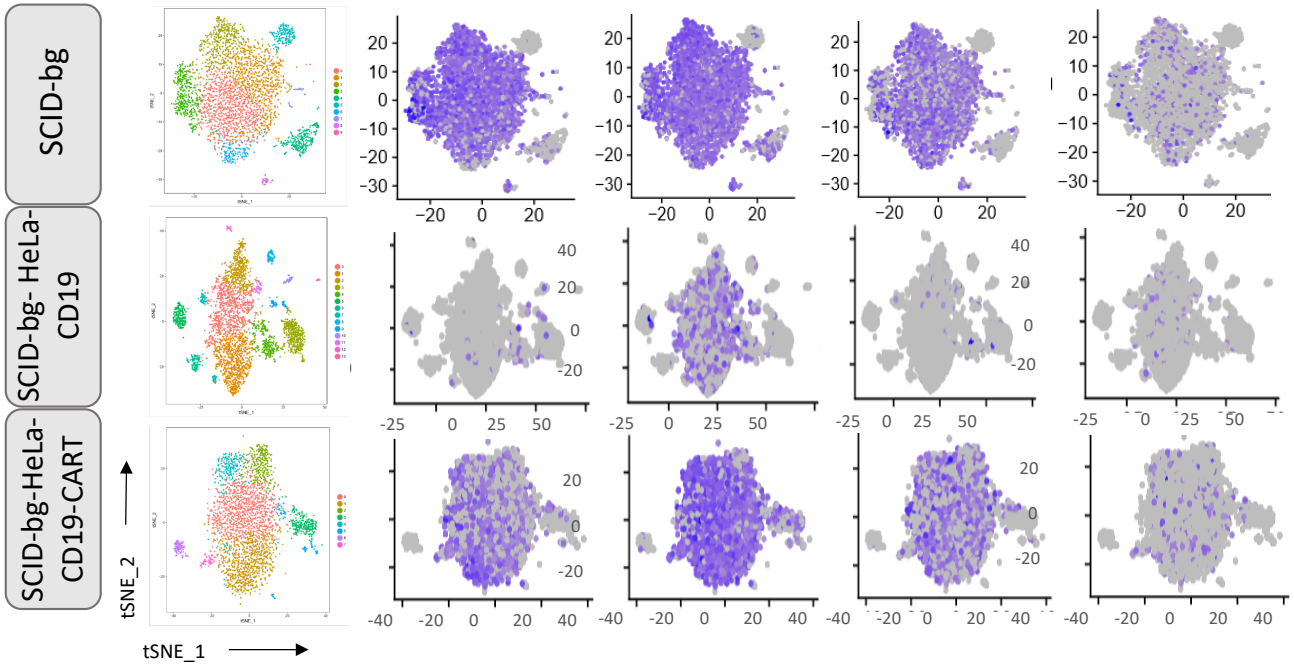
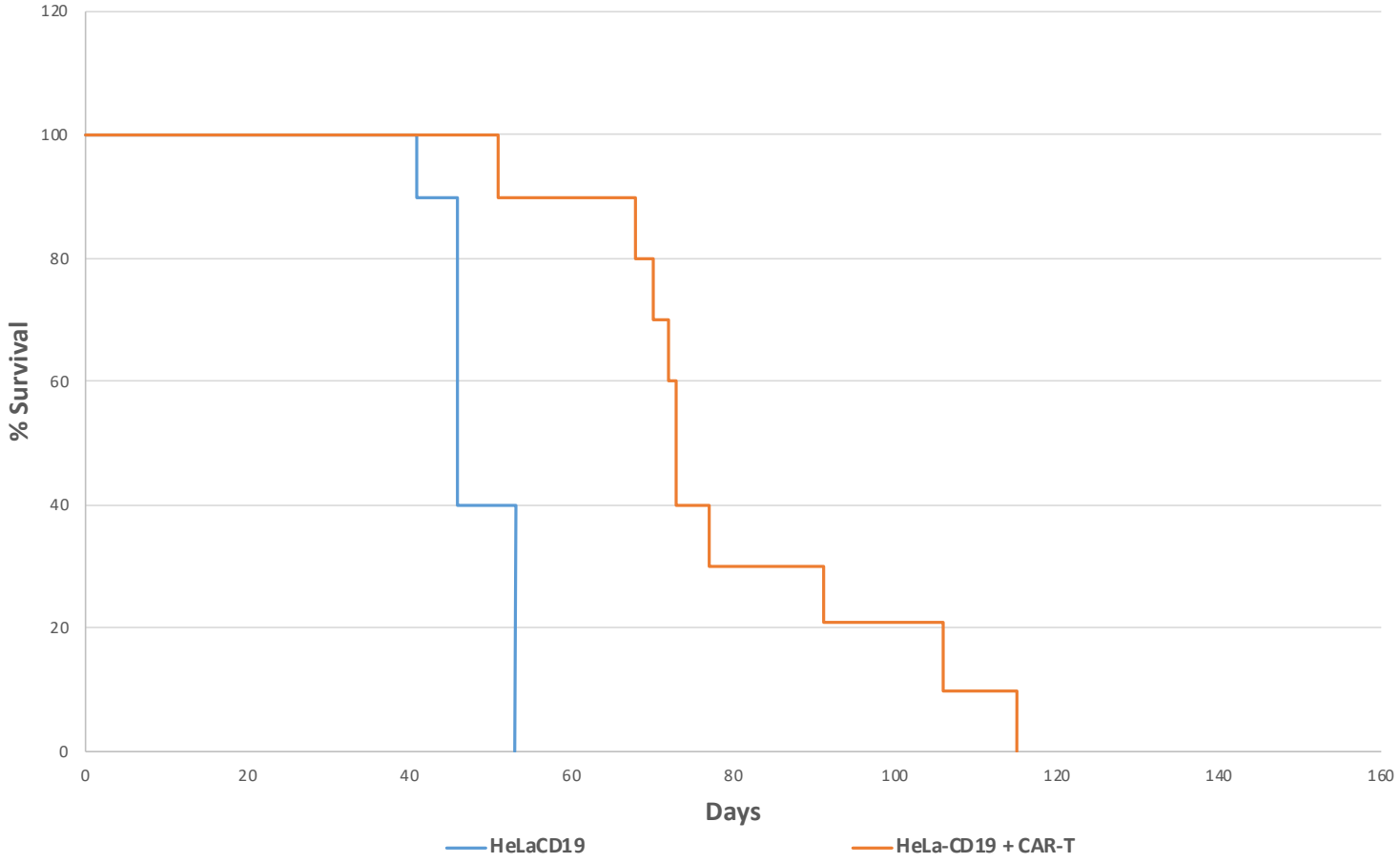
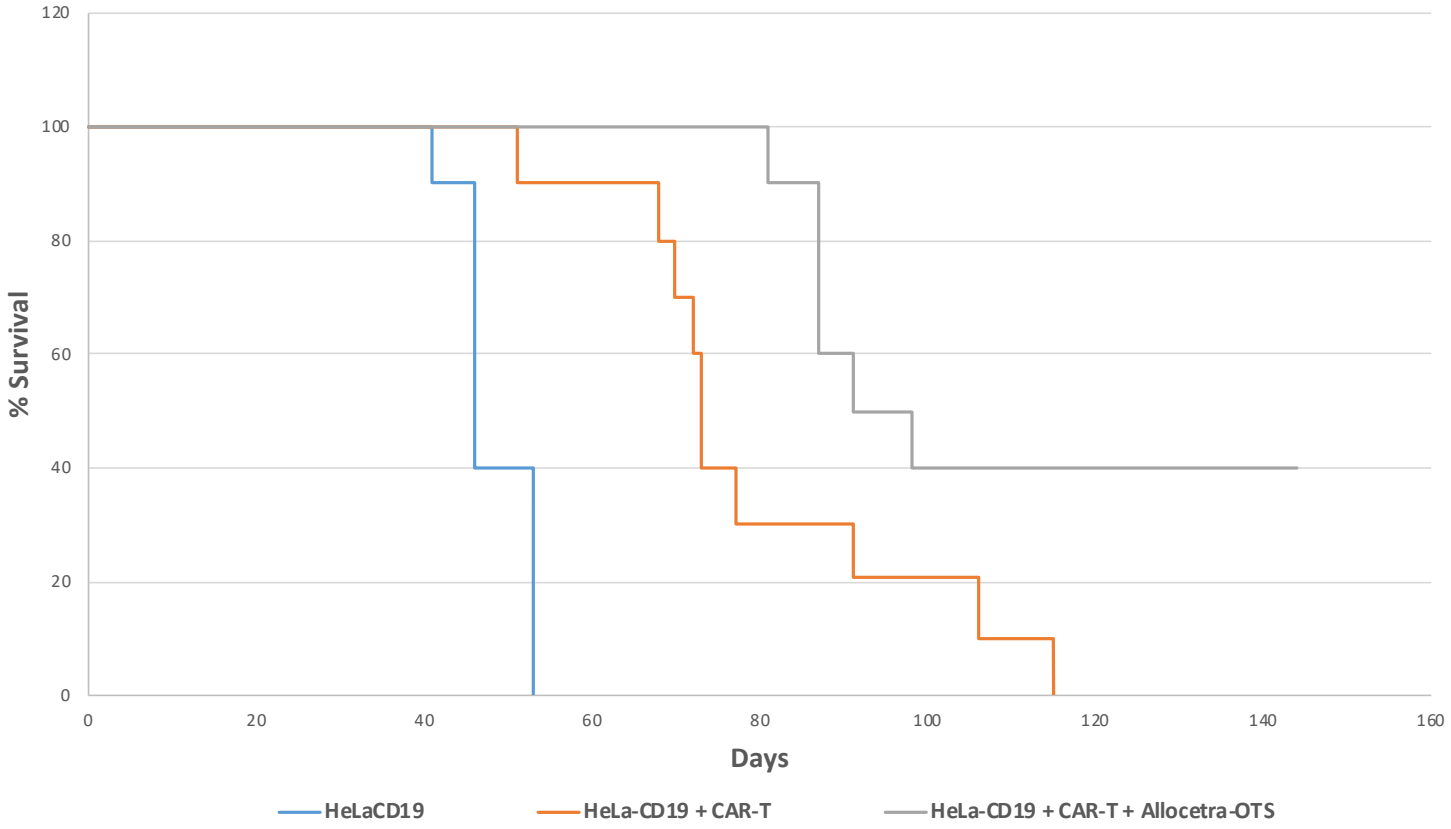


Figure 2A

SCID-Bg mice were injected intra-peritoneally with
human HeLa-CD19-luciferase cells, followed by CD19-CAR T cells.

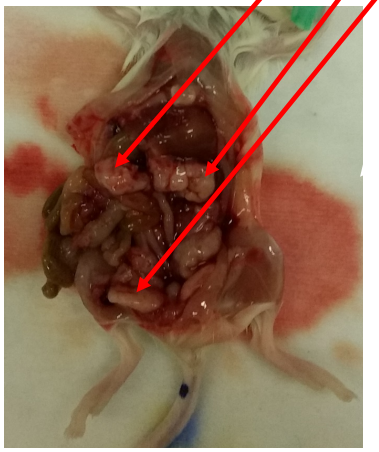


SCID-Bg mice were injected intra-peritoneally with human HeLa-CD19-luciferase cells, followed by CD19-CAR T cells, with or without apoptotic cells (Allocetra-OTS)

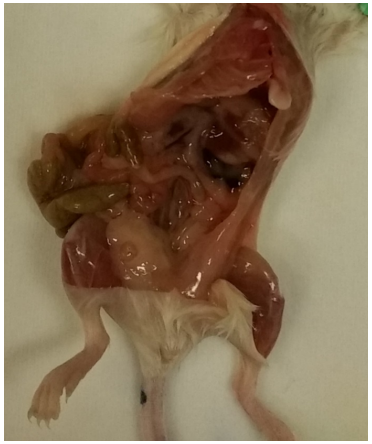


Tumor

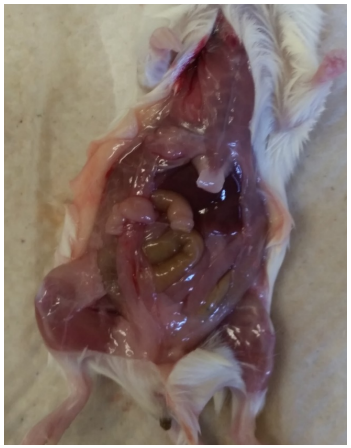
HeLa-CD19

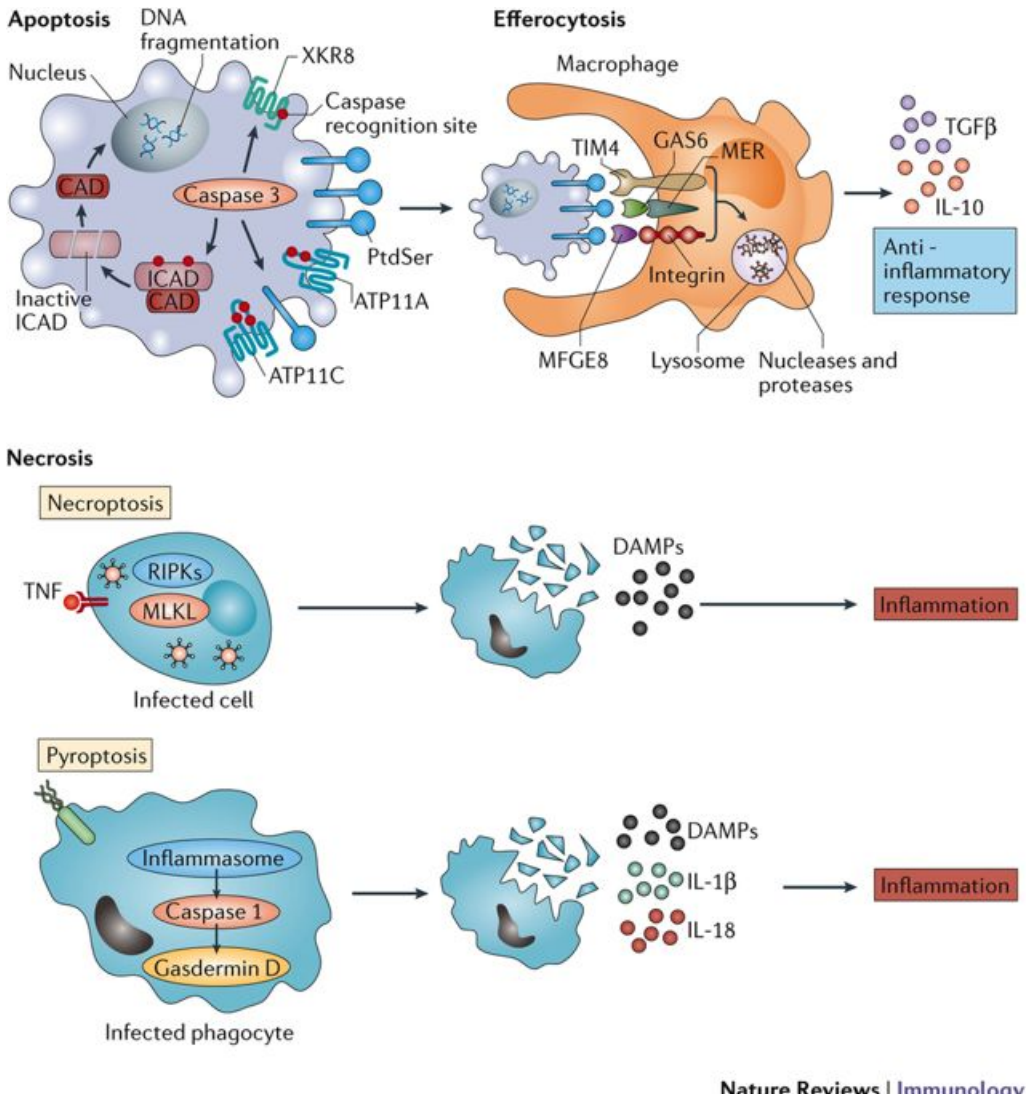


HeLa-CD19
+ CAR-T



HeLa-CD19
+ CAR-T +
ApoCell





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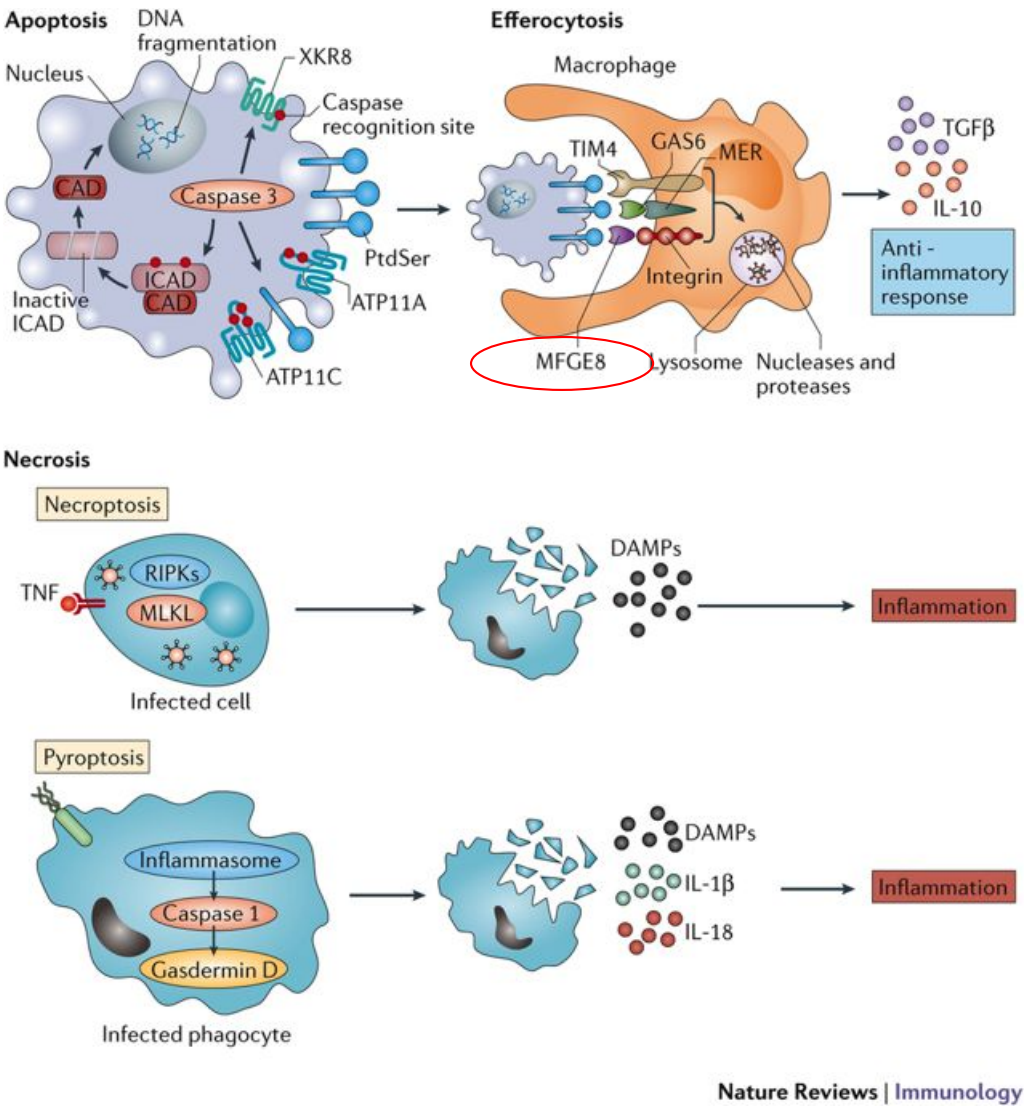

Milk fat globule-EGF-factor 8 (MFG-E8) was originally identified as a component of milk fat globules and is also produced and secreted by activated macrophages and specifically binds to PS exposed on apoptotic cells via COOH-terminal factor VIII homologous domains.

When MFG-E8 is engaged by apoptotic cells, it binds to $\alpha_v\beta_3$ integrin expressed in phagocytes via a NH_2-terminal EGF-like domain, and promotes the phagocytosis of apoptotic cells.

MFG-E8 mutant protein, **D89E** carrying a mutation in the RGD sequence, masks PS on apoptotic cells and specifically avoids their clearance and interactions with macrophages.

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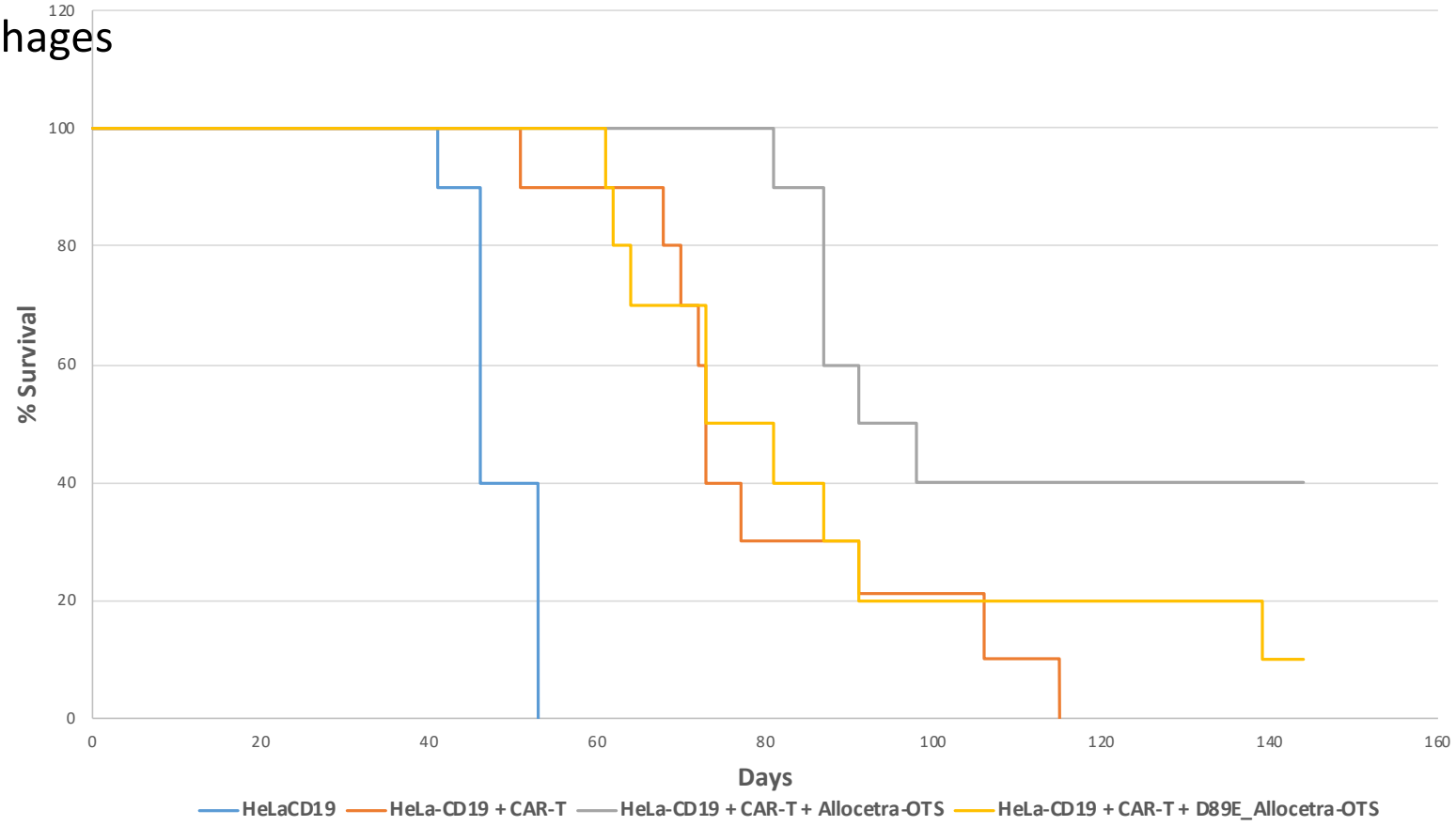
SCID-Bg mice were injected intra-peritoneally with human HeLa-CD19-luciferase cells, followed by CD19-CAR T cells, with or without apoptotic cells (Allocetra-OTS), or opsonized apoptotic cells (D89E_Allocetra-OTS) that avoids clearance of apoptotic cells by resident macrophages



Summary

- Mice survived 30±5 days, and mock treatment non significantly ameliorated their survival to 34±4 days.
- CAR T cell therapy significantly ($p<0.001$) ameliorated their survival to 55±11 days.
- Apoptotic cells injected during tumor progression were able to stabilize the presence of macrophages as confirmed by single cell and flow cytometry analysis and synergize with the anti-tumor CAR-T cell effect, resulting in significantly increased anti-tumor macrophage population and increased survival to 75±10 days ($p<0.01$).
- We are now analyzing at the level of single cell, the characterizations of macrophages during tumor progression and following apoptotic cell treatment.