3rd MEETING ON T-CELL AND NK-CELL BASED IMMUNOTHERAPIES FOR LYMPHOID MALIGNANCIES

Jean Lemoine, MD

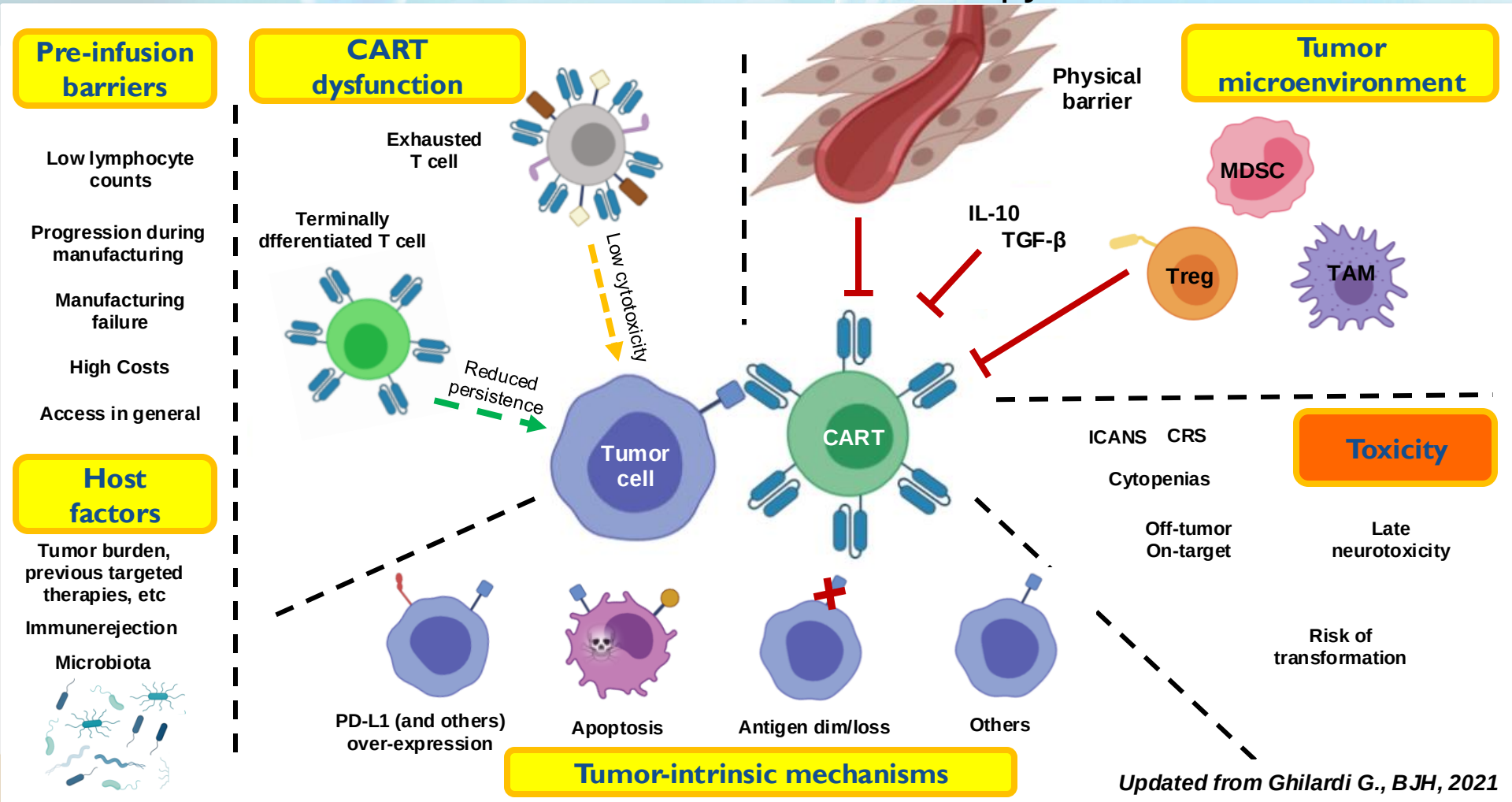
Mechanisms of resistance: laboratory evidence

Ruella lab, Center for Cellular Immunotherapies, University of Pennsylvania

BOLOGNA, ROYAL HOTEL CARLTON
September 13-14, 2024

Disclosures of Jean Lemoine

Causes of Failure of CART Immunotherapy in the Clinic

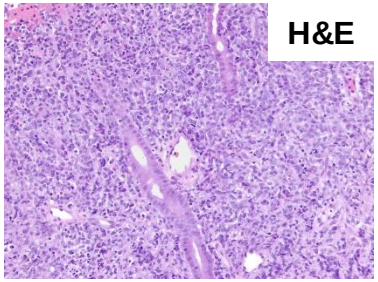


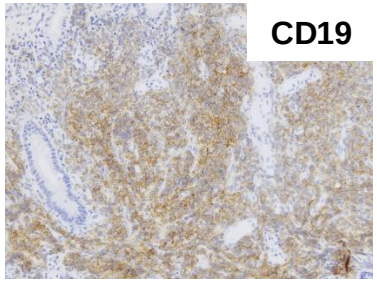
Strategies to overcome CART resistance

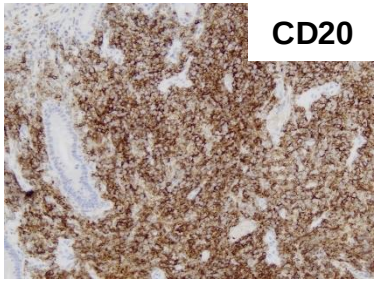
Mechanism of resistance	Potential strategies to overcome	References
Antigen loss or modulation		
Antigen heterogeneity		

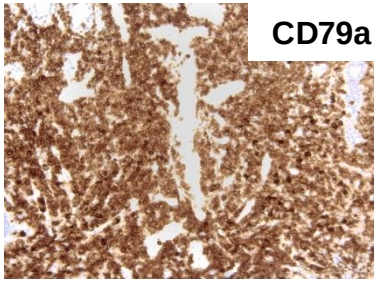
Antigen loss or modulation

Pt #1









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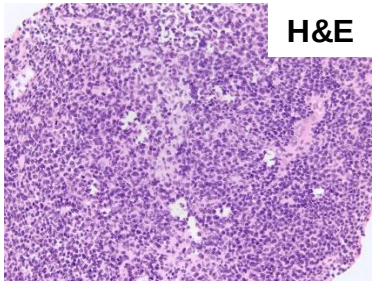
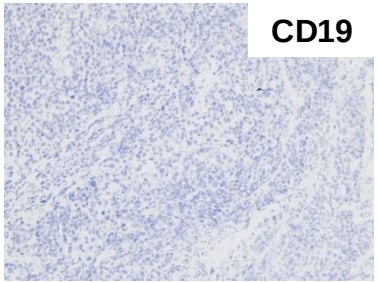
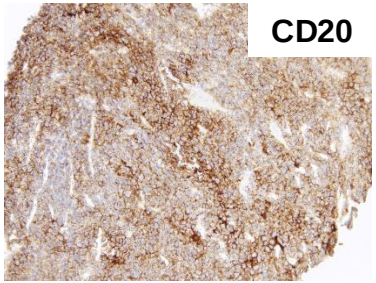
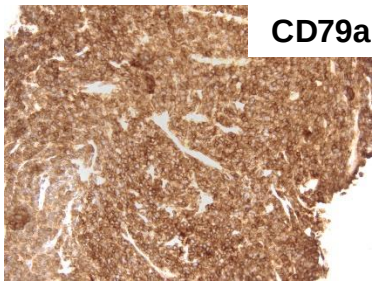


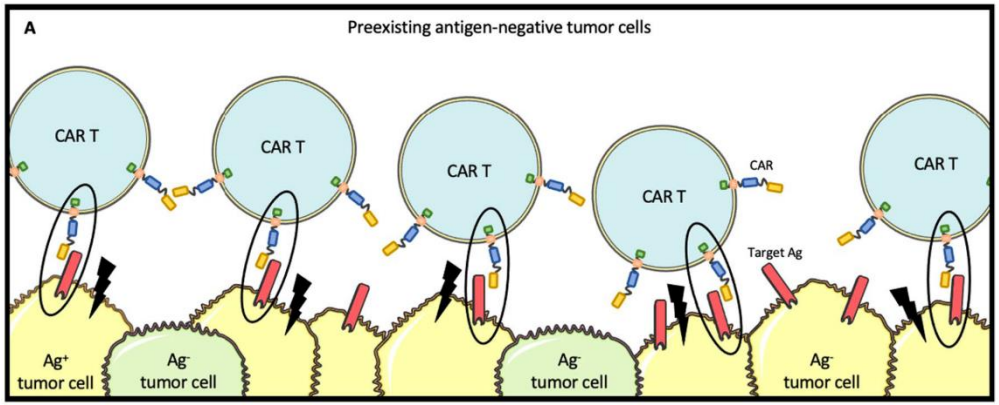






Siddhartha Bhattacharyya

Mechanisms of Antigen loss or modulation



Lemoine, J., Ruella, M. & Houot, *J Hematol Oncol,* 2021

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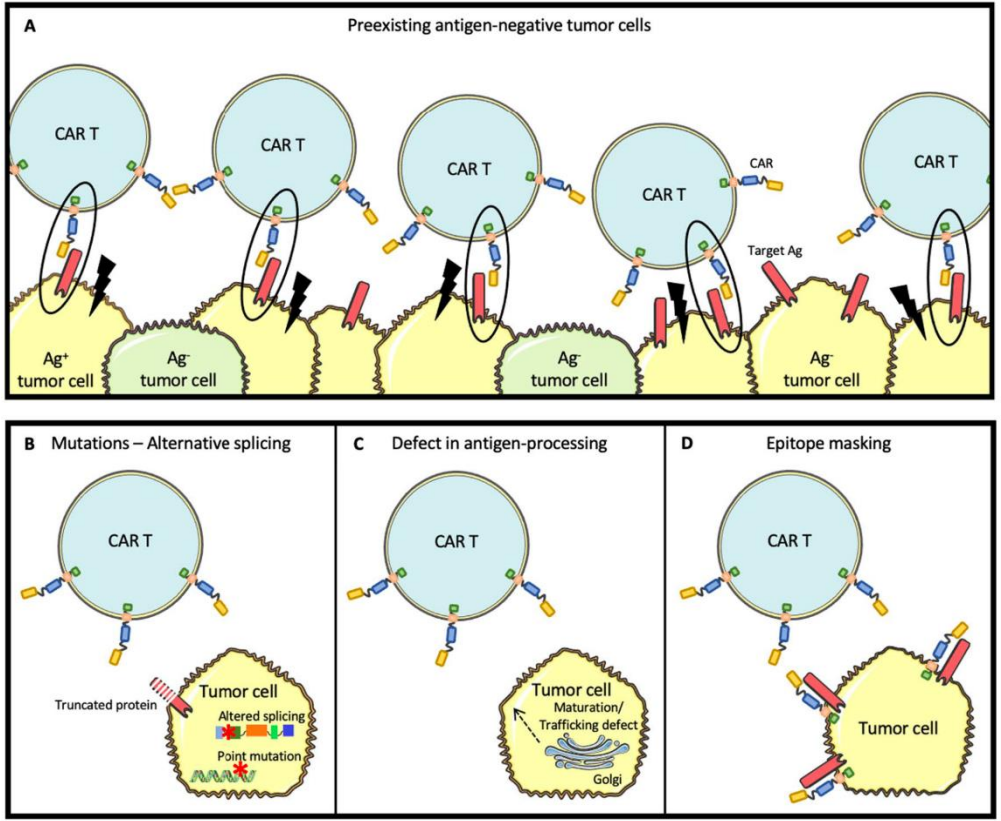

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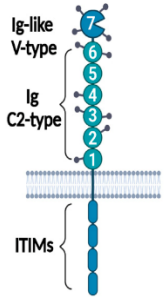
CD19 and CD22 targeting with CART

CD22 structure



Kokalaki, E. *et al. Mol Ther*, 2023

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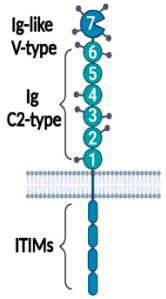

Sample Name	Immunisation	Extracellular Domain(s) of CD22 bound	Ka (1/Ms) x10^4	Kd (1/s) x10^{-4}	K_D (nM)
9F9-6	Wistar rats	5 – 6	20.00	2.01	1.00
7E1-2	Wistar rats	5 – 6	10.88	1.25	1.24
9A8-1	Wistar rats	5 – 6	9.57	1.73	1.90
9F8-2	Wistar rats	5 – 6	69.60	87.80	12.60
10C11-6	Wistar rats	5 – 6	12.80	46.70	36.50
9G11-2	Wistar rats	4	2.06	1.57	7.65
8E7-3	Wistar rats	4	2.89	2.56	8.85
7E3-5	Wistar rats	4	2.70	4.53	16.80
10C1-D9	Hyper-Immune mice	3	0.87	0.002	0.03
1G3-4	Wistar rats	3	79.02	3.85	0.52
5H4-9	Hyper-Immune mice	3	4.21	4.57	10.90
4D9-12	Hyper-Immune mice	3	14.60	11.40	8.99

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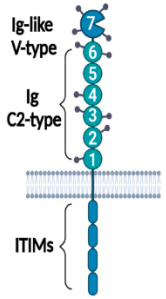

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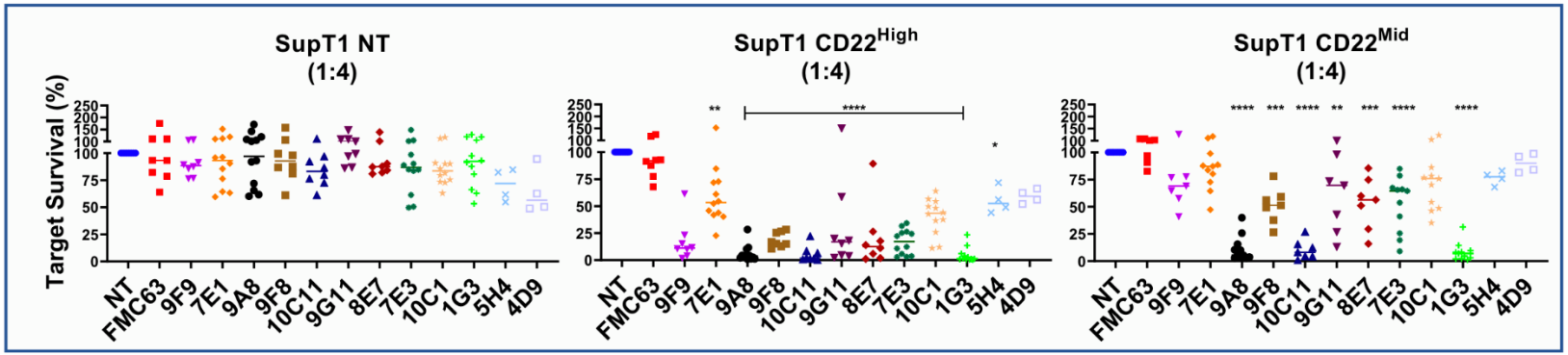

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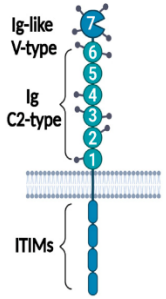

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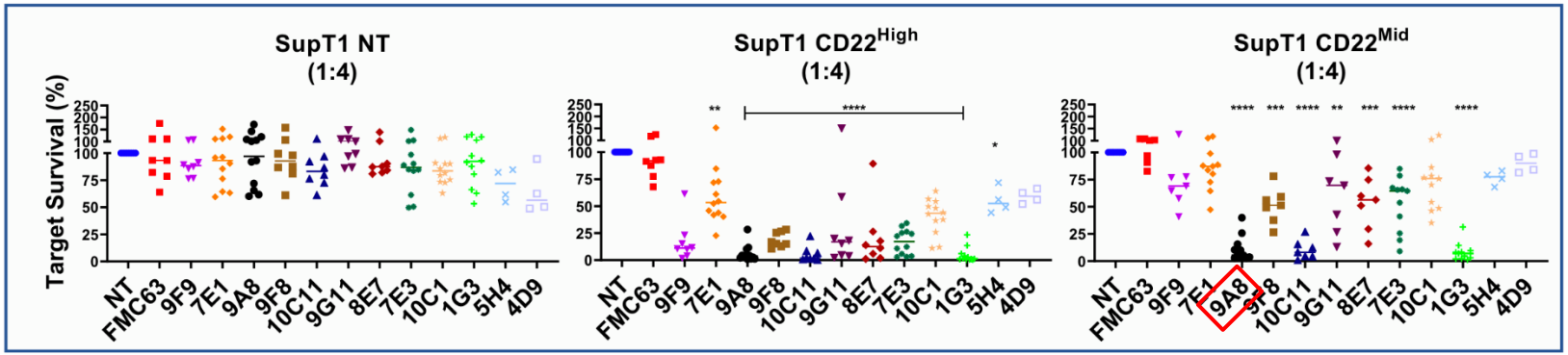

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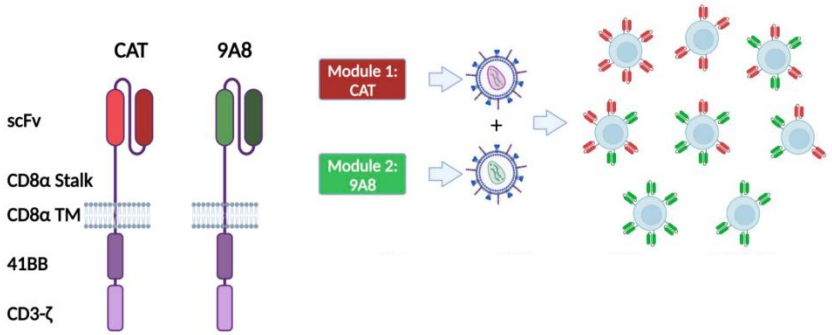
CD19 and CD22 targeting with CART



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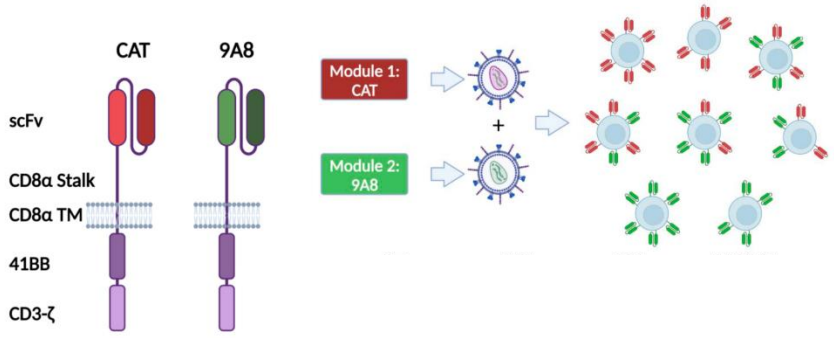

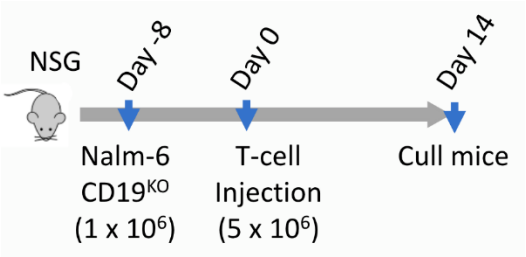

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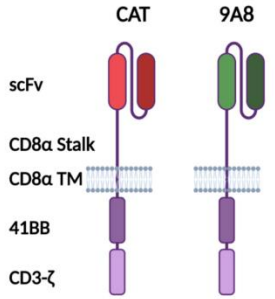

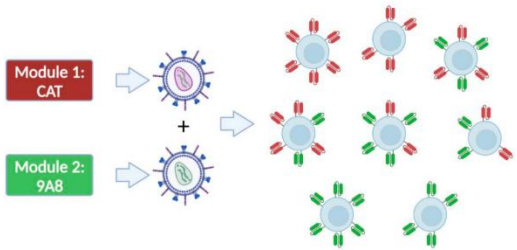

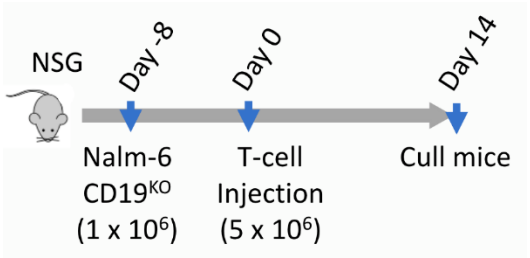

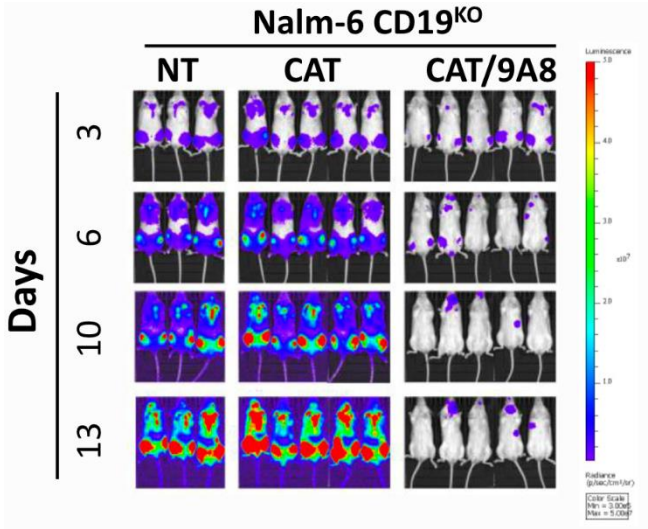

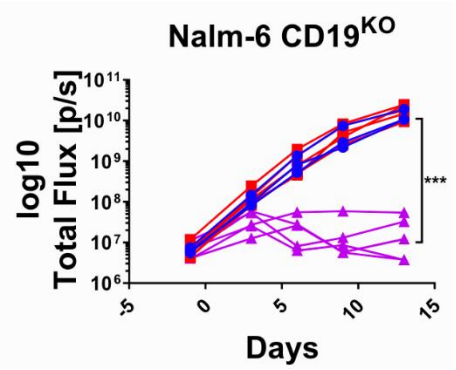

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CD19 and CD22 targeting with CART in the clinic

blood®
Brief Report

CLINICAL TRIALS AND OBSERVATIONS

CD19/CD22 targeting with cotransduced CAR T cells to prevent antigen-negative relapse after CAR T-cell therapy for B-cell ALL

Sara Ghorashian,[1,2,*] Giovanna Lucchini,[3,*] Rachel Richardson,[4] Kyvi Nguyen,[4] Craig Terris,[4] Aleks Guvenel,[4] Macarena Oporto-Espuelas,[4] Jenny Yeung,[4] Danielle Pinner,[3] Jan Chu,[3] Lindsey Williams,[3] Ka-Yuk Ko,[3] Chloe Walding,[5] Kelly Watts,[6] Sarah Inglott,[1] Rebecca Thomas,[1] Christopher Connor,[1] Stuart Adams,[1] Emma Gravett,[1] Kimberly Gilmour,[7] Alka Lal,[8] Sangeetha Kunaseelan,[8] Bilyana Popova,[8] Andre Lopes,[8] Yenting Ngai,[8] Allan Hackshaw,[8] Evangelia Kokalaki,[9] Milena Balasch Carulla,[3] Khushnuma Mullanfiroze,[3] Arina Lazareva,[3] Vesna Pavasovic,[1] Anupama Rao,[1] Jack Bartram,[1] Ajay Vora,[1] Robert Chiesa,[3] Juliana Silva,[3] Kanchan Rao,[4] Denise Bonney,[6] Robert Wynn,[6] Martin Pule,[9] Rachael Hough,[5] and Persis J. Amrolia[3,4]

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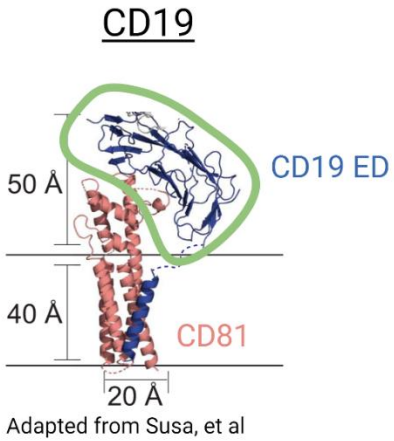
	- Use CAR targeting epitopes less prone to mutation	- Cohen, IJ. *et al.* Chimeric Antigen Receptor T Cells (CART) against the IGHV4-34 B cell Receptor Eliminate Neoplastic B Cells and Reduce Antigen-Negative Escape while Sparing the 2 Healthy B cell Repertoire. Submitted

CART targeting epitopes less prone to mutation



Adapted from Susa, et al

Cohen IJ *et al.* Submitted
Susa, K *et al.* Science 2021
Su, Q *et al.* Science, 2022

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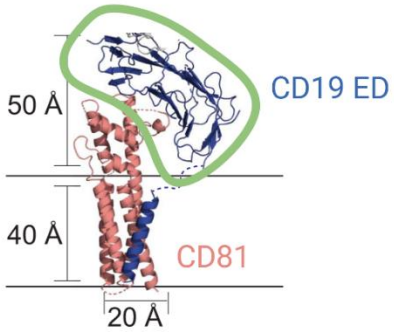

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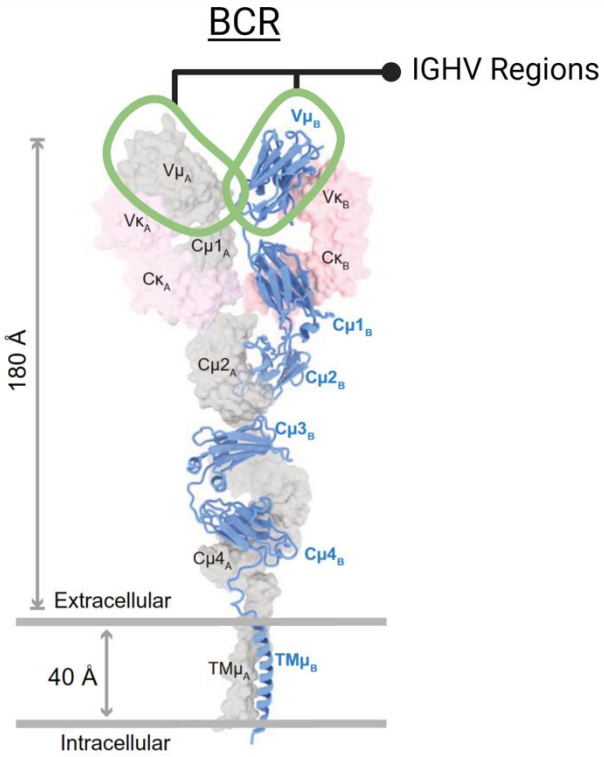

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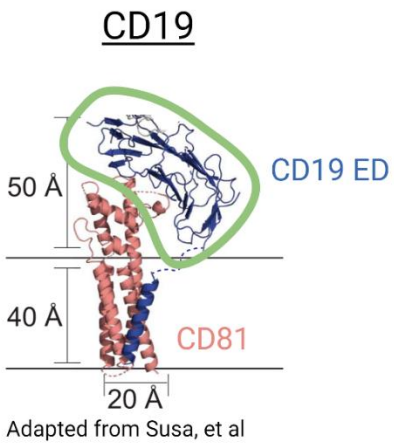

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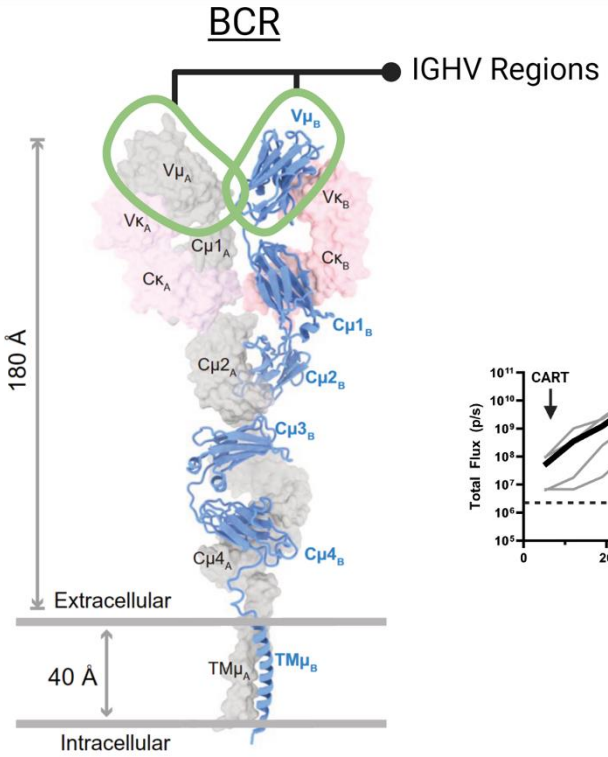

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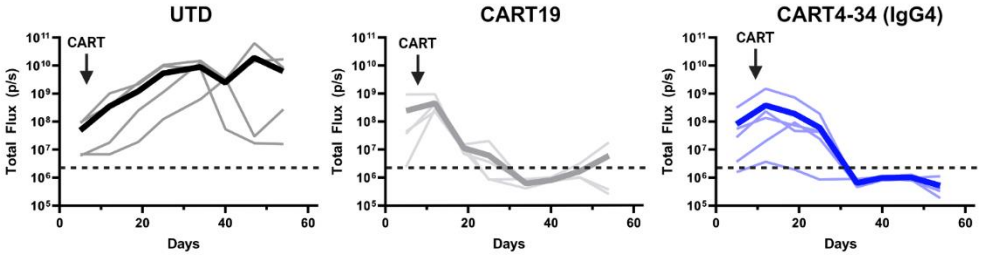
In vivo Anti-tumor Activity



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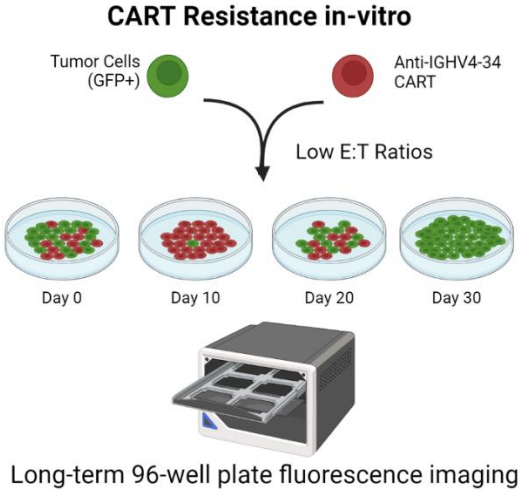

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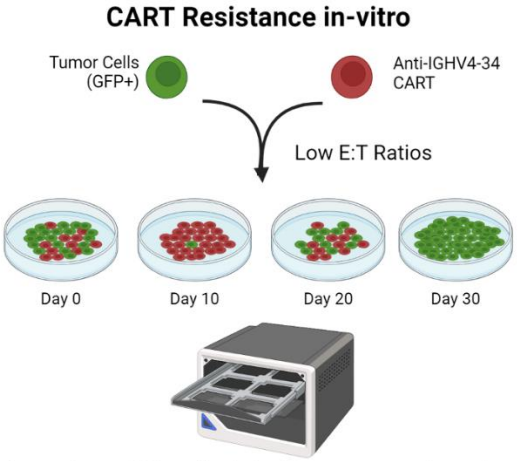
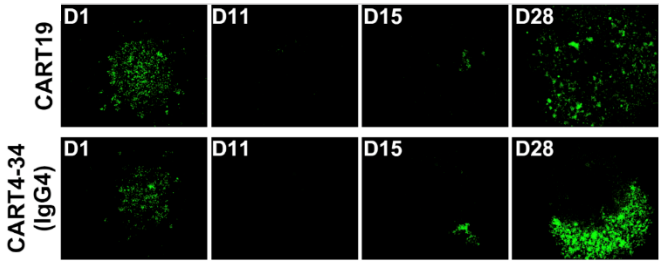
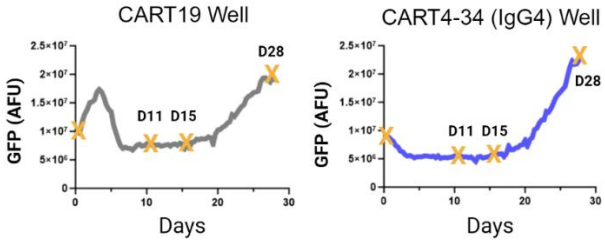


Long-term 96-well plate fluorescence imaging



HBL1-GFP



Longitudinal GFP Intensity (Individual Wells)

Cohen IJ *et al.* submitted

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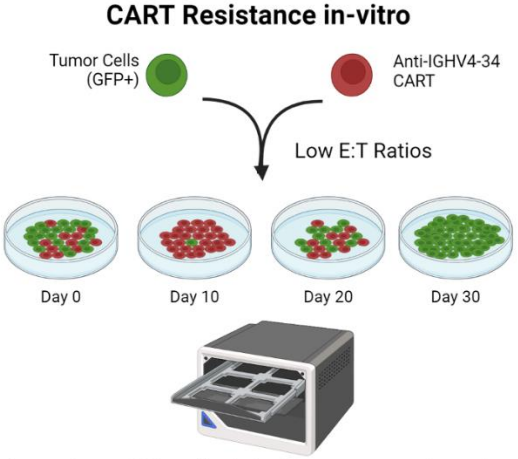

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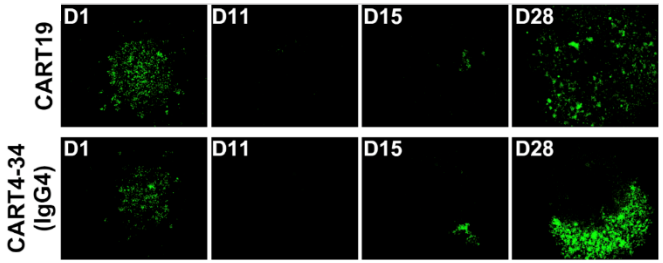

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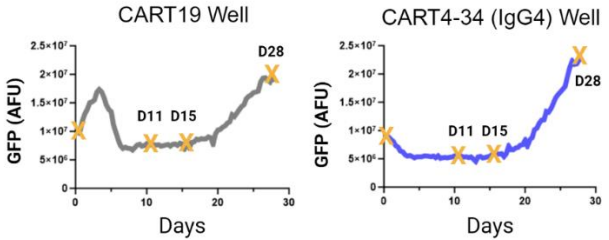

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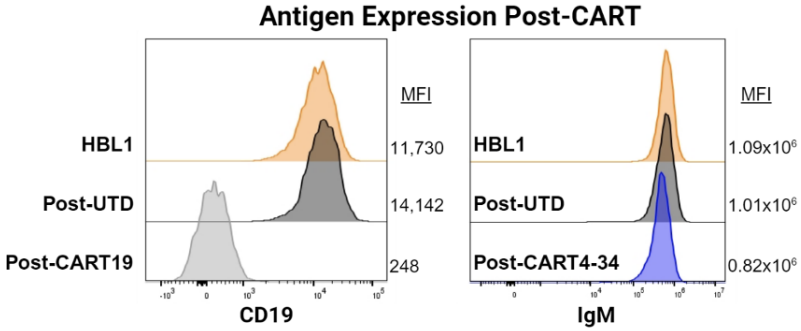

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Immunosuppressive microenvironment		

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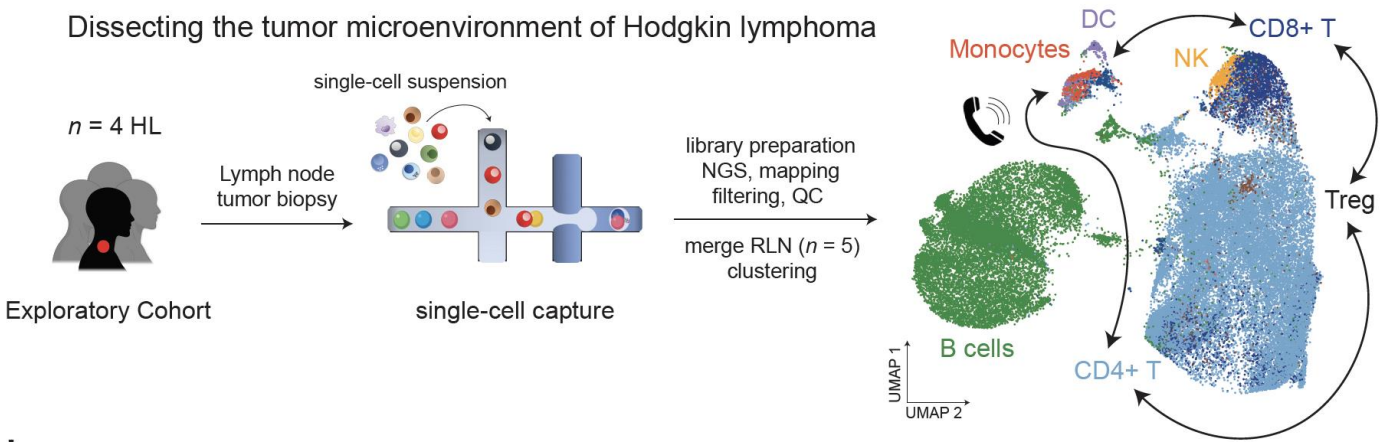
The BTLA-HVEM axis is enriched in Hodgkin Lymphoma



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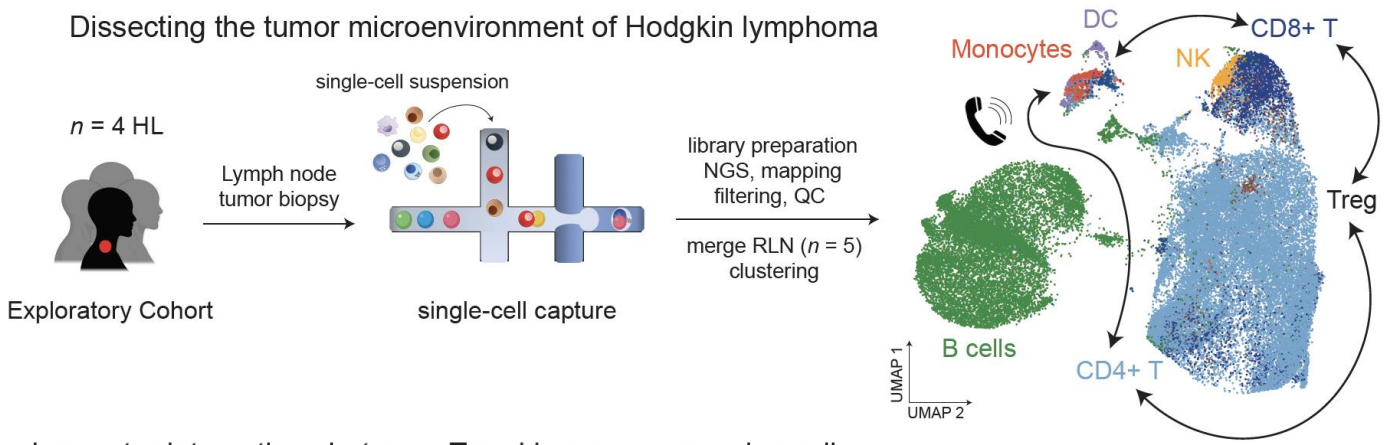
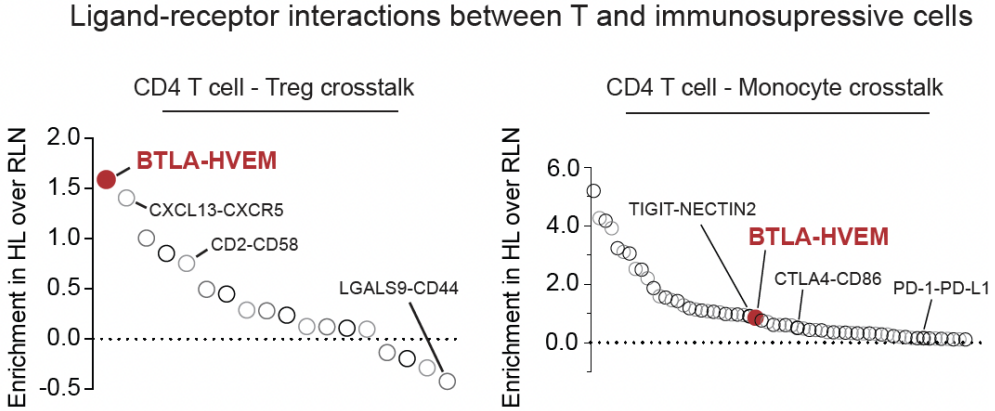
Guruprasad, P. *et al. Nat Immunol* (2024)

The BTLA-HVEM axis is enriched in Hodgkin Lymphoma





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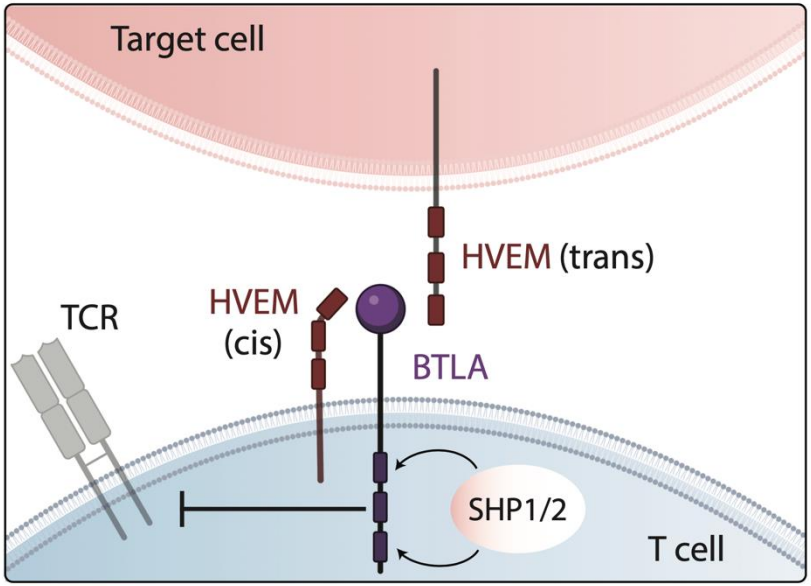

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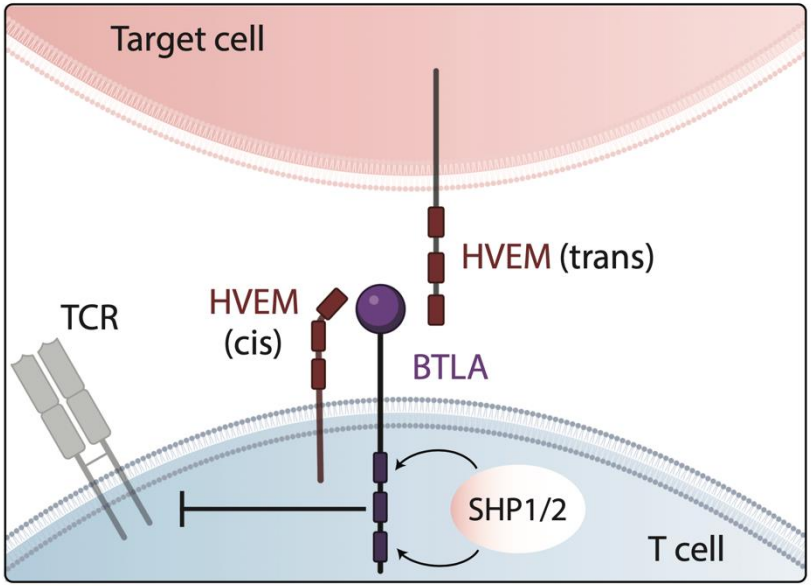
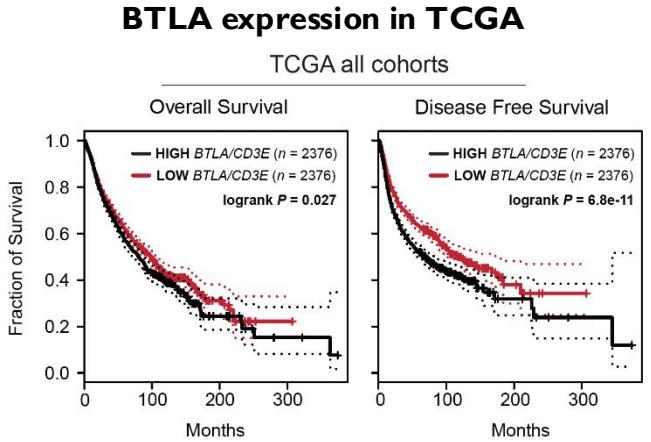
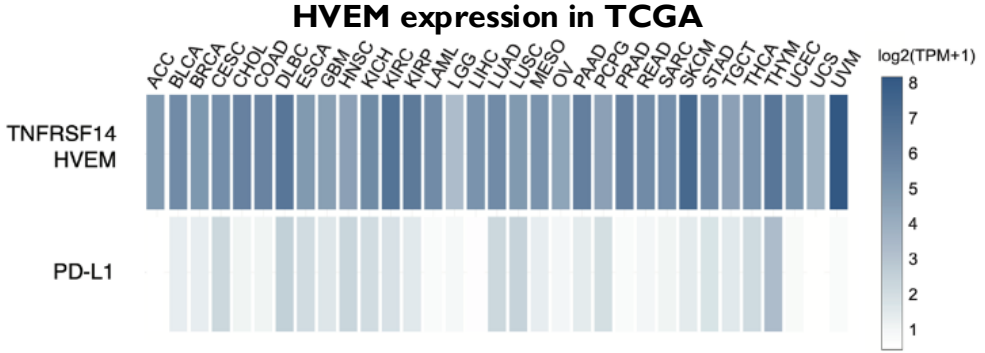
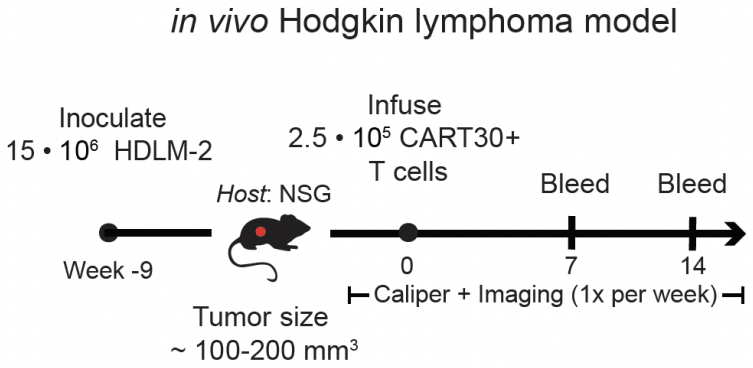


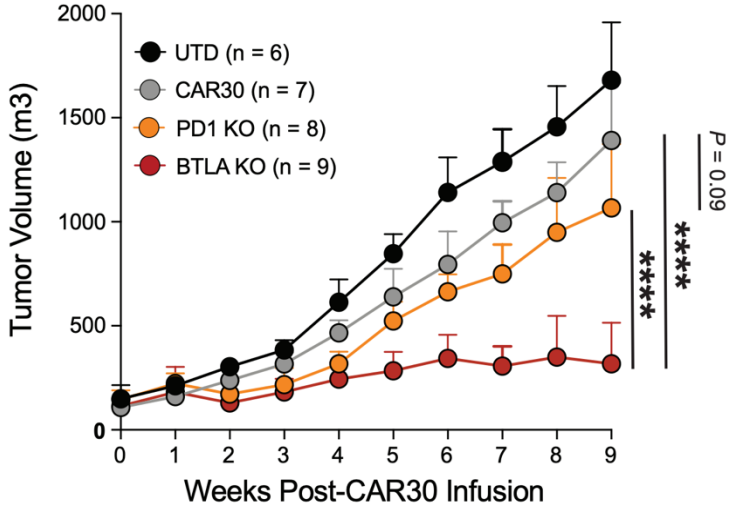




Guruprasad, P. *et al. Nat Immunol* (2024)

BTLA KO compared to PD-1 KO in CART30 for Hodgkin Lymphoma





Guruprasad, P. *et al. Nat Immunol* (2024)

Strategies to overcome CART resistance

Mechanism of resistance	Potential strategies to overcome	References
CART exhaustion	- CART gene editing for improved activation - Optimize co-stimulatory domains	- Guruprasad, P. *et al.* The BTLA-HVEM axis restricts CAR T cell efficacy in cancer. *Nat Immunol,* (2024) - Patel, R. P. *et al.* CD5 deletion enhances the antitumor activity of adoptive T cell therapies. *Sci Immunol,* (2024) - Doan, A. E. *et al.* FOXO1 is a master regulator of memory programming in CAR T cells. *Nature,* (2024)
Lack of costimulatory signals		
Immunosuppressive microenvironment	- Combination with immune checkpoint blockers	- Rupp, L. J. *et al.* CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. *Sci Rep*, (2017) - Pérez-Moreno, M. A. *et al.* Combined or Sequential Treatment with Immune Checkpoint Inhibitors and Car-T Cell Therapies for the Management of Haematological Malignancies: A Systematic Review. *Int J Mol Sci,* (2023)

Combination with checkpoint blockers

Author, Year	Pathology	Study Treatment	Patients (N)	Response N (%)
Li et al. [28], 2017	B-ALL	Anti-CD19 + Nivolumab or Pembrolizumab	14	ORR: 6 (42.9%) - CR: 2 (14.3%) - PR: 4 (28.6%) PD: 1 (7.1%)
Maude et al. [29], 2017	B-ALL	Tisa-cel+ Pembrolizumab	4	ORR: 2 (50.0%) - CR: 1 (25.0%) - PR: 1 (25.0%)
Cao et al. [30], 2019	B cell-NHL	Anti-CD19+ Nivolumab	11	ORR: 9 (81.8%) - CR: 5 (45.5%) - PR: 4 (36.4%)
Siddiqi et al. [31], 2019 (PLATFORM study)	B cell-NHL	Liso-cel + Durvalumab	18 (11 *)	ORR: 10 (90.9%) - CR: 7 (63.6)
Jacobson et al. [33], 2020 (ZUMA-6 trial)	B cell-NHL	Axi-cel + Atezolizumab	28	ORR: 21 (75.0%) - CR: 13 (46.4%)
Ramakrishnan et al. [34], 2020 (ALEXANDER trial)	B cell-NHL	AUTO3 + Pembrolizumab	33 (29 *)	ORR: 20 (69.0%) - CR: 15 (51.7%)
Chong et al. [35], 2022	B cell-NHL	Tisa-cel + Pembrolizumab	12	ORR: 3 (25.0%) - CR: 1 (8.3%) - PR: 2 (16.7%) PD: 8 (66.7%) SD: 1 (8.3%)
Hirayama et al. [36], 2022	B cell-NHL	Anti-CD19+ Durvalumab	29 (26 *)	ORR: 9 (34.6%) - RC: 7 (26.9%)
Jaeger et al. [37], 2023 (PORTIA trial)	B cell-NHL	Tisa-cel + Pembrolizumab	12	ORR: 6 (50.0%) - CR: 4 (33.3%) - PR: 2 (16.7%) PD: 6 (50.0%)

Pérez-Moreno, M. A. *et al. Int J Mol Sci*, (2023)

Strategies to overcome CART resistance

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CART exhaustion	- CART gene editing for improved activation	- Guruprasad, P. *et al.* The BTLA-HVEM axis restricts CAR T cell efficacy in cancer. *Nat Immunol,* (2024)
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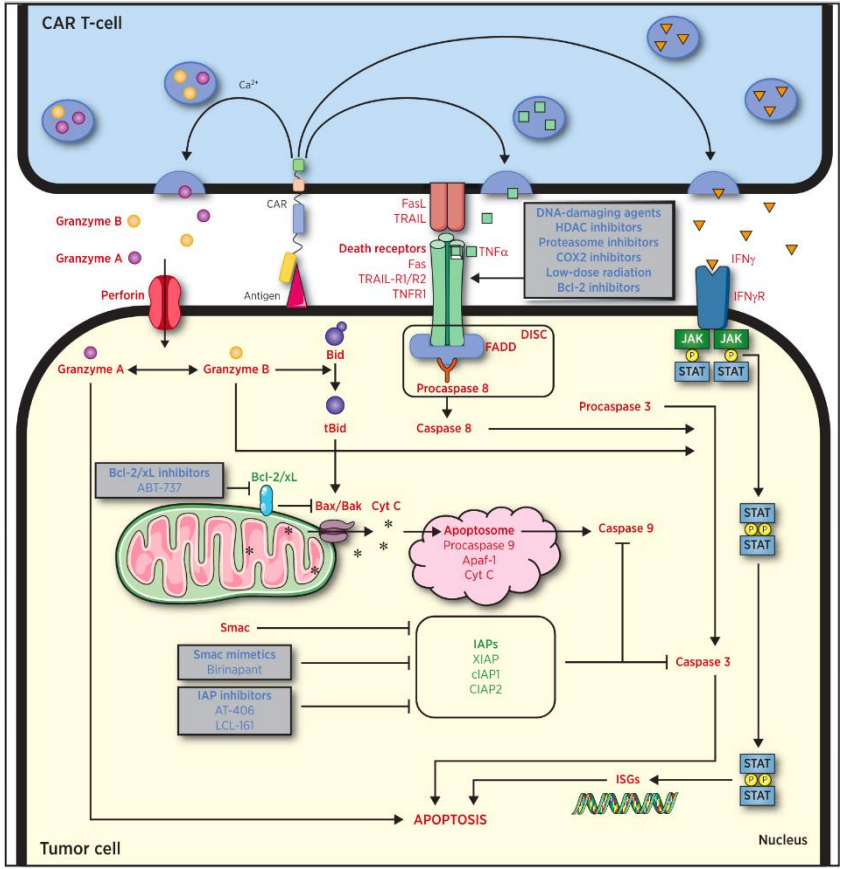
	- Combination with small molecules	- Lemoine, J., Ruella, M. & Houot, R. Overcoming intrinsic resistance of cancer cells to CAR T-cell killing. *Clin Cancer Res,* (2021) -Michie, J. *et al.* Antagonism of IAPs Enhances CAR T-cell Efficacy. *Cancer Immunology Research*, (2019)

Combination with pro-apoptotic small molecules

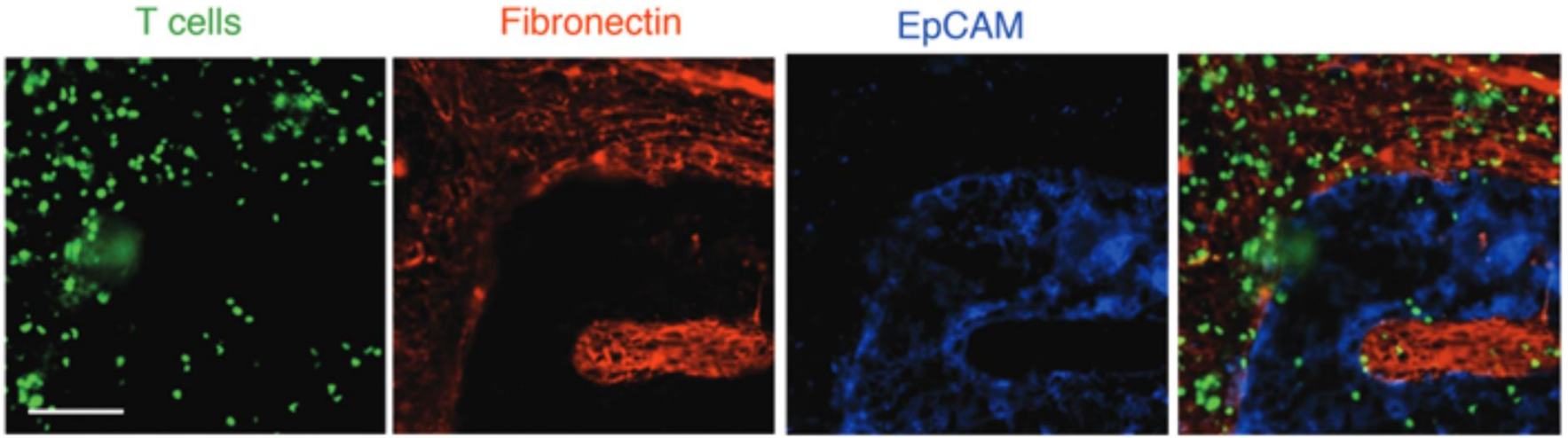


Lemoine, J., Ruella, M. & Houot, R. *Clin Cancer Res*, (2021)

Strategies to overcome CART resistance

Mechanism of resistance	Potential strategies to overcome	References
Poor tumor infiltration		

Poor tumor infiltration



Salmon, H. *et al. J Clin Invest,* (2012)

Strategies to overcome CART resistance

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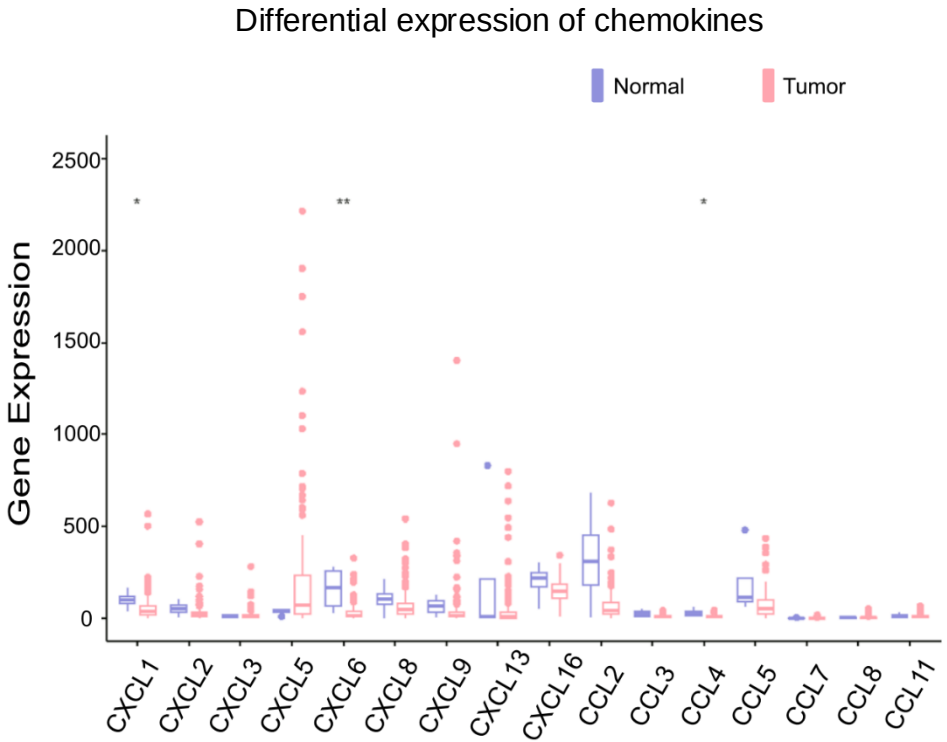
CXCR2 expressing CAR to enhance infiltration in pancreatic adenocarcinoma



Proudfoot, A. E. I. *Nat Rev Immunol,* (2002)
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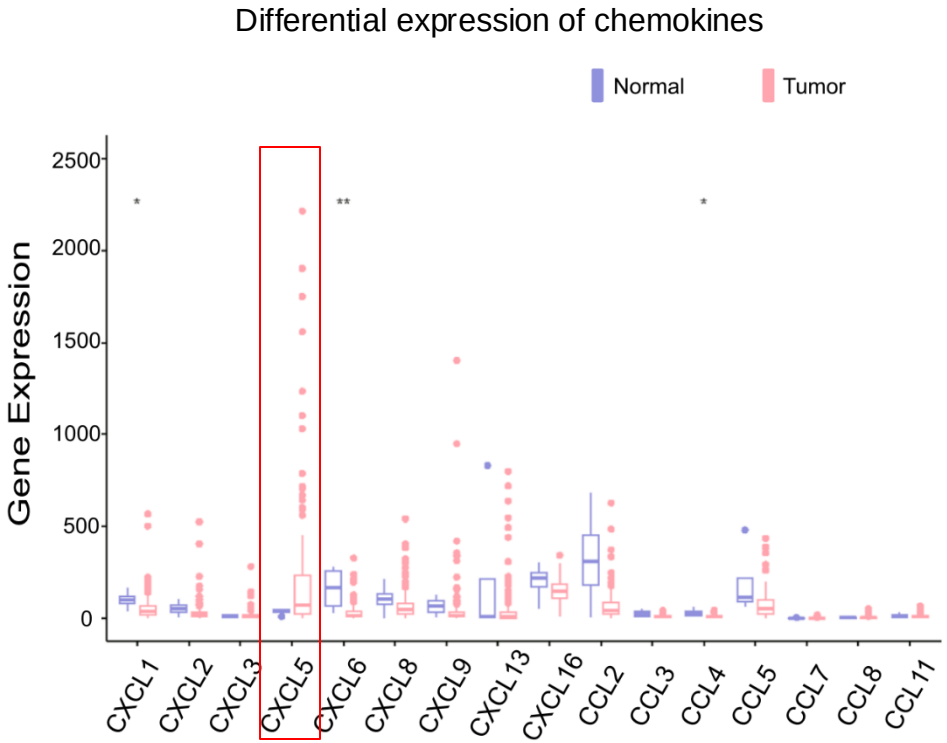

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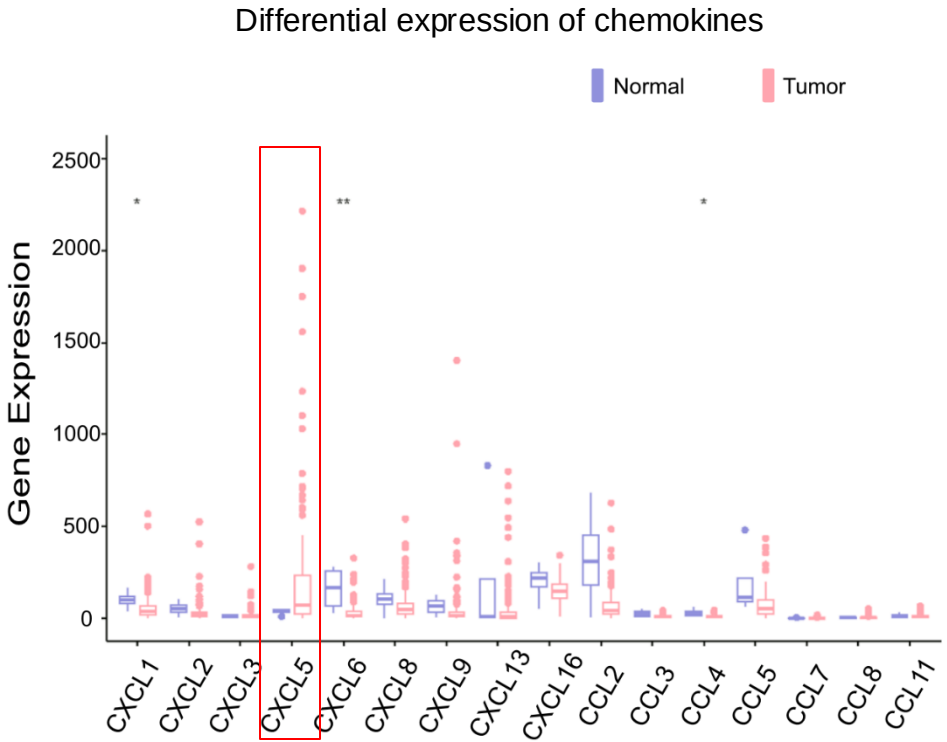

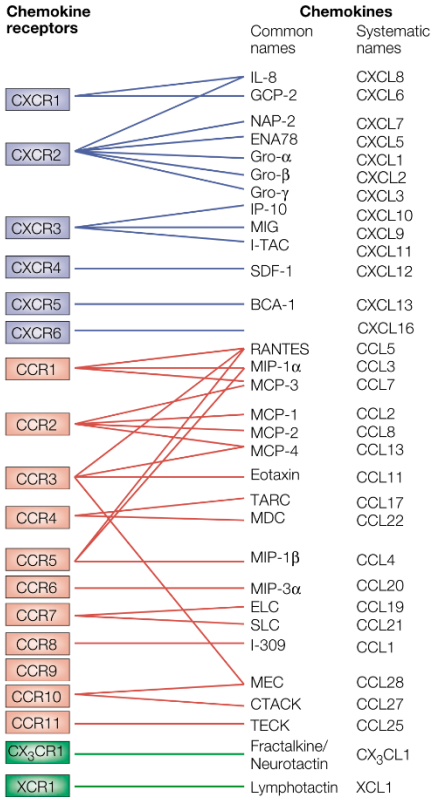

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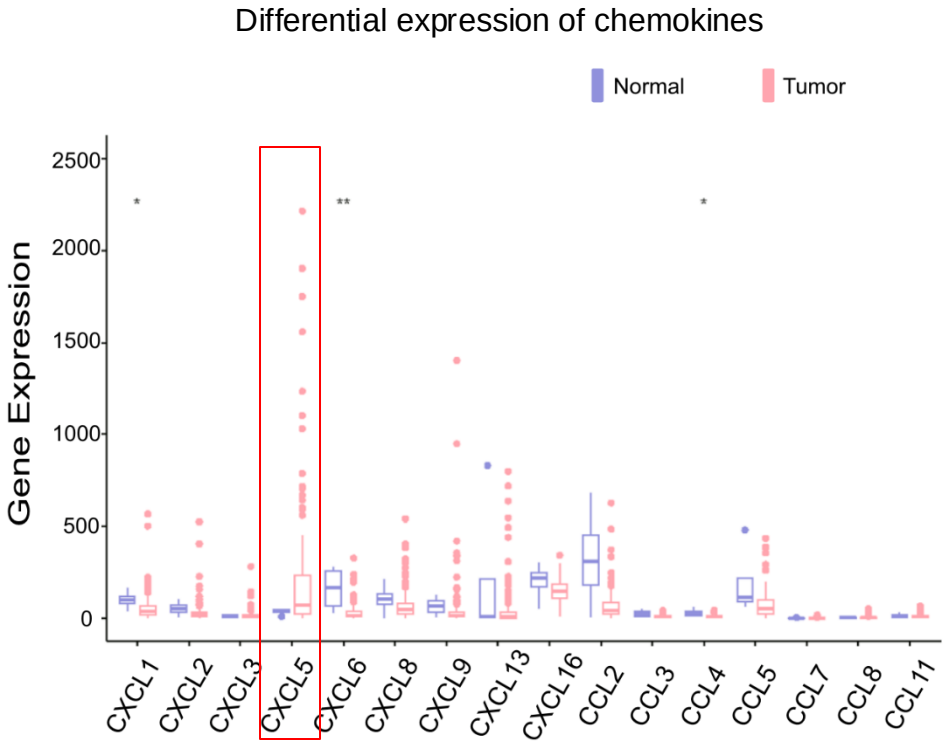

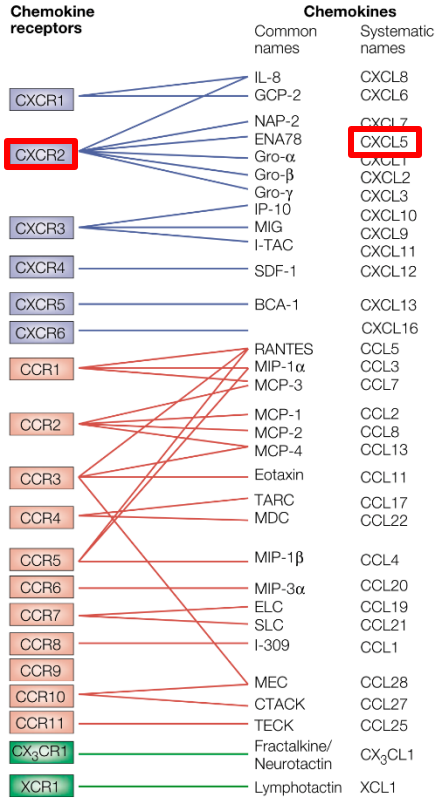

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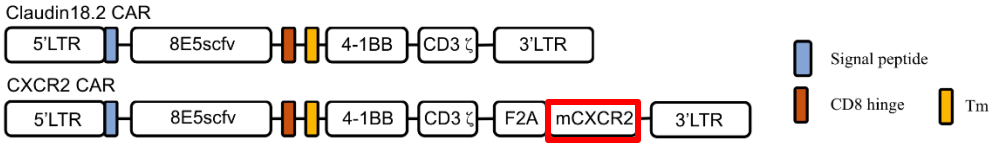

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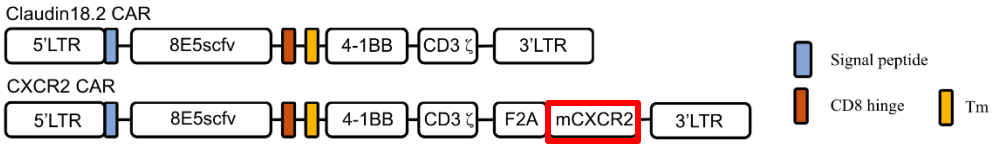

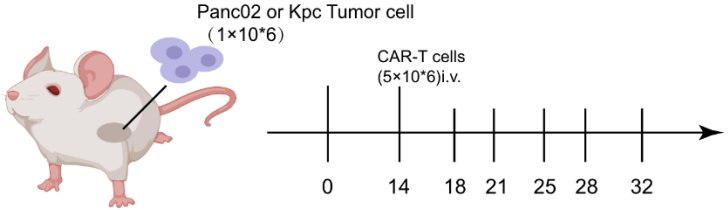

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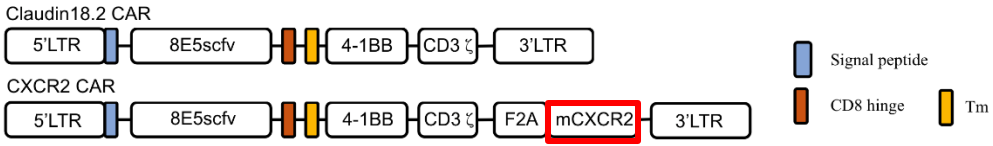

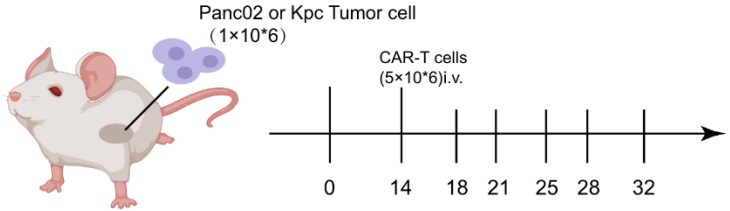

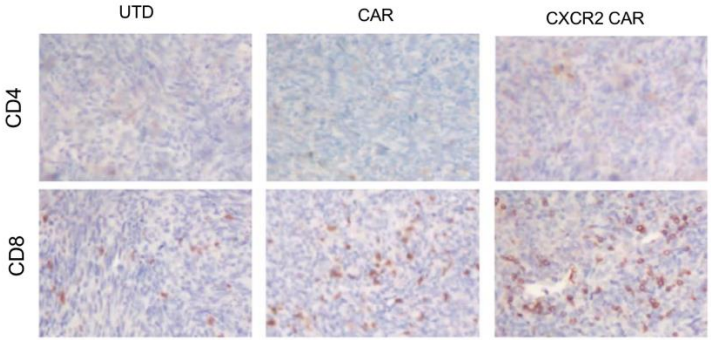

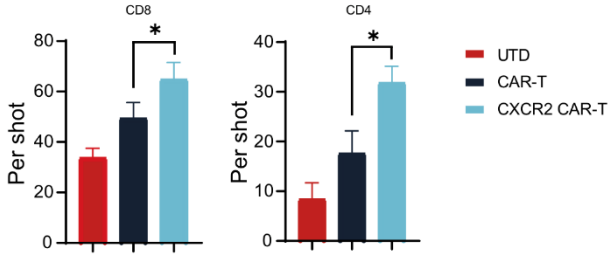

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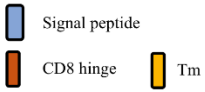

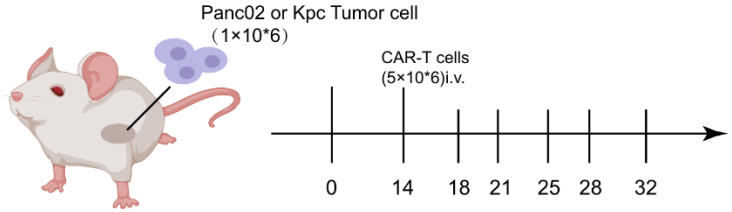

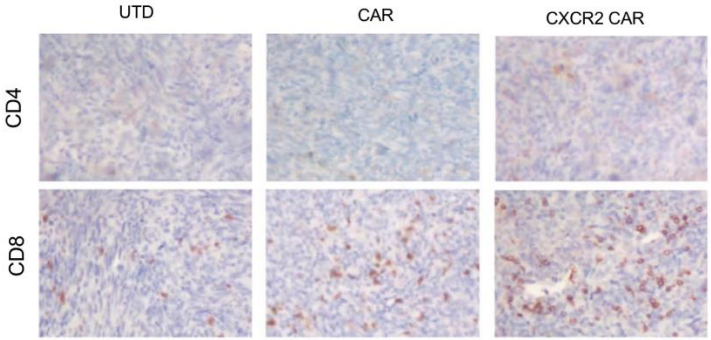

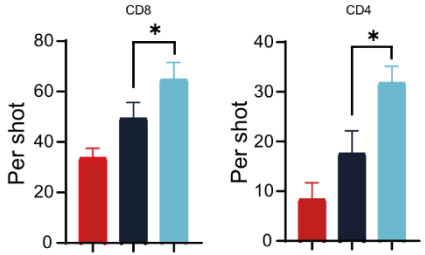

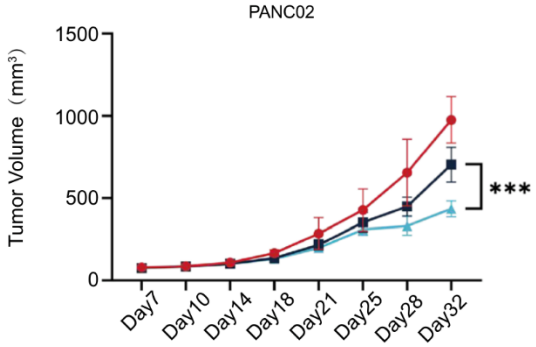

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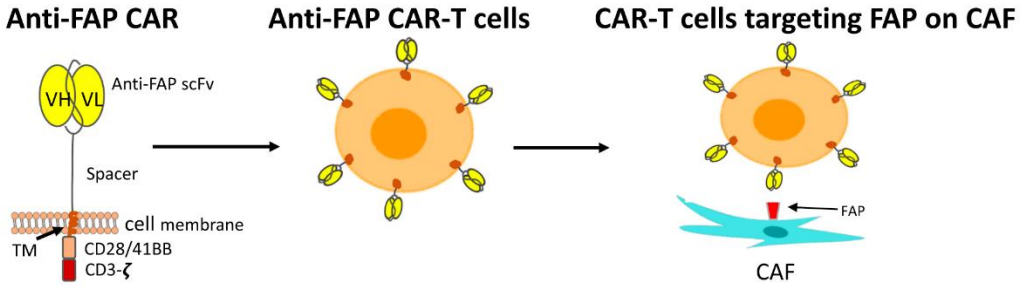
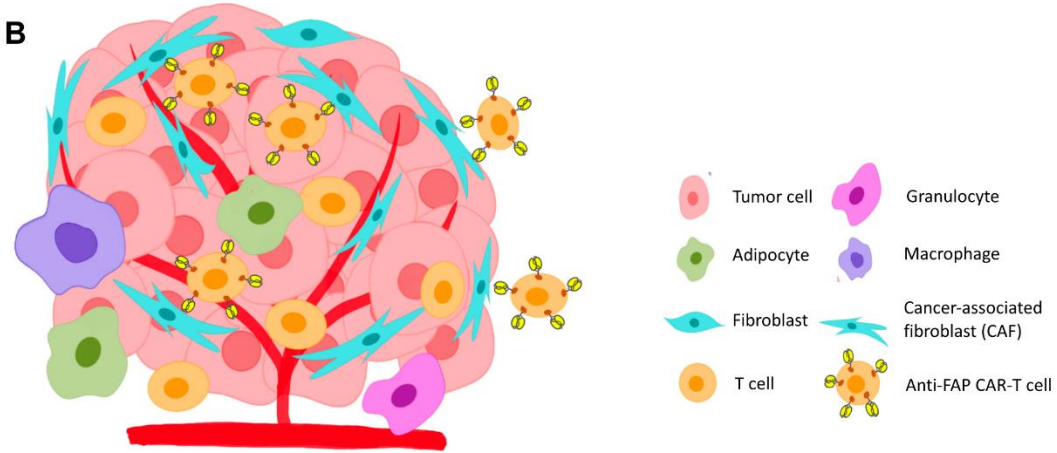
	- Enhancing tumor infiltration targeting extra-cellular matrix fibers or fibroblasts	- Caruana, I. *et al.* Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat Med*, (2015) - Wang, L.-C. S. *et al.* Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. *Cancer Immunol Res*, (2014) - Wehrli, M. *et al.* Mesothelin CAR T Cells Secreting Anti-FAP/Anti-CD3 Molecules Efficiently Target Pancreatic Adenocarcinoma and its Stroma. *Clin Cancer Res* **30**, 1859–1877 (2024).

Targeting Cancer Associated Fibroblast





Bughda, R. *et al. Immunotargets Ther* (2021)
Wang, L.-C. S. *et al. Cancer Immunol Res*, (2014)

Strategies to overcome CART resistance

Mechanism of resistance	Potential strategies to overcome	References
Unfavorable host factors		

Strategies to overcome CART resistance

Mechanism of resistance	Potential strategies to overcome	References
Unfavorable host factors	- Pre- and post-infusion optimization of host factors	- Iacoboni, G. *et al.* Recent Bendamustine Treatment Before Apheresis Has a Negative Impact on Outcomes in Patients With Large B-Cell Lymphoma Receiving Chimeric Antigen Receptor T-Cell Therapy. *J Clin Oncol,* (2024). - Smith, M. *et al.* Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. *Nat Med,* (2022)

Strategies to overcome CART resistance

Mechanism of resistance	Potential strategies to overcome	References
Access to CART therapy		

Strategies to overcome CART resistance

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Access to CART therapy	- Improving access to CART therapy	- Ghilardi, G. *et al.* CAR T-Cell Immunotherapy in Minority Patients with Lymphoma. *NEJM Evid,* (2024)
	- Expanding CART indications	- Snook, A. E. *et al.* Senza5 TM CART5: An Autologous CD5-Deleted Anti-CD5 CART Product with Enhanced Anti-T-Cell Lymphoma Activity. *Blood* (2023)

Strategies to overcome CART resistance

Mechanism of resistance	Potential strategies to overcome	References
Antigen loss or modulation	- Develop CART targeting multiple antigens - Develop CAR with high sensitivity for the antigen	- Kokalaki, E. *et al*. Dual targeting of CD19 and CD22 against B-ALL using a novel high-sensitivity aCD22 CAR. *Mol Ther*, (2023) - Chu, F. *et al*. Chimeric antigen receptor T cells to target CD79b in B-cell lymphomas. *J Immunother Cancer,* (2023) - Zhang, Y. *et al*. Safety and efficacy of a novel anti-CD19 chimeric antigen receptor T cell product targeting a membrane-proximal domain of CD19 with fast on- and off-rates against non-Hodgkin lymphoma: a first-in-human study. *Mol Cancer,* (2023)
Antigen heterogeneity	- Use CAR targeting epitopes less prone to mutation	- Cohen, I.J. *et al*. Chimeric Antigen Receptor T Cells (CART) against the IGHV4-34 B cell Receptor Eliminate Neoplastic B Cells and Reduce Antigen-Negative Escape while Sparing the 2 Healthy B cell Repertoire. Submitted
CART exhaustion	- CART gene editing for improved activation - Optimize co-stimulatory domains	- Guruprasad, P. *et al*. The BTLA-HVEM axis restricts CAR T cell efficacy in cancer. *Nat Immunol,* (2024) - Patel, R. P. *et al*. CD5 deletion enhances the antitumor activity of adoptive T cell therapies. *Sci Immunol,* (2024) - Doan, A. E. *et al*. FOXO1 is a master regulator of memory programming in CAR T cells. *Nature,* (2024)
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Poor tumor infiltration	- Local delivery - Enhancing tumor infiltration through chemokine axis - Enhancing tumor infiltration targeting extra-cellular matrix fibers or fibroblasts	- Adusumilli, P. S. *et al*. Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. *Sci Transl Med,* (2014) - Brown, C. E. *et al*. Optimization of IL13Rα2-Targeted Chimeric Antigen Receptor T Cells for Improved Anti-tumor Efficacy against Glioblastoma. *Mol Ther,* (2018) - Dai, Z. *et al*. Ectopic CXCR2 expression cells improve the anti-tumor efficiency of CAR-T cells and remodel the immune microenvironment of pancreatic ductal adenocarcinoma. *Cancer Immunol Immunother,* (2024) - Caruana, I. *et al*. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat Med*, (2015) - Wang, L.-C. S. *et al*. Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. *Cancer Immunol Res,* (2014) - Wehrli, M. *et al*. Mesothelin CAR T Cells Secreting Anti-FAP/Anti-CD3 Molecules Efficiently Target Pancreatic Adenocarcinoma and its Stroma. *Clin Cancer Res* **30**, 1859–1877 (2024).
Unfavorable host factors	- Pre- and post-infusion optimization of host factors	- Iacoboni, G. *et al*. Recent Bendamustine Treatment Before Apheresis Has a Negative Impact on Outcomes in Patients With Large B-Cell Lymphoma Receiving Chimeric Antigen Receptor T-Cell Therapy. *J Clin Oncol,* (2024). - Smith, M. *et al*. Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. *Nat Med,* (2022)
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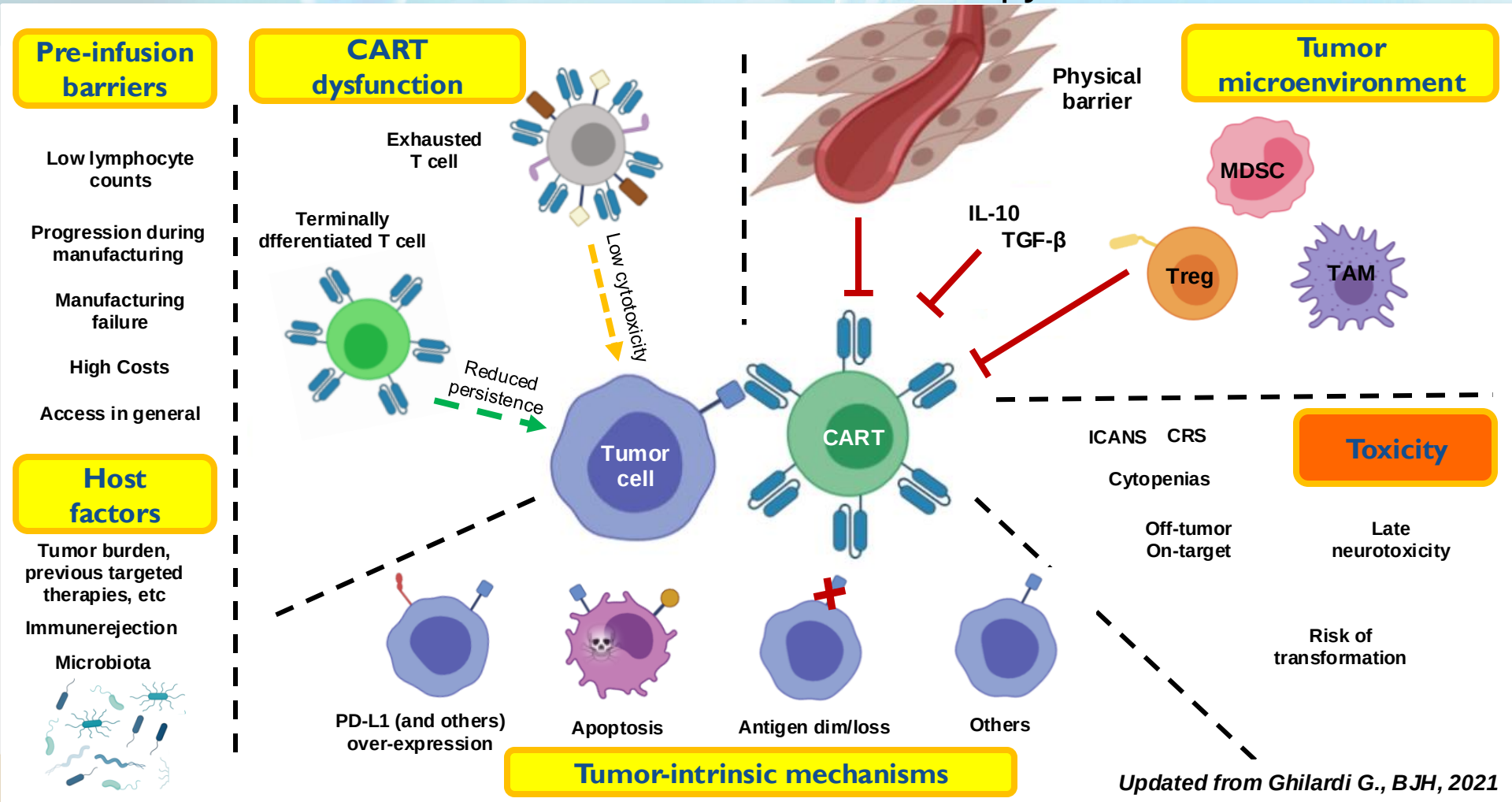
Causes of Failure of CART Immunotherapy in the Clinic



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3rd MEETING ON T-CELL AND NK-CELL BASED IMMUNOTHERAPIES FOR LYMPHOID MALIGNANCIES

Jean Lemoine, MD

Mechanisms of resistance: laboratory evidence

Ruella lab, Center for Cellular Immunotherapies, University of Pennsylvania

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