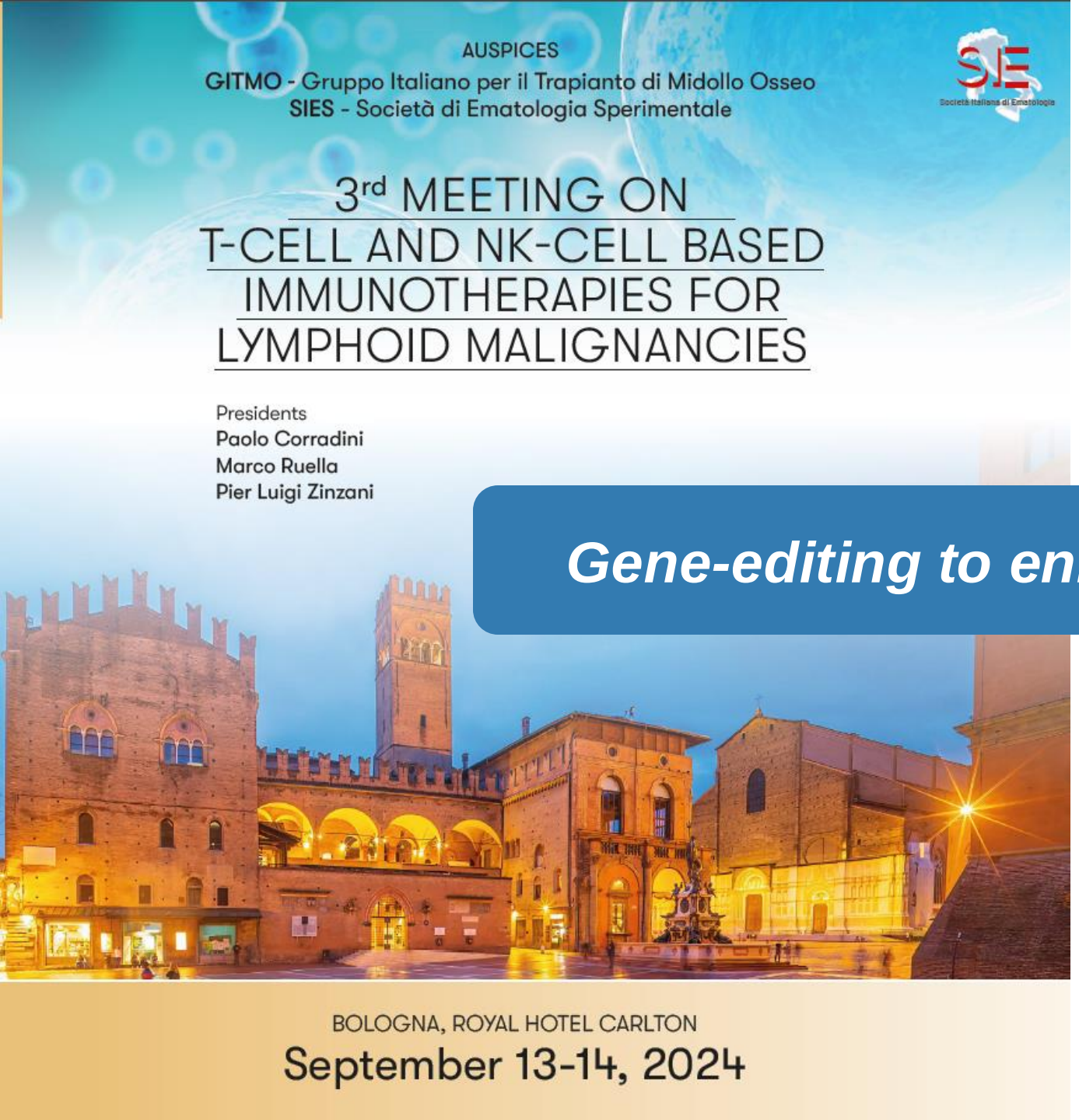


Penn Medicine
Center for Cellular Immunotherapies

Gene-editing to enhance CAR-T immunotherapies

Marco Ruella, MD

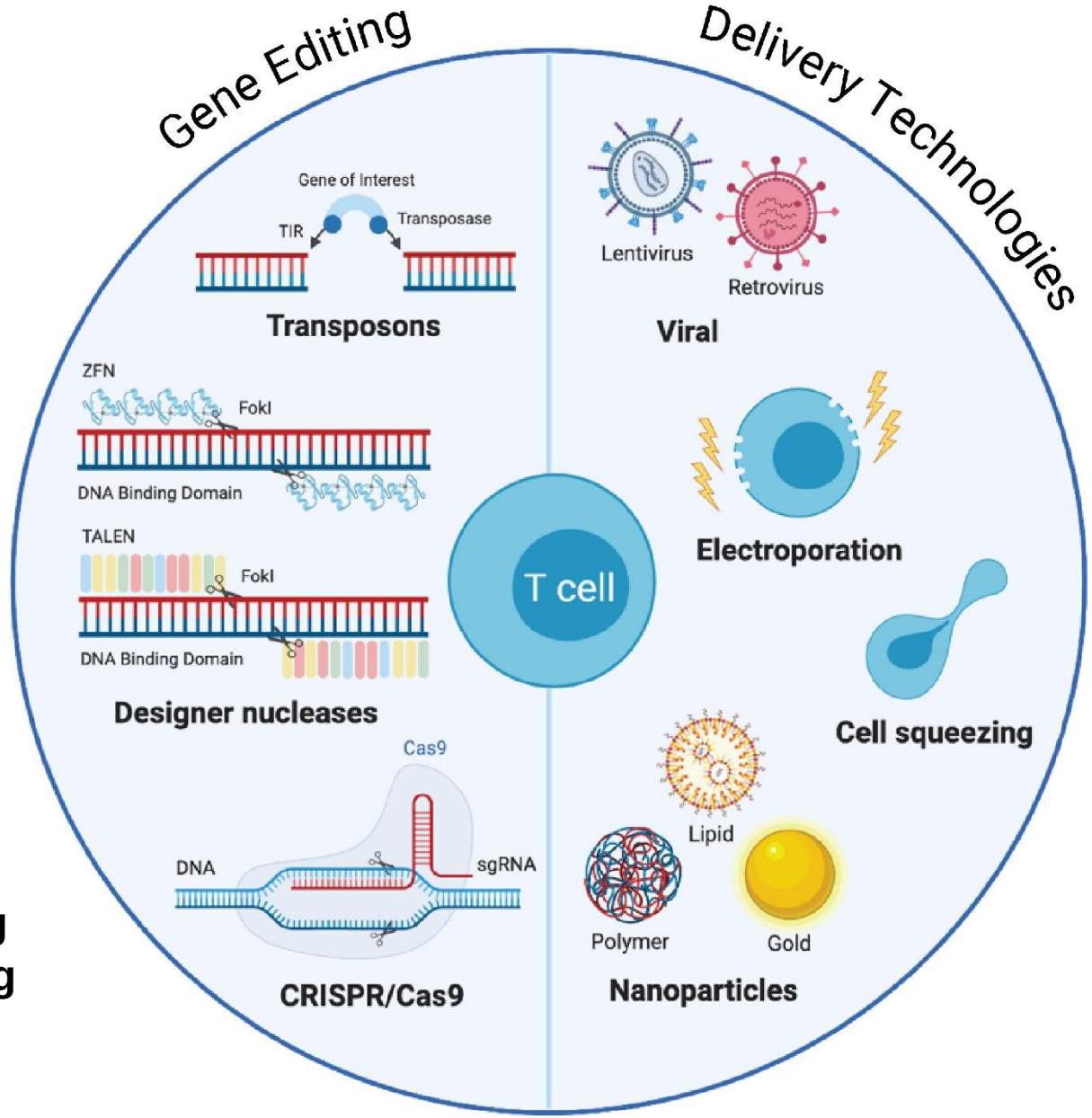
Assistant Professor of Medicine

Scientific Director, Lymphoma Program

Disclosures and Conflicts of Interest

- ***Inventor:*** *CART technologies (including the ones presented in this talk), Univ. of Pennsylvania, partly licensed to Novartis, Tmunity, and viTToria biotherapeutics*
- ***Research Funding:*** *AbClon, Beckman-Coulter, Lumicks, ONI, CurioX,*
- ***Consultancy/Honoraria:*** *nanoString, GLG, Bayer, Sana, BMS, GSK*
- ***Advisory Board:*** *AbClon, viTToria bio, Lumicks*
- ***Scientific Founder:*** *viTToria biotherapeutics*

What is gene editing?



Base editing
Prime editing

Causes of Failure of CART Immunotherapy in the Clinic

Pre-infusion barriers

CART dysfunction

Tumor microenvironment

Enhance T cell function:

E.g.: PD-1 KO and many others

Endow CART resistance to viruses/drugs:

E.g.: HIV CCR5 KO

Study resistance mechanisms:

E.g.: functional genomics

Avoid T cell fratricide:

E.g.: CD7 KO CART7 and others

Host factors

Toxicity

Generate allogeneic CART:

E.g.: TCR KO, MHC KO

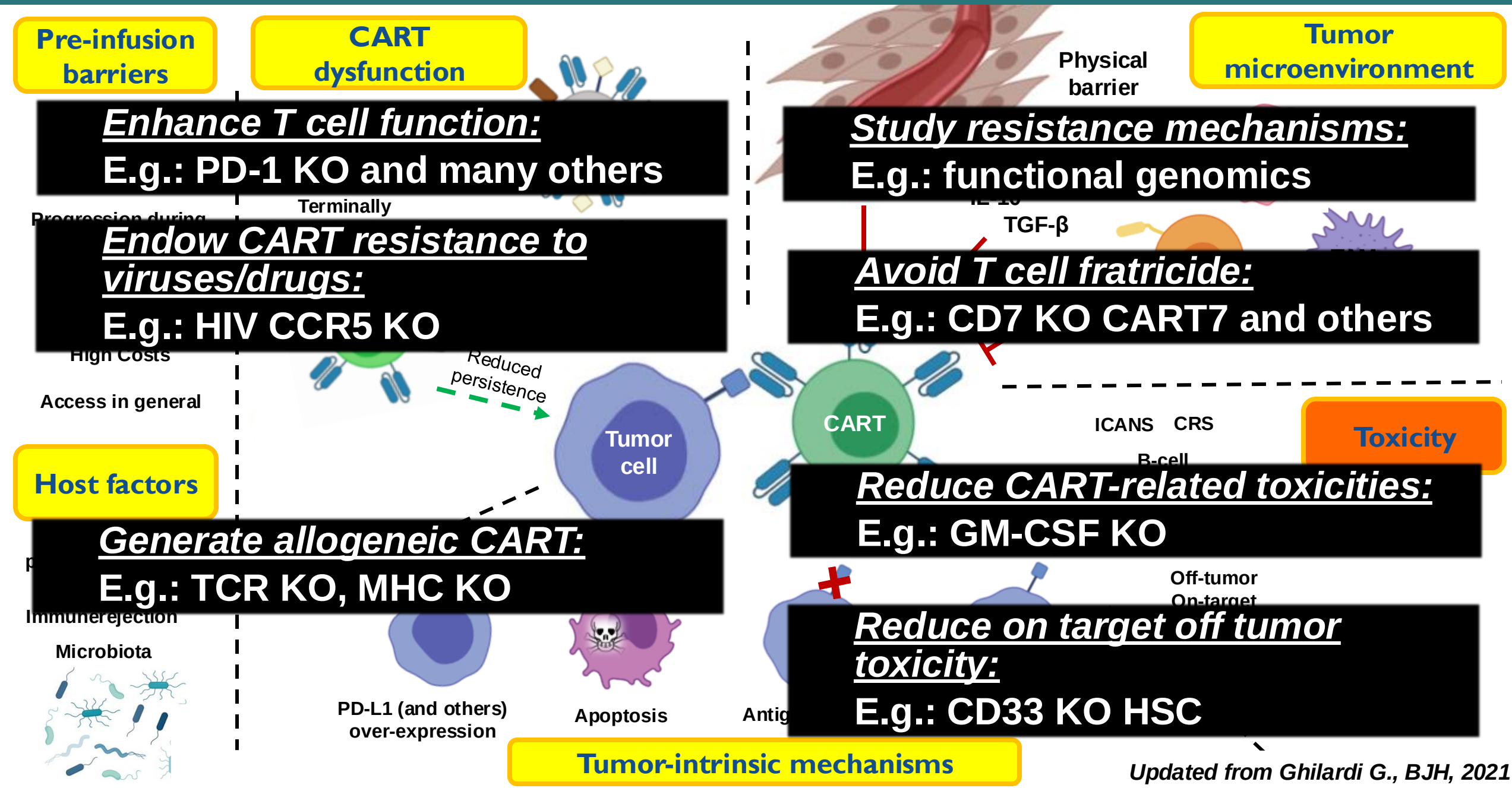
Reduce CART-related toxicities:

E.g.: GM-CSF KO

Reduce on target off tumor toxicity:

E.g.: CD33 KO HSC

Tumor-intrinsic mechanisms



Updated from Ghilardi G., BJH, 2021

Target Genes to Enhance CAR T cells in Preclinical Studies

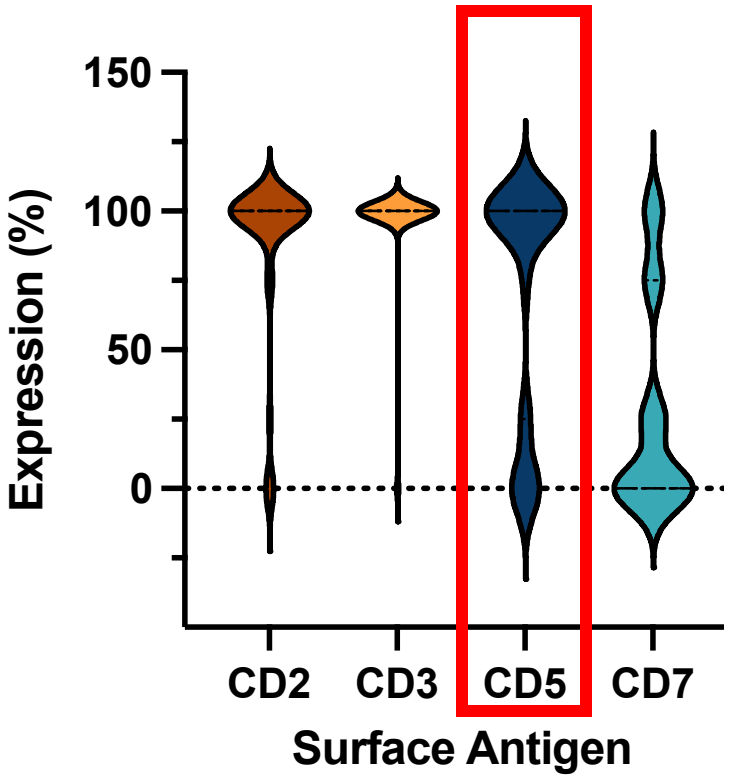
GENE KO/KD	MOA	PMID (selected)	GENE KO/KD	MOA	PMID (selected)
PD-1	exhaustion	28389661	**BTLA**	TME/exhaust	38831106
DGKα	activation	29967261	**NRA4**	multiple	31527257
RASA-2	activation	36002574	**REGNASE**	multiple	35323877
CBL-B	activation	33462140	**TIM-3**	exhaustion	34450537
TET-2	multiple	29849141	**LAG-3**	exhaustion	28625015
TIGIT	exhaustion	34628052	**FOXO1**	multiple	37649096
PTPN2	activation	31803974	**HPK1**	activation	32860752
PTP1B	activation	34794959	**TGFBR2**	multiple	31999649
Fas	AICD	28199983	**Dhx37**	multiple	31442407
DNMT3A	persistence	34788079	**Nr2f6**	multiple	31937317
CTLA-4	exhaustion	28888577	**CD5**	signaling	39028827
SUV39H1	Eff function	37934007	**Adenosine A2A receptor**	Eff function	34050151
BATF	Eff function	36240777	**IKZF3**	Eff function	34687790
ID3	Eff function	34861191	**PRDM1**	Eff function	34861037

Source: everywhere I could find information on this topic, as of 2024

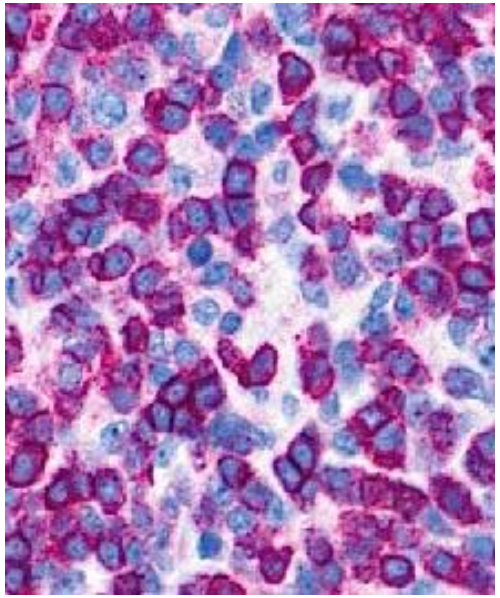
Expanding CART therapy: targeting CD5 for T-cell lymphomas

CD5 is highly and homogeneously expressed in:

- **85% T-cell Lymphoma** (10K+/year US)
- **Also expressed in other hematological malignancies**

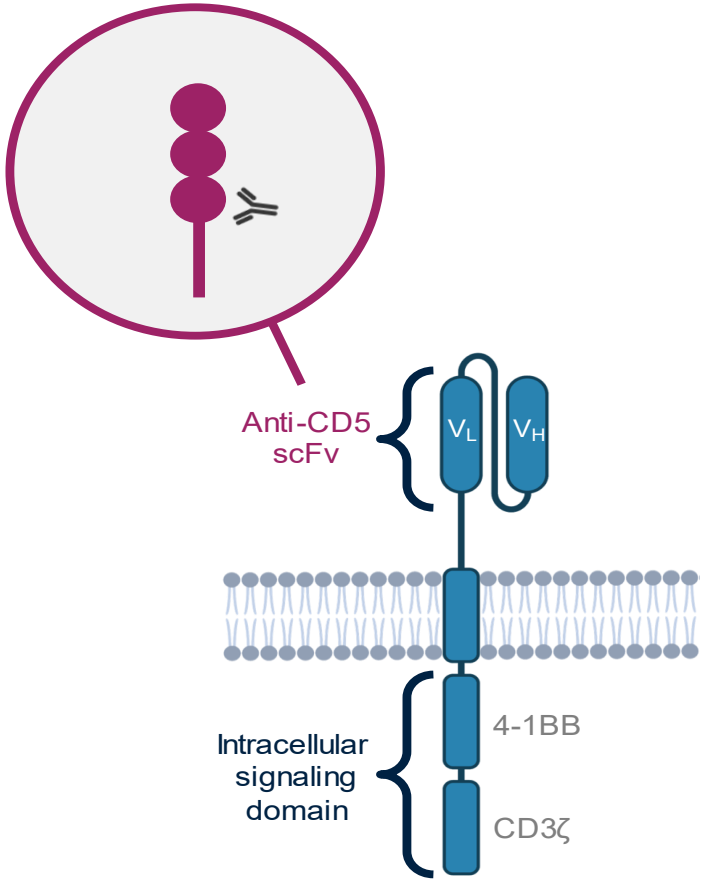


T cell lymphoma



CD5

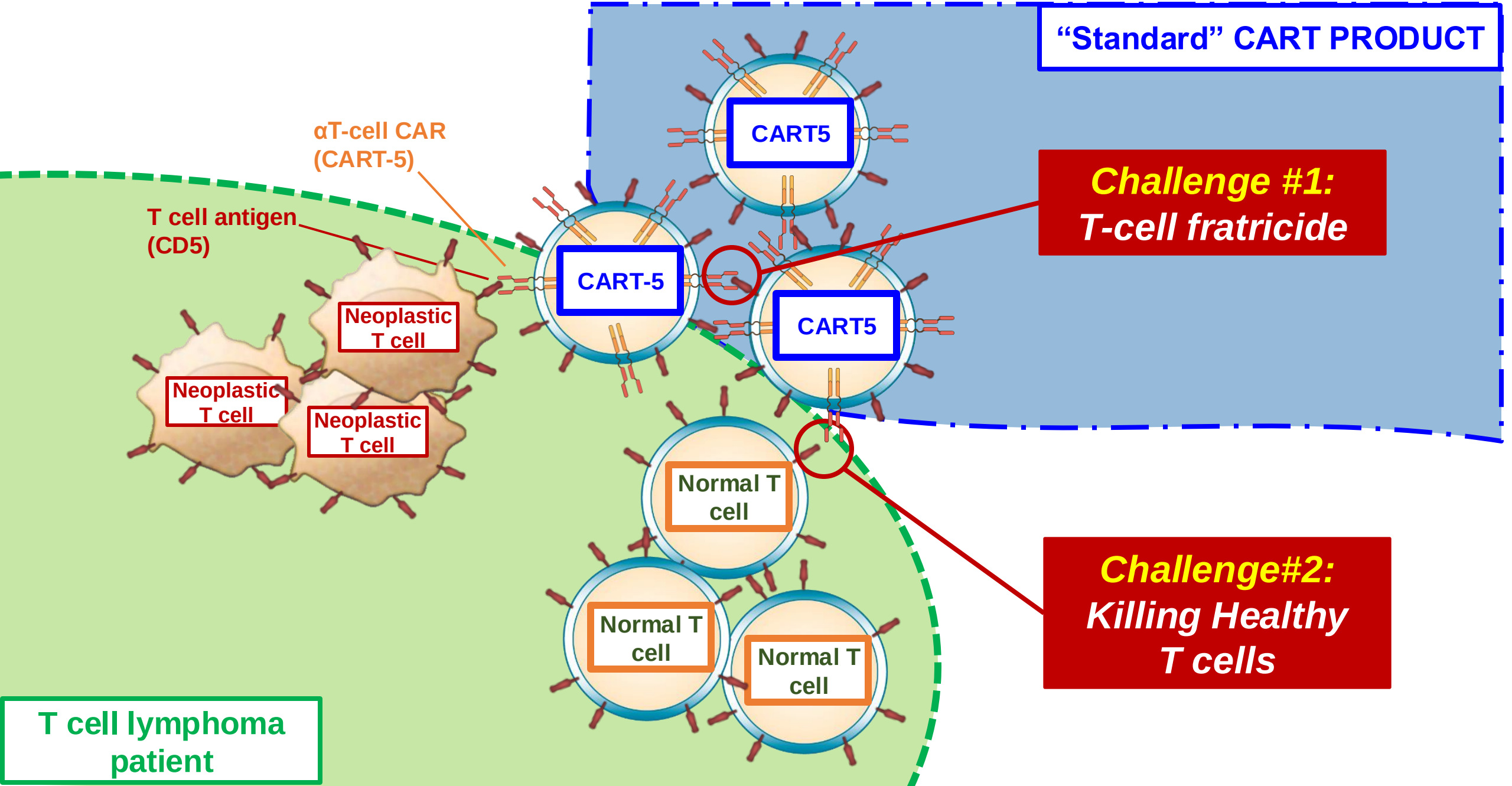
Anti-CD5 CAR



Courtesy: Stefano Pileri

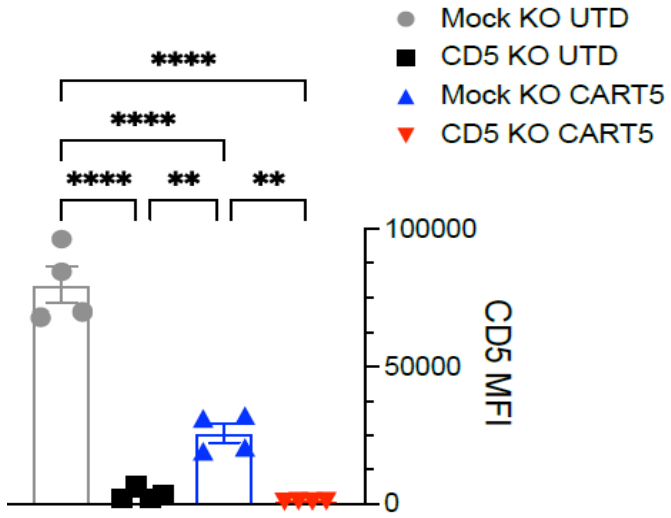
Patel RP, Science Immunology, 2024

T CELL LYMPHOMAS POSE UNIQUE CHALLENGES FOR CAR-T DESIGN

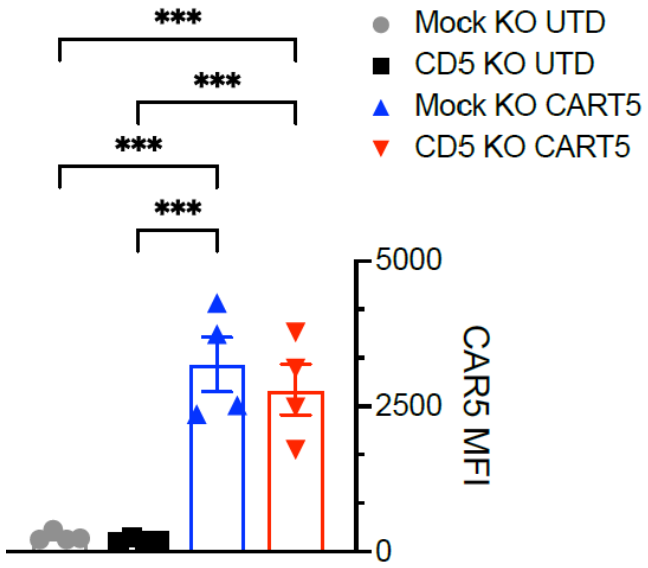


CD5 Knock Out as a Strategy to Enhance CART Efficacy

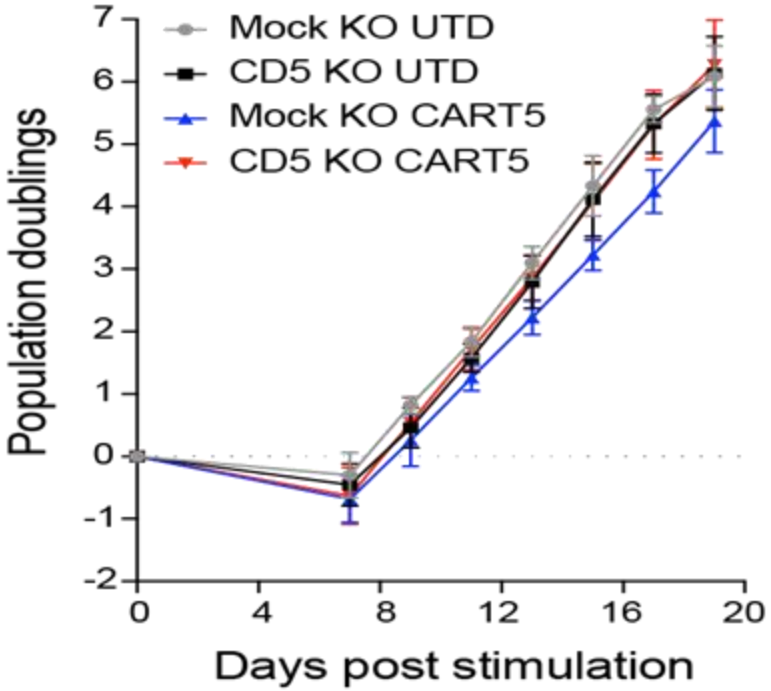
CD5 expression



CAR5 expression

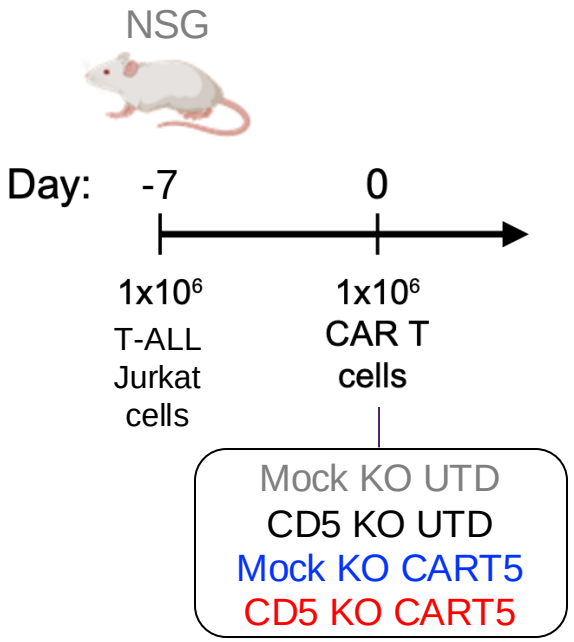


CAR T cell expansion



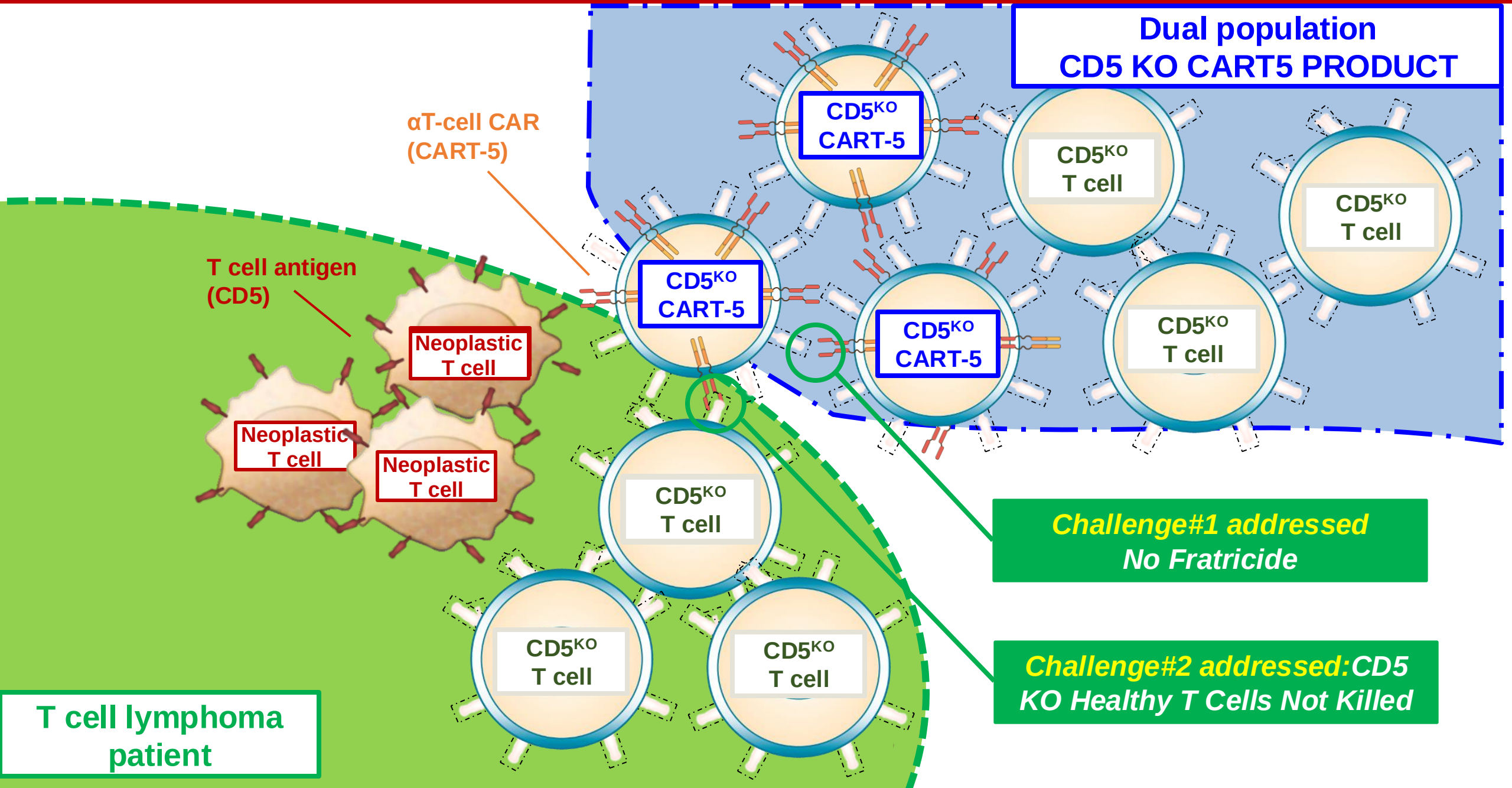
Patel RP, Science Immunology, 2024

CD5 KO enhances CART5 against T-cell Lymphoma



Patel RP, Science Immunology, 2024

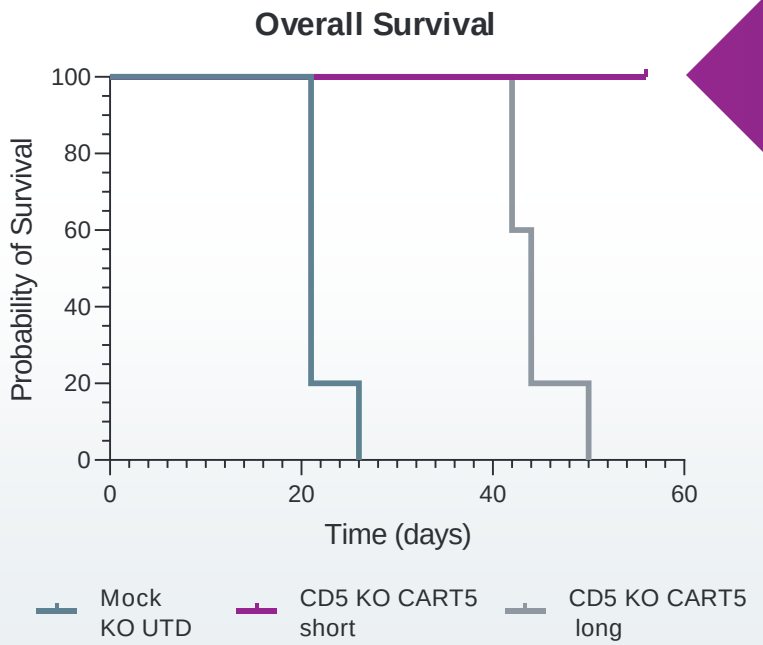
Novel CD5 knock out Dual-Population CART5 Product
ENABLES ANTI-TUMOR EFFECT AND PREVENTION OF T CELL TOXICITY



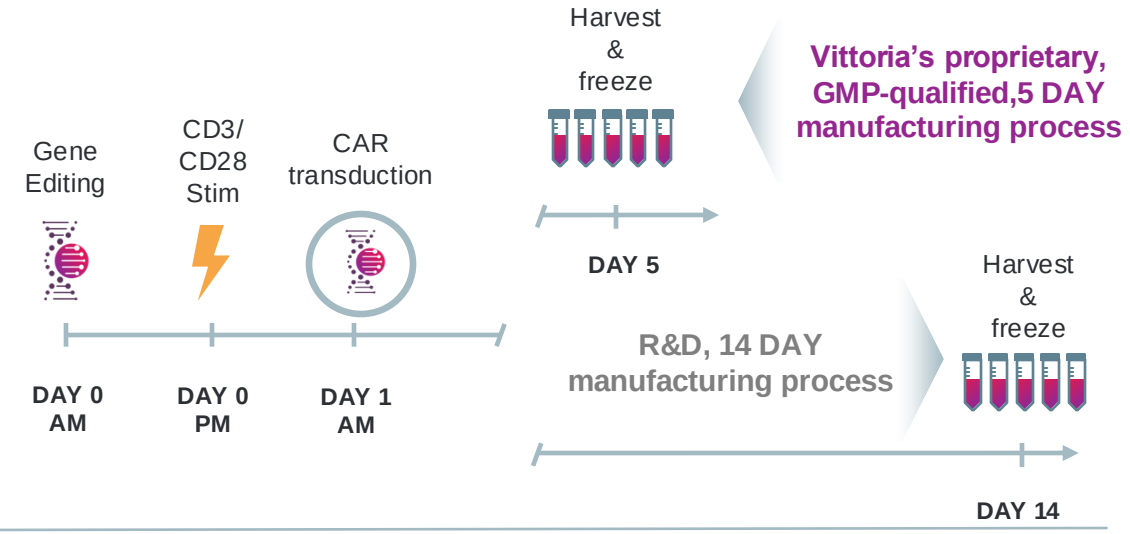
Viper 101: Senza5 CART5 Clinical Embodiment Further Enhanced by 5-Day, Proprietary GMP Manufacturing Process

Senza5 CART5

Clinical embodiment proves to be curative in aggressive T-cell lymphoma murine model **at sub-optimal dose** of CAR+ cells

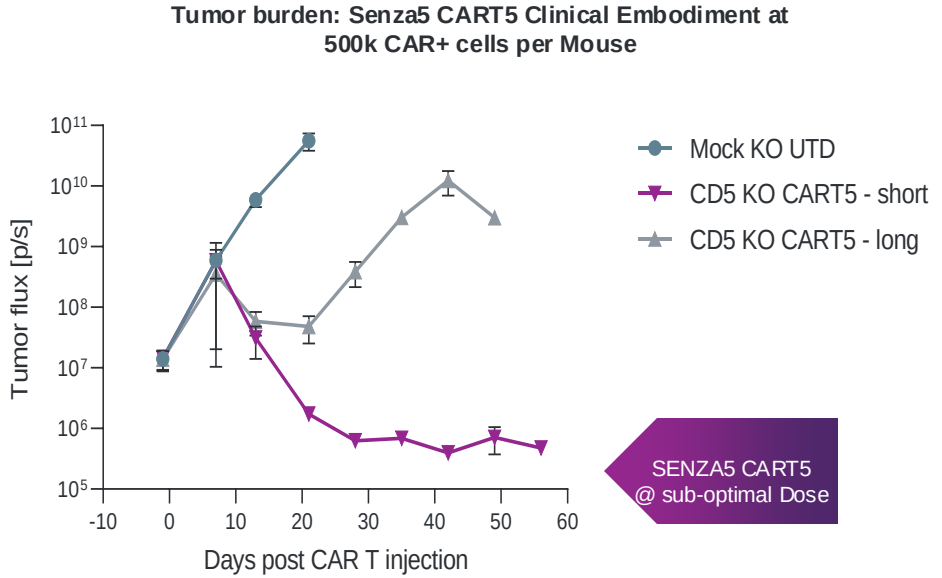


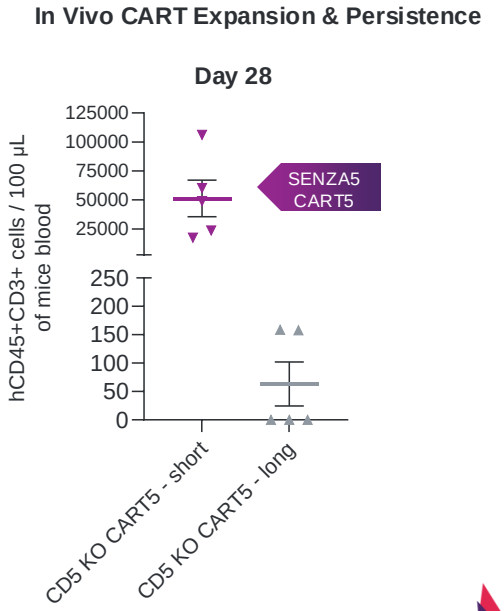
SENZA5 CART5 SHOWS UNPRECEDENTED CURATIVE POTENTIAL



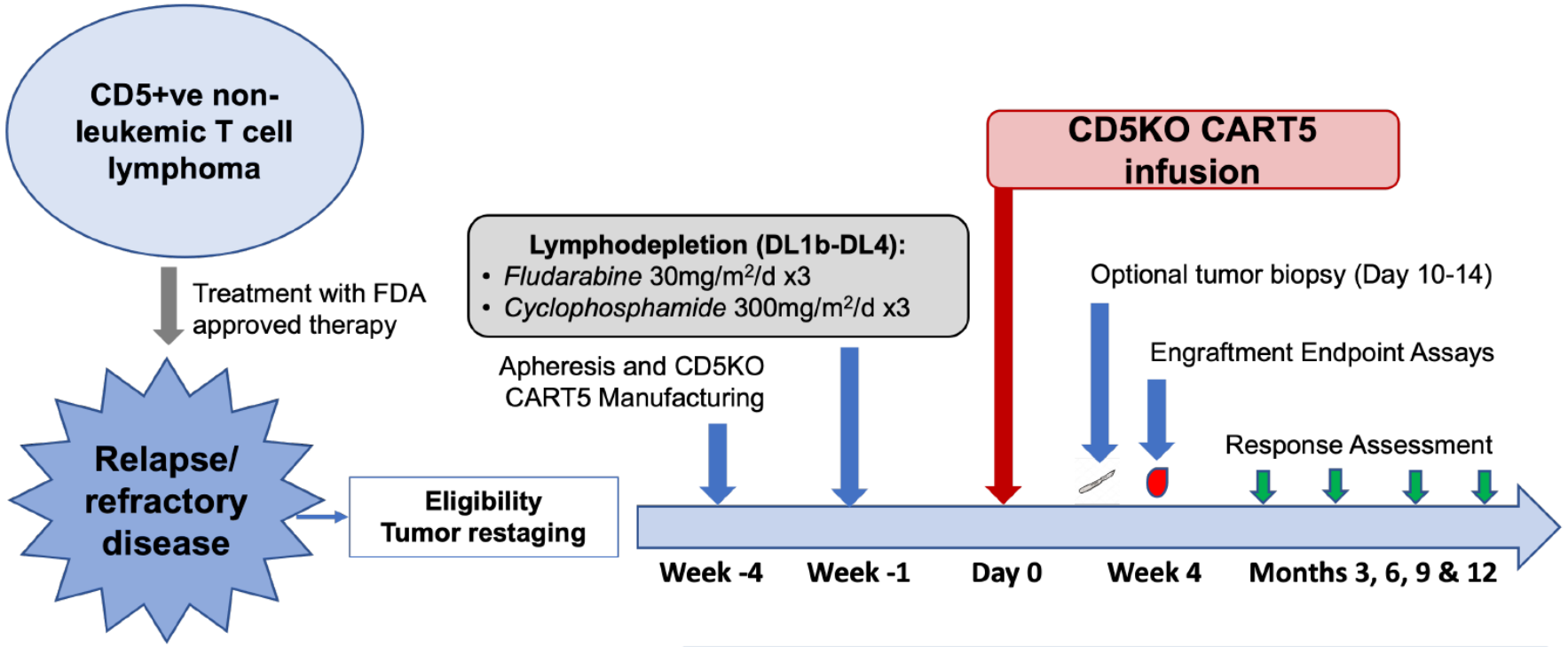
LEAD PROGRAM: VIPER-101

IND FDA cleared





A Phase I Clinical trial of CD5 KO CART5 for T cell lymphomas



Phase I:
Dose as per assigned dose level using a **BOIN design and** 6 **dose levels;** n≤21

Opportunistic infection prophylaxis:
- Levofloxacin and fluconazole during periods of neutropenia
- PJP and HSV/VZV ppx for 3 months or until CD4 count >200
- CMV and EBV viral load monitoring initially weekly (months 1-3), then monthly (months 4-12)

Post-infusion monitoring	
Months 1-6 Post-infusion	Monthly observation/ monitoring
Months 7-12 Post-infusion	Quarterly observation/ monitoring
Year 2+: Rollover to destination protocol for 15-year follow-up monitoring for delayed AEs related to gene transfer	

RECRUITING

NCT06420089 NEW

CD5-deleted Chimeric Antigen Receptor Cells (**Senza5** CART5) for T Cell Non-Hodgkin Lymphoma (NHL)

Conditions

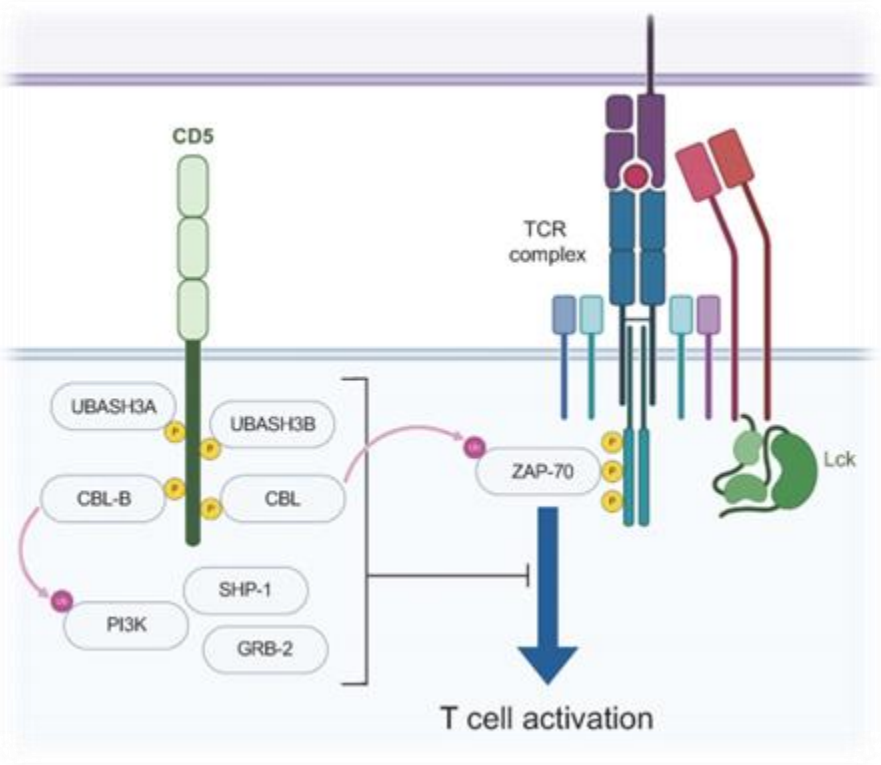
T Cell Non-Hodgkin Lymphoma

Locations

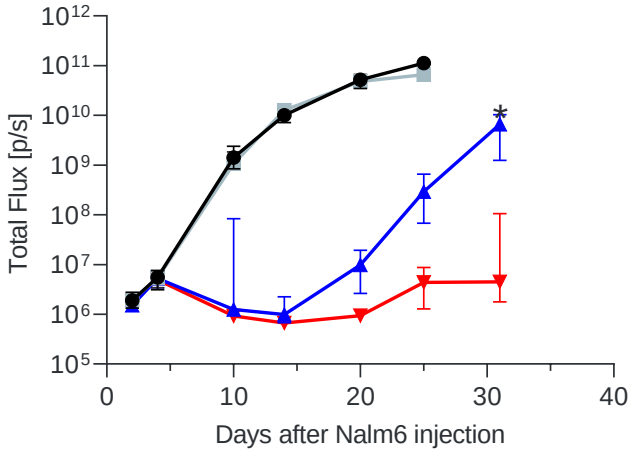
Philadelphia, Pennsylvania, United States

- **NOW OPEN AT PENN, SLOTS AVAILABLE**
- **Reach out to: PI: Stefan.barta@pennmedicine.upenn.edu**
  Rosemary Mazanet: RMazanet@vittoriabio.com

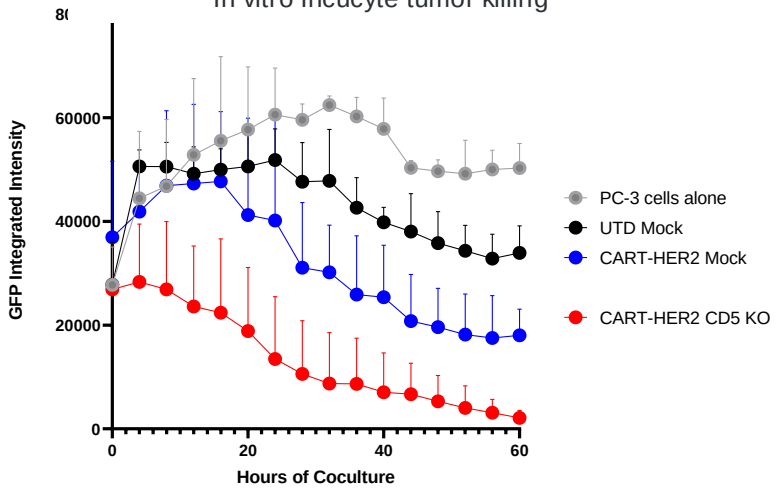
CD5 KO CAR T cells Enhance Efficacy in Multiple Liquid + Solid tumor Models



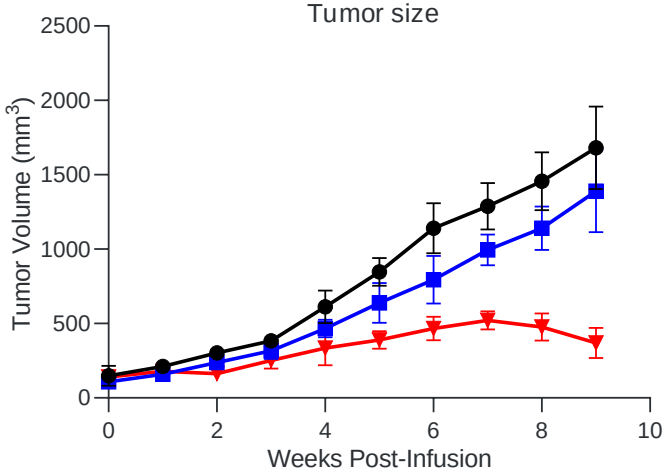
B-CELL LEUKEMIA AND LYMPHOMA
CD5 KO vs Traditional CD19 CAR-T
Tumor burden



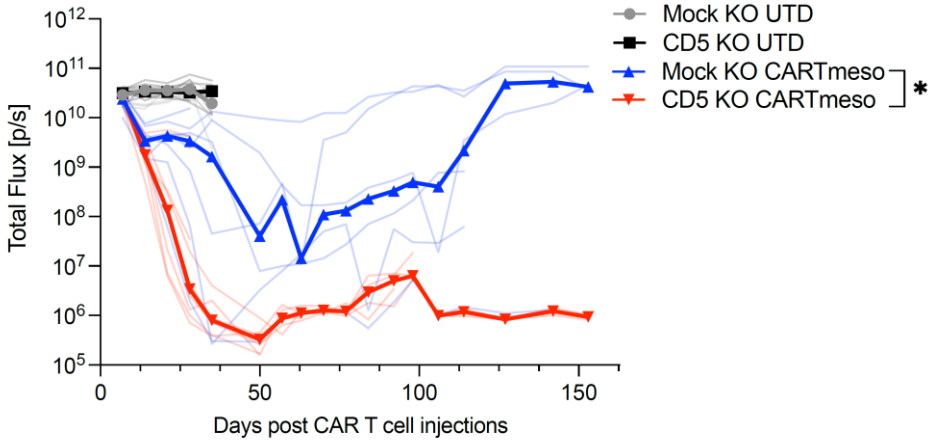
OVARIAN CANCER
CD5 KO vs Traditional HER2 CAR-T
In vitro Incucyte tumor killing



HODGKIN LYMPHOMA
CD5 KO vs Traditional CD30 CAR-T
Tumor size



PANCREATIC CANCER
CD5 KO vs Traditional Mesothelin CAR-T
Tumor size



Patel R., Science Immunology, 2024

Target Genes to Enhance CAR T cells in Clinical Studies: CRISPR

NCT Number	Study Title	Study Status	Conditions
NCT04244656	A Safety and Efficacy Study Evaluating CTX120 in Subjects With Relapsed or Refractory Multiple Myeloma	ACTIVE_NOT_RECRUITING	Multiple Myeloma
NCT04438083	A Safety and Efficacy Study Evaluating CTX130 in Subjects With Relapsed or Refractory Renal Cell Carcinoma (COBALT-RCC)	ACTIVE_NOT_RECRUITING	Renal Cell Carcinoma
NCT03545815	Study of CRISPR-Cas9 Mediated PD-1 and TCR Gene-knocked Out Mesothelin-directed CAR-T Cells in Patients With Mesothelin Positive Multiple Solid Tumors.	UNKNOWN	Solid Tumor, Adult
NCT03398967	A Feasibility and Safety Study of Universal Dual Specificity CD19 and CD20 or CD22 CAR-T Cell Immunotherapy for Relapsed or Refractory Leukemia and Lymphoma	UNKNOWN	B Cell Leukemia|B Cell Lymphoma
NCT05643742	A Safety and Efficacy Study Evaluating CTX112 in Subjects With Relapsed or Refractory B-Cell Malignancies	RECRUITING	B-cell Lymphoma|Non-Hodgkin Lymphoma|B-cell Malignancy|Chronic Lymphocytic Leukemia (CLL)/Small Lymphocytic Lymphoma (SLL)|Follicular Lymphoma|Mantle Cell Lymphoma|Marginal Zone Lymphoma|Large B-cell Lymphoma
NCT04037566	CRISPR (HPK1) Edited CD19-specific CAR-T Cells (XYF19 CAR-T Cells) for CD19+ Leukemia or Lymphoma.	UNKNOWN	Leukemia Lymphocytic Acute (ALL) in Relapse|Leukemia Lymphocytic Acute (All) Refractory|Lymphoma, B-Cell|CD19 Positive
NCT05722418	CRISPR-Edited Allogeneic Anti-BCMA CAR-T Cell Therapy in Patients With Relapsed/Refractory Multiple Myeloma	RECRUITING	Relapsed/Refractory Multiple Myeloma
NCT04502446	A Safety and Efficacy Study Evaluating CTX130 in Subjects With Relapsed or Refractory T or B Cell Malignancies (COBALT-LYM)	NOT RECRUITING	T Cell Lymphoma
NCT06014073	TRAC and Power3 Genes Knock-out Allogeneic CD19-targeting CAR-T Cell Therapy in r/r B-NHL	RECRUITING	Non Hodgkin's Lymphoma
NCT03166878	A Study Evaluating UCART019 in Patients With Relapsed or Refractory CD19+ Leukemia and Lymphoma	UNKNOWN	B Cell Leukemia|B Cell Lymphoma
NCT04767308	Safety and Efficacy of CT125A Cells for Treatment of Relapsed/Refractory CD5+ Hematopoietic Malignancies	UNKNOWN	CD5+ Relapsed/Refractory Hematopoietic Malignancies|Chronic Lymphocytic Leukemia (CLL)|Mantle Cell Lymphoma (MCL)|Diffuse Large B-cell Lymphoma (DLBCL)|Follicular Lymphoma (FL)|Peripheral T-cell Lymphomas (PTCL)

Target Genes to Enhance CAR T cells in Clinical Studies: CRISPR

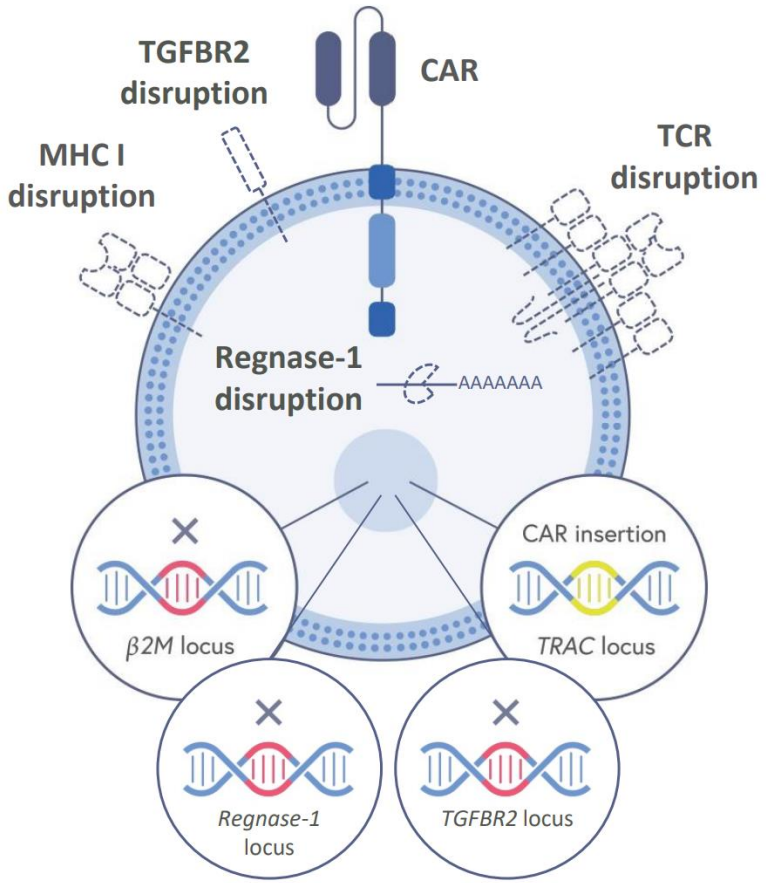
NCT Number	Study Title	Study Status	Conditions
NCT05812326	PD-1 Knockout Anti-MUC1 CAR-T Cells in the Treatment of Advanced Breast Cancer	COMPLETED	Advanced Breast Cancer|Breast Neoplasm Malignant Female
NCT04637763	CRISPR-Edited Allogeneic Anti-CD19 CAR-T Cell Therapy for Relapsed/Refractory B Cell Non-Hodgkin Lymphoma (ANTLER)	RECRUITING	Lymphoma, Non-Hodgkin|Relapsed Non Hodgkin Lymphoma|Refractory B-Cell Non-Hodgkin Lymphoma|Non Hodgkin Lymphoma|Lymphoma|B Cell Lymphoma|B Cell Non-Hodgkin's Lymphoma
NCT03747965	Study of PD-1 Gene-knocked Out Mesothelin-directed CAR-T Cells With the Conditioning of PC in Mesothelin Positive Multiple Solid Tumors	UNKNOWN	Solid Tumor, Adult
NCT05795595	A Safety and Efficacy Study Evaluating CTX131 in Adult Subjects With Relapsed or Refractory Solid Tumors	RECRUITING	Clear Cell Renal Cell Carcinoma|Cervical Carcinoma|Esophageal Carcinoma|Pancreatic Adenocarcinoma|Malignant Pleural Mesothelioma
NCT04976218	TGFÎ²R-KO CAR-EGFR T Cells in Previously Treated Advanced EGFR-positive Solid Tumors	RECRUITING	Solid Tumor, Adult|EGFR Overexpression
NCT04035434	A Safety and Efficacy Study Evaluating CTX110 in Subjects With Relapsed or Refractory B-Cell Malignancies (CARBON)	RECRUITING	B-cell Malignancy|Non-Hodgkin Lymphoma|B-cell Lymphoma|Adult B Cell ALL
NCT04557436	TT52CAR19 Therapy for B-cell Acute Lymphoblastic Leukaemia (B-ALL)	COMPLETED	B Acute Lymphoblastic Leukemia
NCT05037669	Programmed Allogeneic CRISPR-edited T Cells Engineered to Express Anti-CD19 Chimeric Antigen Receptor (PACE CART19) in Patients With Relapsed Or Refractory CD19+ Leukemia and Lymphoma	WITHDRAWN	Acute Lymphoblastic Leukemia|Chronic Lymphocytic Leukemia|Non Hodgkin Lymphoma
NCT06208878	A Long-term Follow-up Study of Subjects Who Received CRISPR CAR T Cellular Therapies	ENROLLING BY INVITATION	Multiple indications
NCT06492304	A Safety and Efficacy Study Evaluating CTX131 in Adult Subjects With Relapsed/Refractory Hematologic Malignancies	RECRUITING	Relapsed/Refractory Hematologic Malignancies
NCT06128044	CRISPR-Edited Allogeneic Anti-CLL-1 CAR-T Cell Therapy in Patients With Relapsed/Refractory Acute Myeloid Leukemia	RECRUITING	AML
NCT05169489	A Study of bbT369 in Relapsed and/or Refractory B Cell Non-Hodgkin's Lymphoma (NHL)	RECRUITING	NHL
NCT05945849	CD33KO-HSPC Infusion Followed by CART-33 Infusion(s) for Refractory/Relapsed AML (CART33)	RECRUITING	AML

CTX112 and CTX131 Incorporate Novel Potency Edits

Next-generation CRISPR gene-edited allogeneic CAR T chassis

Novel Potency Edits

- **Regnase-1 KO:** Increase functional persistence, cytokine secretion and sensitivity, and effector function
- **TGFBR2 KO:** Reduce tumor microenvironment inhibition of multiple CAR T cell functions



- **TCR KO:** Prevent GvHD
- **CAR KI:** Site-specific insertion into TRAC locus without using lentivirus
- **MHC I KO:** Improve persistence in the allogeneic setting and avoid need for more toxic lymphodepletion

CTX112 and CTX131 utilize the same CRISPR-edited allogeneic T cell chassis, *but CTX112 incorporates a CD19-targeted CAR while CTX131 incorporates a CD70-targeted CAR and knock-out of CD70*

RESEARCH ARTICLE

CRISPR-engineered T cells in patients with refractory cancer

EDWARD A. STADTMAUER, JOSEPH A. FRAIETTA, MEGAN M. DAVIS, ADAM D. COHEN, [...], AND CARL H. JUNE +36 authors Authors Info & Affiliations

SCIENCE • 6 Feb 2020 • Vol 367, Issue 6481 • DOI: 10.1126/science.aba7365

Target Genes to Enhance CAR T cells in Clinical Studies: TALEN

NCT Number	Study Title	Study Status	Conditions
NCT04150497	Phase 1/2 Study of UCART22 in Patients With Relapsed or Refractory CD22+ B-cell Acute Lymphoblastic Leukemia (BALLI-01)	RECRUITING	B-cell Acute Lymphoblastic Leukemia
~~NCT04142619~~	~~Study Evaluating Safety and Efficacy of UCART Targeting CS1 in Patients With Relapsed/Refractory Multiple Myeloma (MELANI-01)~~	~~TERMINATED~~	~~Relapsed/Refractory Multiple Myeloma~~
NCT05607420	Study Evaluating UCART20x22 in B-Cell Non-Hodgkin Lymphoma	RECRUITING	B-cell Non-Hodgkin Lymphoma (B-NHL)
NCT04416984	Safety and Efficacy of ALLO-501A Anti-CD19 Allogeneic CAR T Cells in Adults With Relapsed/Refractory Large B Cell Lymphoma (ALPHA2)	RECRUITING	Relapsed/Refractory Large B Cell Lymphoma
~~NCT04106076~~	~~Phase I Study of UCART123 in Patient With Adverse Genetic Risk Acute Myeloid Leukemia~~	~~WITHDRAWN~~	~~Acute Myeloid Leukaemia~~
NCT03190278	Study Evaluating Safety and Efficacy of UCART123v1.2 in Patients With Relapsed/Refractory Acute Myeloid Leukemia (AMELI-01)	RECRUITING	Acute Myeloid Leukaemia

Target Genes to Enhance CAR T cells in Clinical Studies: Base-editing

NCT Number	Study Title	Study Status	Conditions
NCT05885464	A Study Evaluating the Safety and Efficacy of BEAM-201 in Relapsed/Refractory T-Cell Acute Lymphoblastic Leukemia (T-ALL) or T-Cell Lymphoblastic Lymphoma (T-LL)	RECRUITING	Lymphoblastic Lymphoma|T-Cell Lymphoblastic Leukemia/Lymphoma|Lymphoblastic Leukemia
NCT05942599	Base Edited CAR T Cells Against AML: Deep Conditioning Ahead of Allogeneic Stem Cell Transplantation	RECRUITING	Relapsed Acute Myeloid Leukaemia
NCT05397184	Base Edited CAR7 T Cells to Treat T Cell Malignancies (TvT CAR7)	RECRUITING	Relapsed/Refractory T-cell Acute Lymphoid Leukaemia

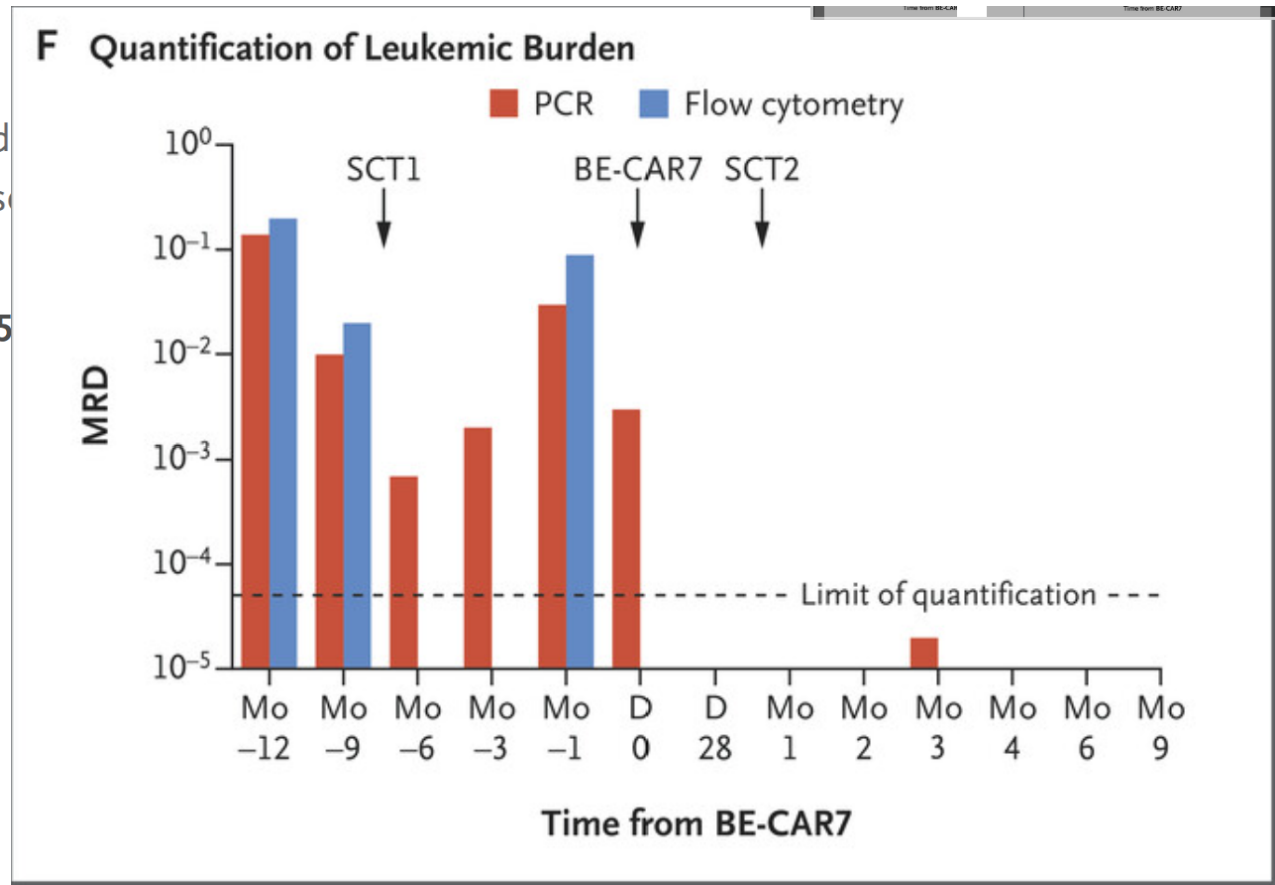
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Base-Edited CAR7 T Cells for Relapsed T-Cell Acute Lymphoblastic Leukemia

Authors: Robert Chiesa, M.D., Christos Georgiadis, Ph.D., Farhatullah Syed Ph.D., Soragia Athina Gkazi, Ph.D., Roland Preece, Ph.D., +10, for the Bas
Affiliations

Published June 14, 2023 | N Engl J Med 2023;389:899-910 | DOI: 10.105



Conclusions and Potential concerns

- Gene editing offers opportunity to modify CART to address efficacy, toxicity and allogeneic use
- Allogeneic approaches and strategies to avoid fratricide are the predominant approaches so far in the clinic
- PD-1, TGFRB, HPK1, CD5, regnase and others have been in clinical trials to enhance efficacy

- Regulatory complexities
- Insertional mutagenesis
- Enhanced toxicity
- Reduced persistence (alloCART)
- Intellectual property
- Costs

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and all lab members

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Michael Cook
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and all clinical staff

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Noelle Frey
Al Garfall
Adam Cohen

Patients and their families

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Adam Snook
Aditya Nimmagadda
Rosemary Mazanet
Stephen Yang

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DKMS We Delete Blood Cancer
Lymphoma Research Foundation
Hairy Cell Leukemia Foundation
Leukemia & Lymphoma Society
American Society of Hematology
Parker Institute for Cancer Immunotherapy
Lymph&Co
The Laffey-McHugh Foundation
Emerson Collective
National Cancer Institute

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https://www.med.upenn.edu/ruella-lab/