Penn Medicine
Center for Cellular Immunotherapies

The Role of Diet and Microbiota on CART Immunotherapy

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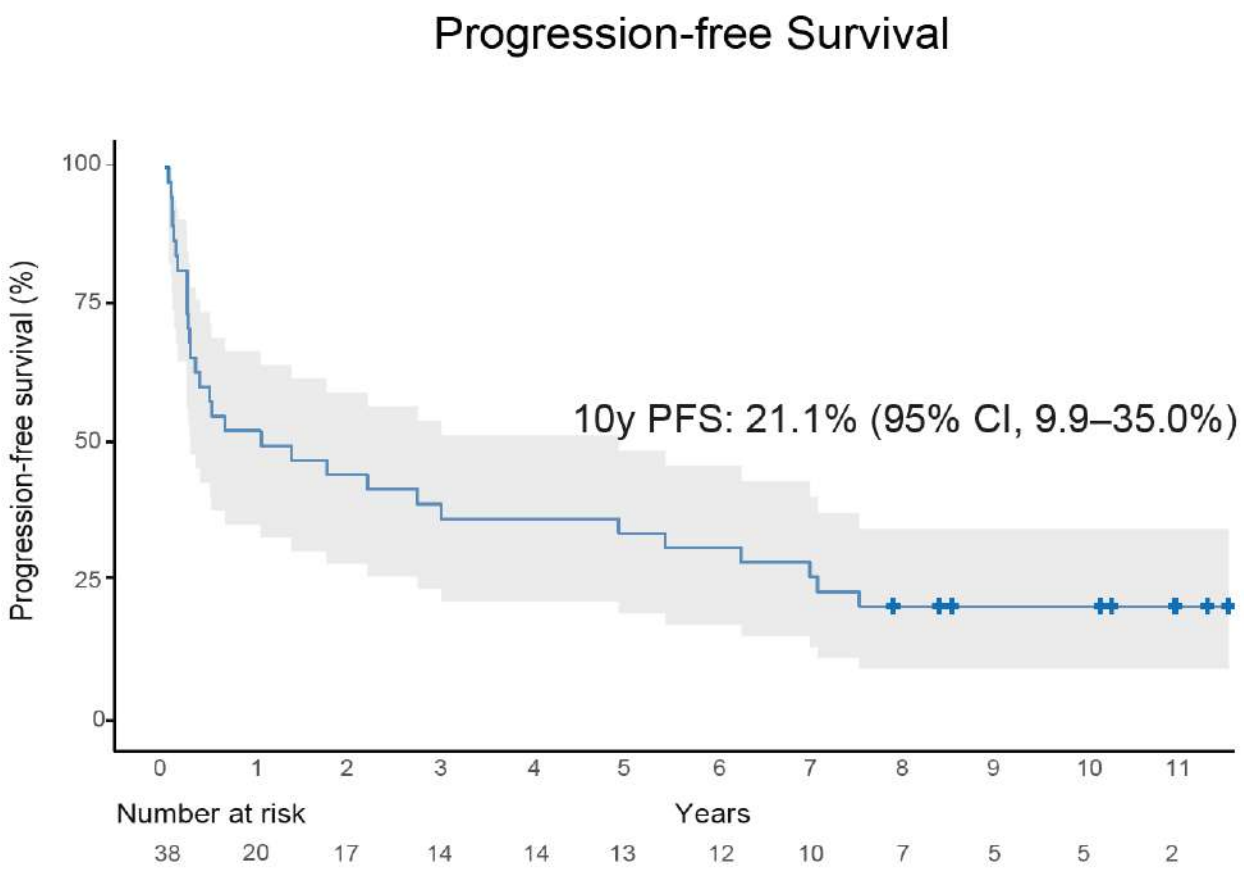
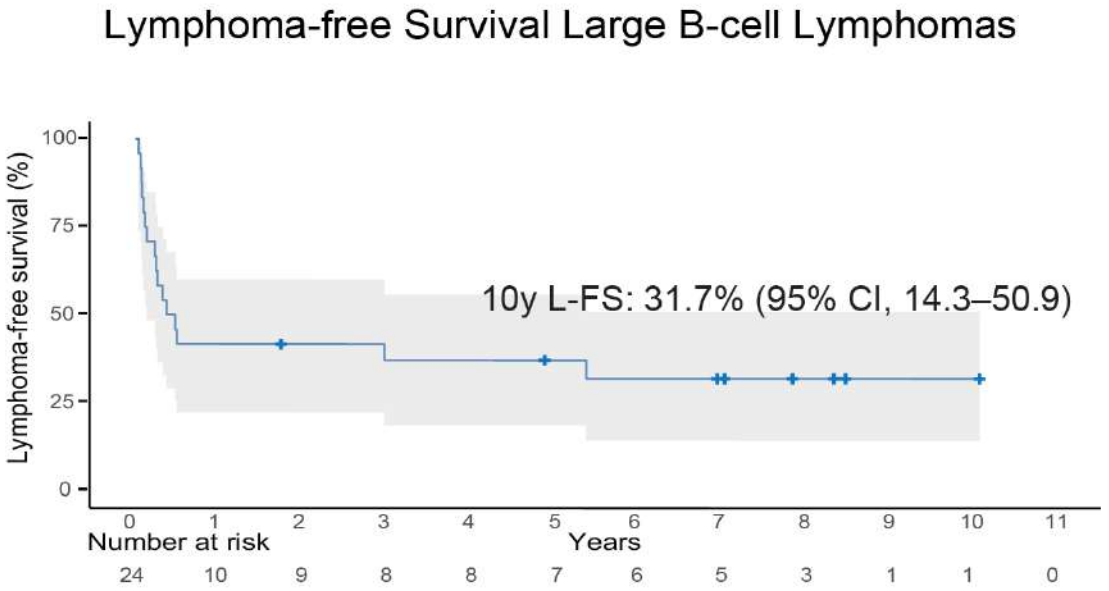
RUELLA LAB

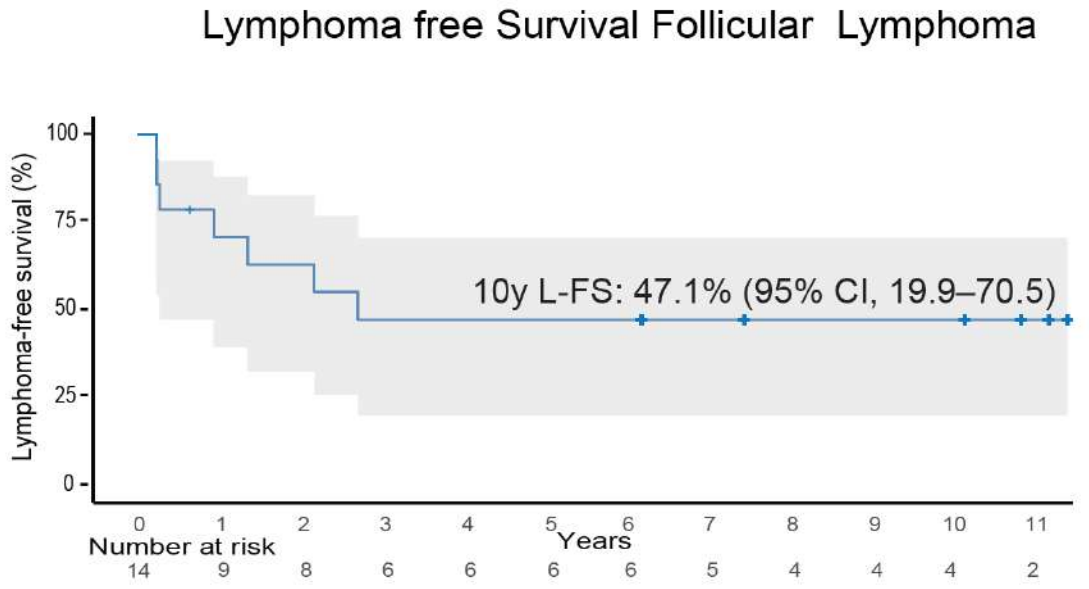
Presenter disclosure information

- ***Inventor:*** CART technologies, Univ. of Pennsylvania, partly licensed to Novartis, Tmunity (Kite), and viTToria biotherapeutics
- ***Research Funding:*** AbClon, Beckman-Coulter, ONI, Lumicks, viTToria bio, ORFLO, CURIOX
- ***DSMB****:* PeproMene
- ***Consultancy/Honoraria:*** GLG, Guidepoint
- ***Advisory Board:*** , AbClon, viTToria bio, BMS, Lumicks, ModeX, Acera tx, Biocartis
- ***Scientific Founder:*** viTToria biotherapeutics, Linfa therapeutics

CTL019 (now tisa-cel) at 10 years: PFS and LFS







Ruella M., Paruzzo L., and Schuster S.J., in revision

-- Mechanism of CART resistance --

Pre-infusion barriers-access

High Costs/Access

Progression pre/ during manufacturing

Manufacturing failure

Low lymphocytes

Lymphodepletion

Host factors

Tumor burden, stage, extra-nodal sites, previous therapies

Age, ECOG, comorbidities

Immunerejection

Diet, Microbiota

Inflammation (CRP, Ferritin, IL-6)

CART dysfunction

Exhausted/ senescent T cell

Terminally differentiated T cell

Low cytotoxicity

Reduced persistence

Physical barrier and stroma

Tumor microenvironment

High LDH, low glucose, low nutrients

Low oxygen

Low pH

TGF-β

IL-10

Tumor cell

CART

MDSC

TAM

Treg

Costim/inhibition (PD-L1 and others)

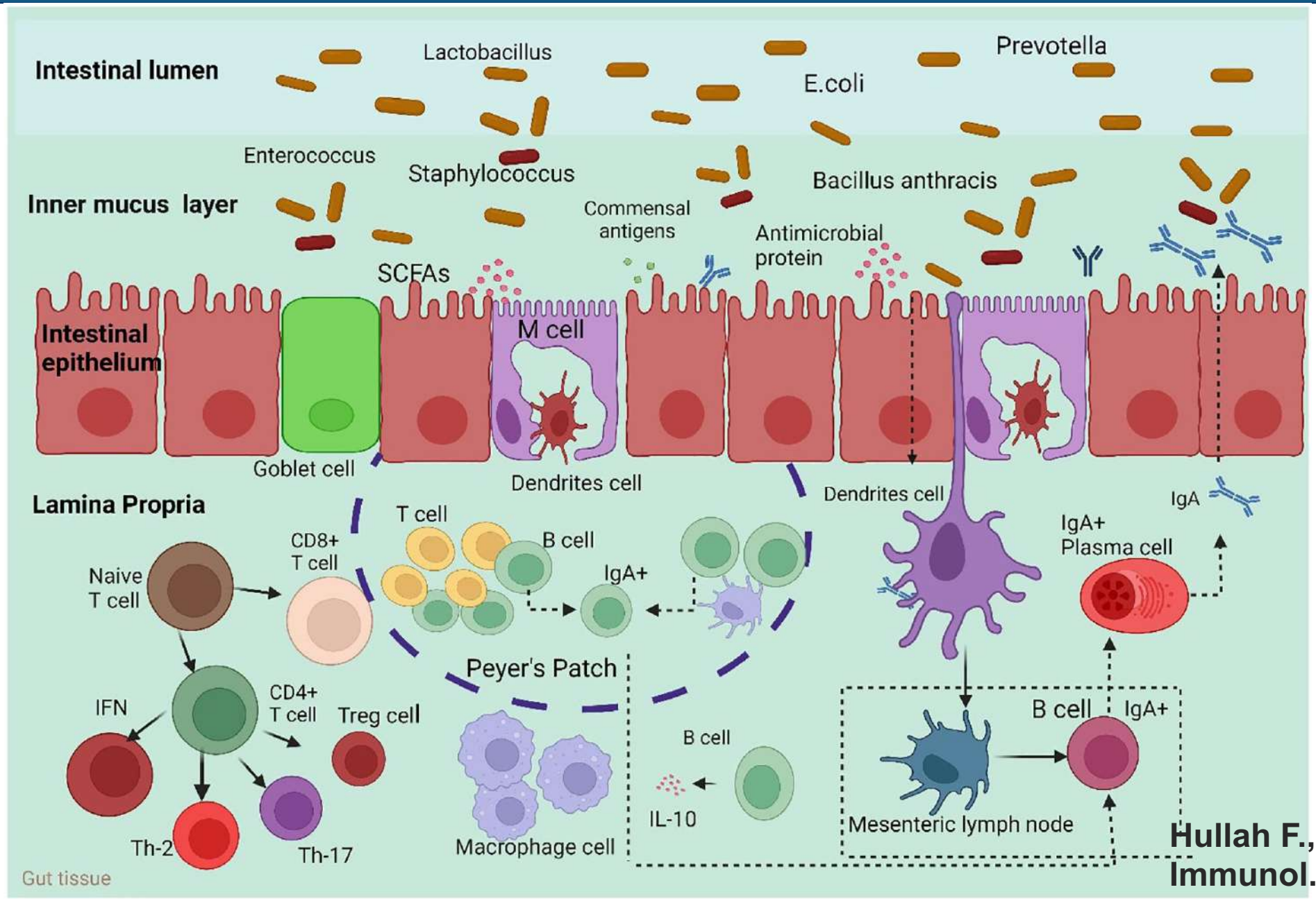
Apoptosis, TP53 mutations, double-hit

Antigen dim/loss

Tumor-intrinsic mechanisms

Updated from Ghilardi G., BJH, 2021

The Gut Microbiota and the Immune System



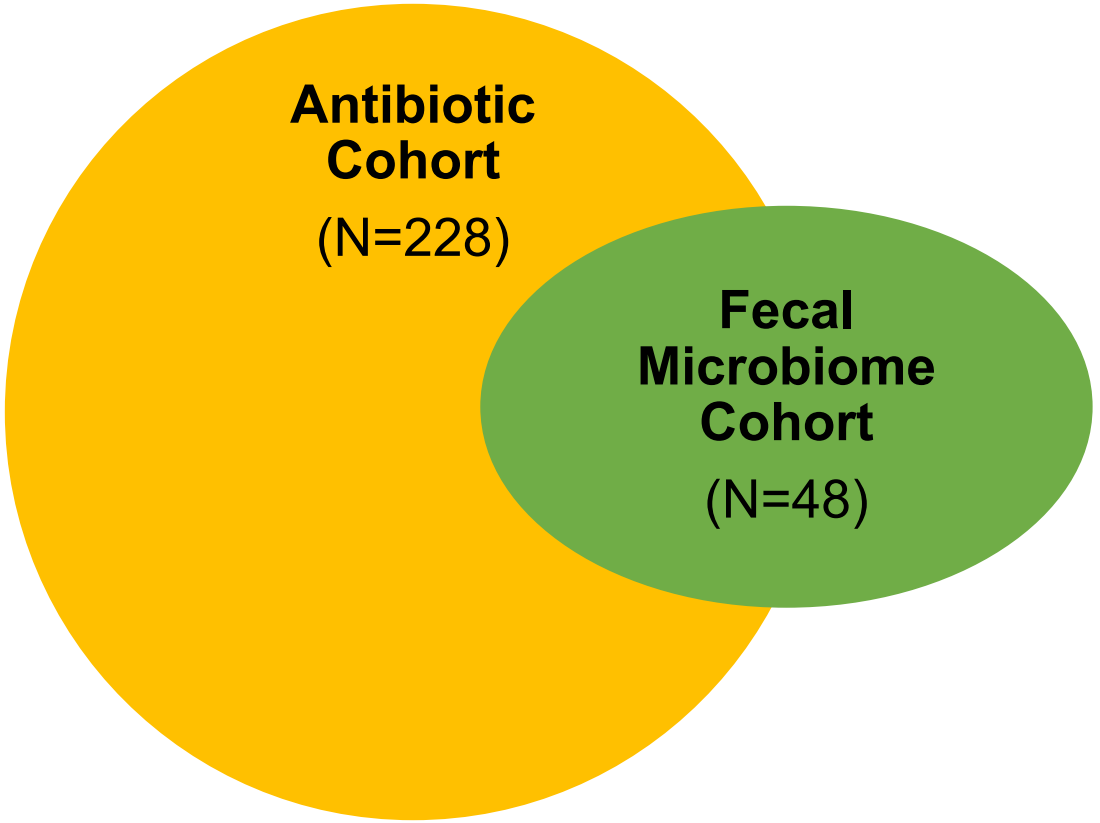
Hullah F., Front. Immunol., 30 July 2024

What is the role of the gut microbiota on CART immunotherapy?

M. van den Brink

M. Smith

Collaboration between Memorial Sloan Kettering Cancer Center (MSK) and the University of Pennsylvania (Penn)

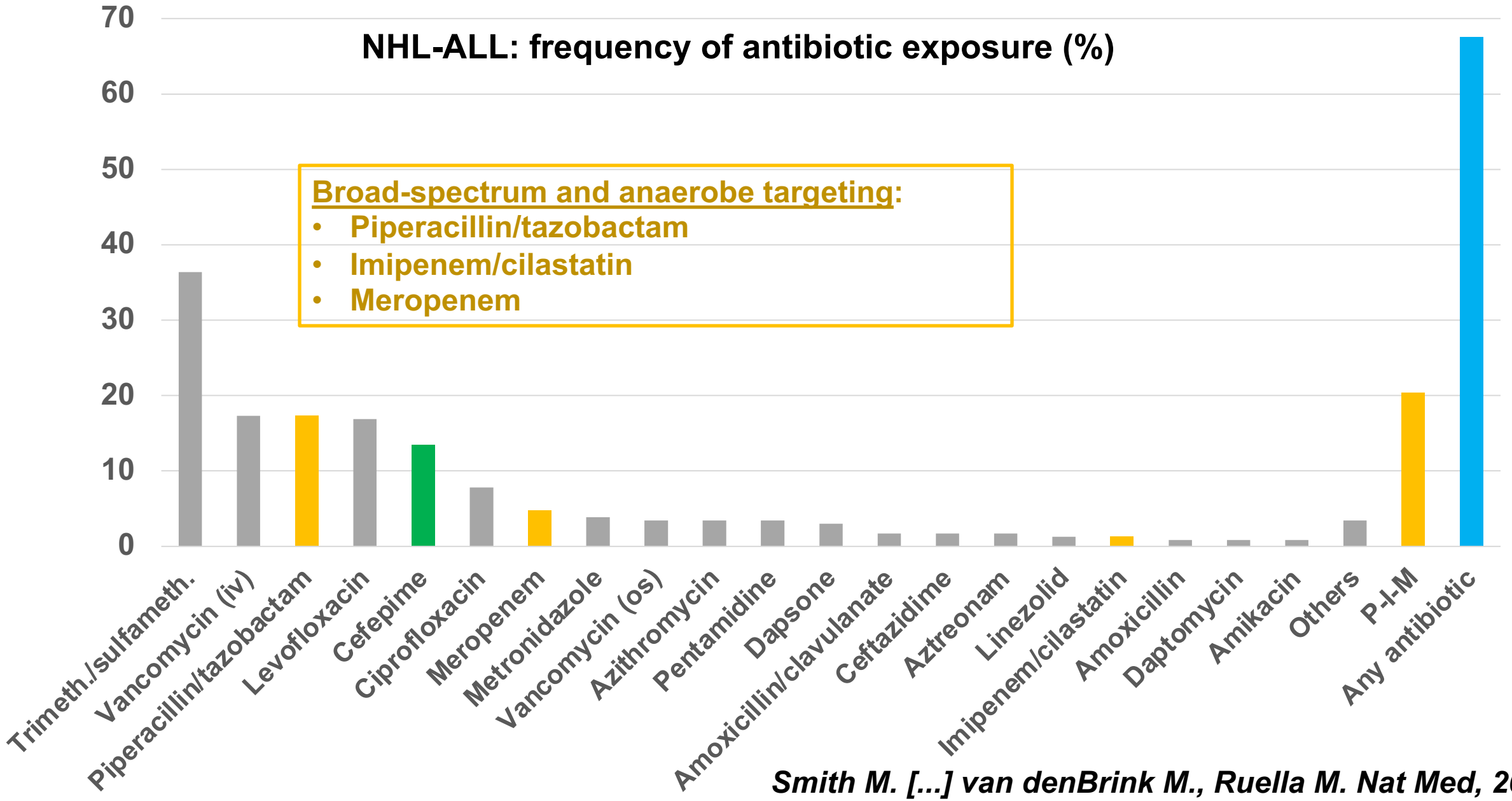


Hypothesis:

We hypothesize that the composition of the intestinal microbiome before CD19 CAR T cell infusion is associated with clinical outcomes in patients with **ALL and NHL**.

Smith M. et al, Nat Med, 2021

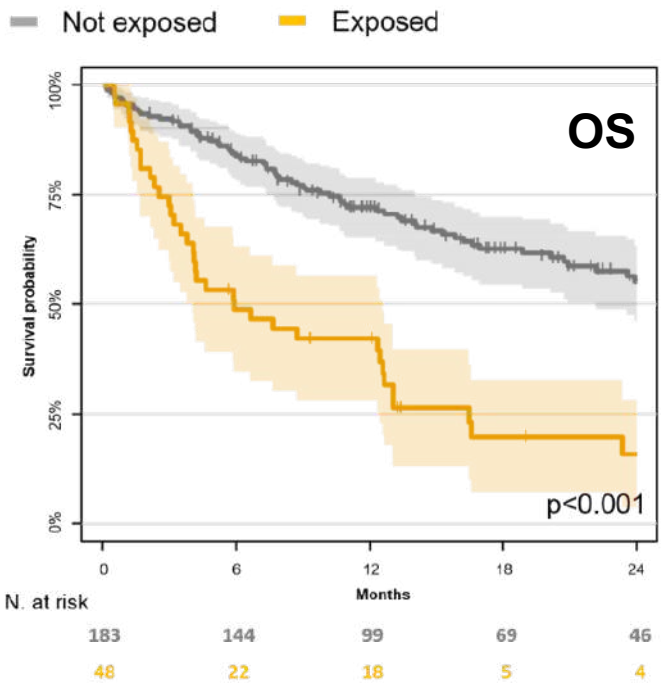
Heavy exposure to antibiotic: pre-CART



Smith M. [...] van denBrink M., Ruella M. Nat Med, 2021

Impact of antibiotics on CART outcomes

NHL-ALL: exposure to P-I-M antibiotics



- **Piperacillin/tazobactam**
- **Imipenem/cilastatin**
- **Meropenem**

Overall Survival					
		Univariable*		Multivariable*	
Variable		HR (95% CI)	P-value	HR (95% CI)	p-value
Gender	Female	(reference)	0.157		
	Male	1.37 (0.89-2.11)			
Age	(Continuous)	1 (0.99-1.01)	0.669		
Disease	ALL	(reference)	0.097	(reference)	0.130
	NHL	0.72 (0.48-1.06)		0.72 (0.46-1.1)	
Performance status (ECOG)	0	(reference)	0.03	(reference)	0.033
	1	1.49 (0.98-2.27)		1.59 (1.0-2.54)	
	2-3	2.49 (1.21-5.15)		2.56 (1.2-5.45)	
Previous lines of therapy	≤4	(reference)	0.404		
	>4	0.85 (0.59-1.24)			
Costimulatory domain	4-1BB	(reference)	0.865		
	CD28	1.04 (0.63-1.73)			
LDH	(Continuous, per 100)	1.33 (1.18-1.5)	<0.001	1.34 (1.18-1.52)	<0.001
P-I-M antibiotic exposure	No	(reference)	<0.001	(reference)	<0.001
	Yes	2.71 (1.76-4.16)		2.58 (1.55-4.3)	

Smith M. [...] van denBrink M., Ruella M. Nat Med, 2021

CAR T cell Therapy: Antibiotic Exposure is Linked with Poor Outcomes

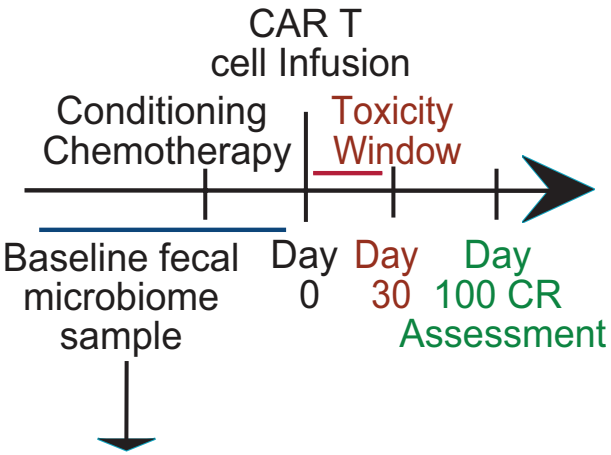
Study	CAR	Antibiotic timepoint	Antibiotics linked to worse survival	Toxicity
Smith 2022[1]	Anti-CD19	4 weeks pre-CAR	Any, P-I-M	ICANS ↑
Stein-Thoeringer 2023[2]	Anti-CD19	3 weeks pre-CAR	Any, high-risk antibiotics	ICANS ↑
Prasad 2025[3]	Anti-CD19	6 weeks pre-CAR	Any, P-I-M, anaerobic antibiotics	
Yin 2025[4]	Anti-BCMA	4 weeks pre-CAR	Any, cephalosporin, fluoroquinolone	NS
Marcos-Kovandzic 2025 [5]	Anti-CD19	4 weeks pre-CAR	No association	

(1) Smith, M et al. *Nat Med* 2022
(2) Stein-Thoeringer, C et al. *Nat Med* 2023
(3) Prasad, R et al. *Blood* 2025
(4) Yin, L et al. *Front Immunol* 2025
(5) Marcos-Kovandzic, L et al. Cancer Discovery, 2025

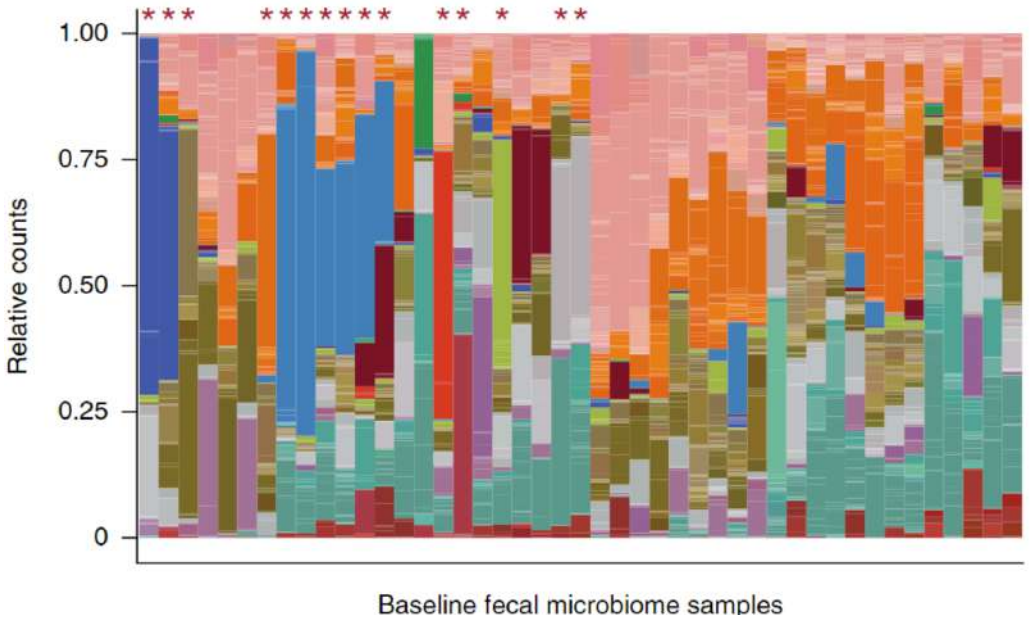
Fecal microbiome in CART patients

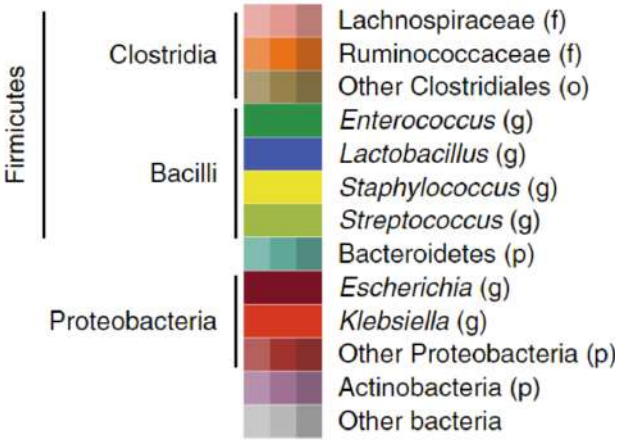
Fecal Microbiome Cohort (N=48)
Prospective fecal collection

MSK (n=28)
Penn (n=20)



Metagenomic Shotgun
Sequencing

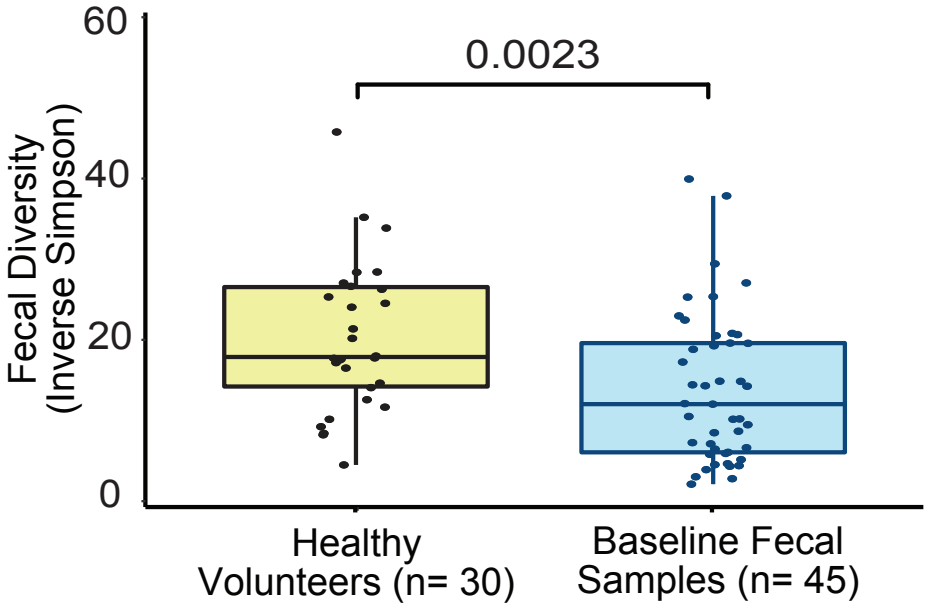




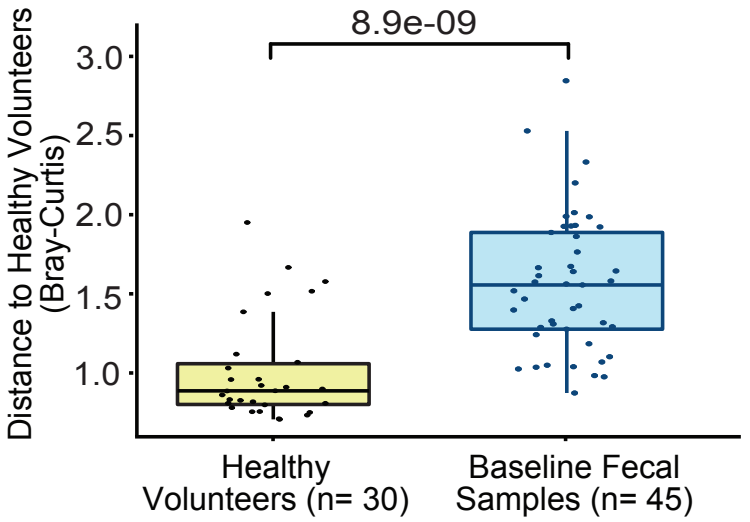
- Samples collected and aliquoted locally
- Sequenced at a central location at MSKCC

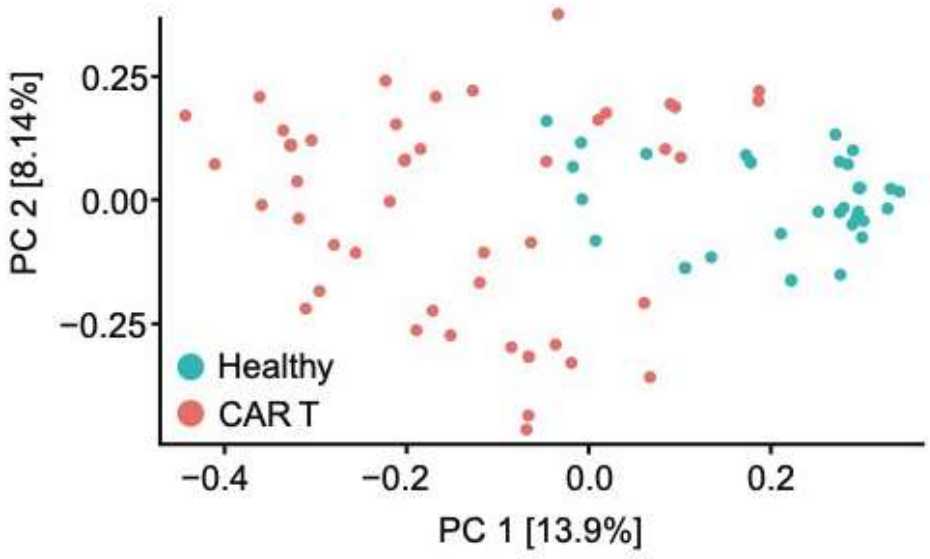
Diversity in CART patients at baseline compared to healthy people

Alpha-diversity at baseline



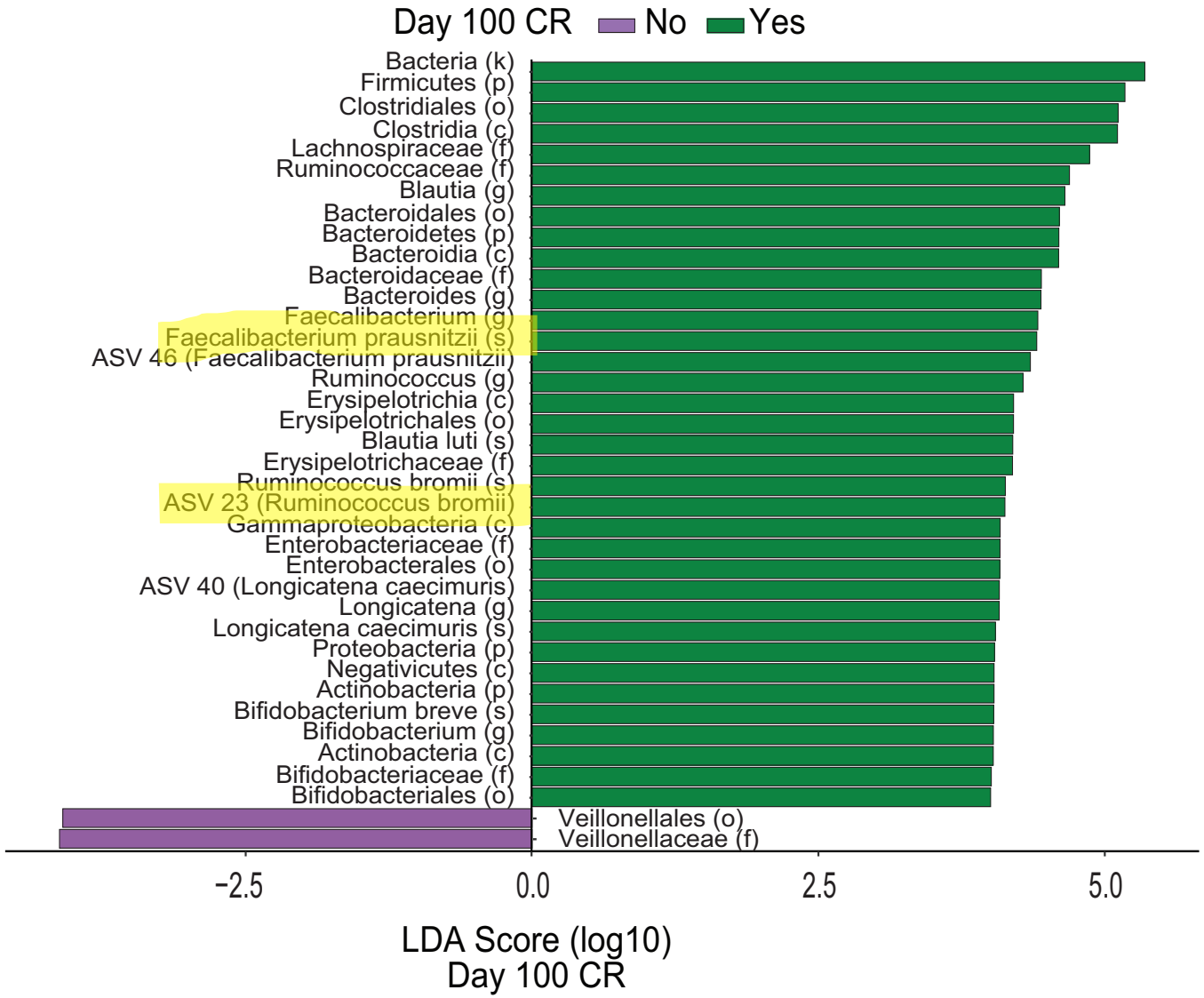
Beta-diversity at baseline





16S sequencing
Wilcoxon rank, PCOA

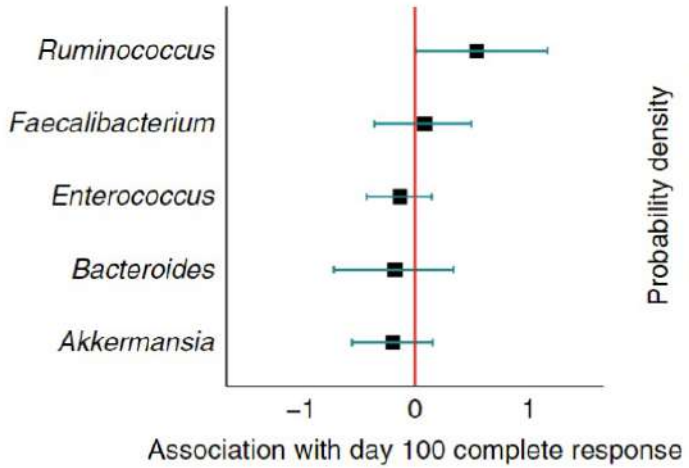
Linear discriminant analysis (LDA) scores computed for differentially abundant bacterial taxa of Day 100 CR

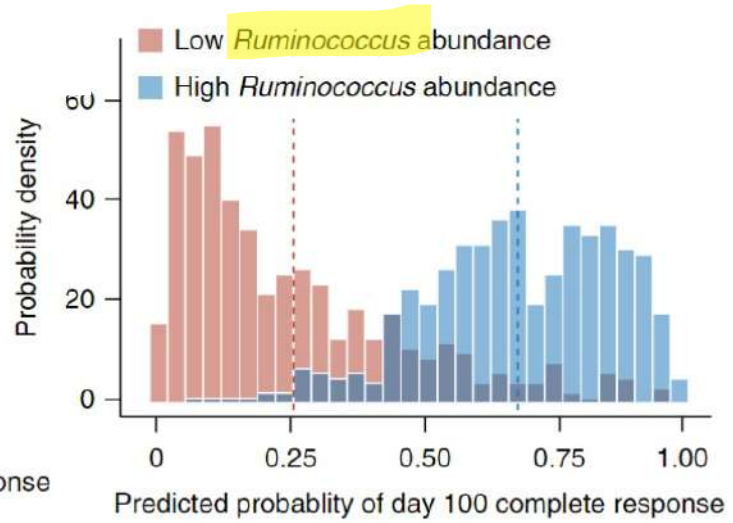


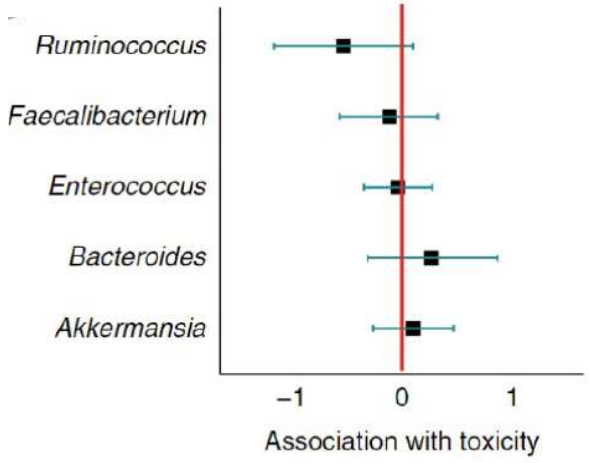
Enrichment of microbial taxa within the class Clostridia, including the genera *Ruminococcus* and *Faecalibacterium*, as well as the species *Faecalibacterium prausnitzii* were associated with Day 100 CR

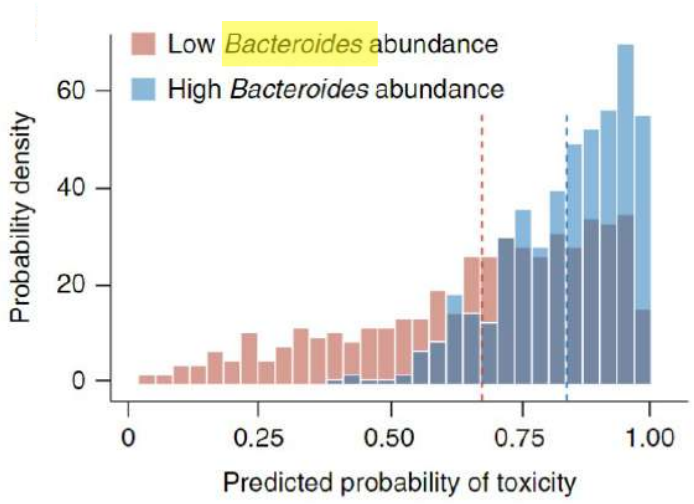
16S sequencing
Linear discriminant analysis of effect size (LEfSe)

Bayesian analysis of relevant genera









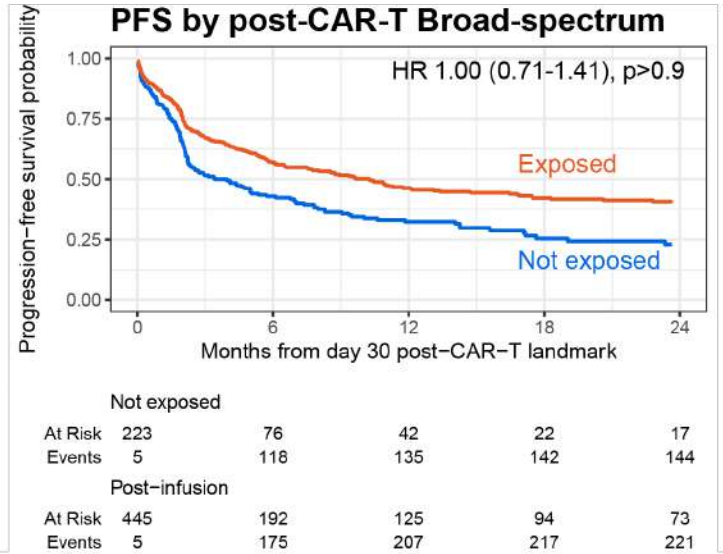
Gopalakrishnan, V., *et al. Science* (2018).
Matson, V., *et al. Science* (2018).
Routy, B., *et al. Science* (2018).
Schluter, J., *et al. Nature* (2020).
Dubin, K., *et al. Nat Commun* (2016).

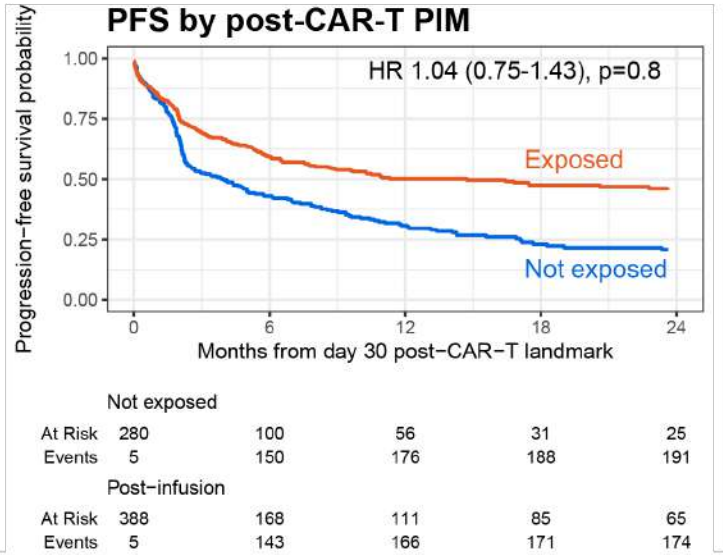
Microbiota composition and CART outcomes

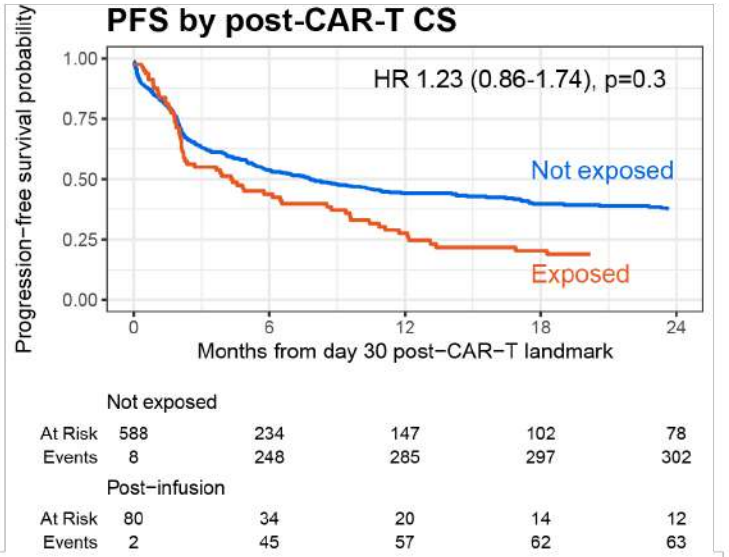
DISEASE/CART	STUDY	N	ENRICHED IN CR	ENRICHED IN CRS
ALL/NHL/CART19	***Smith M, Nat Med, 2022***	48	PRE: *Ruminococcus* *Faecalibacterium* *Bacteroidetes*	*Bacteroides*
MM/CARTBCMA ALL/CART9 NHL/CART19	***Hu Y, Nat Comm, 2022***	43+38	MM PRE and POST: *Prevotella, Collinsella, Bifidobacterium, and Sutterella* NHL PRE and POST: *Faecalibacterium, Bifidobacterium, and Ruminococcus*	*Bifidobacterium, and Leuconostoc*
NHL/CART19	***Stein-Thoeringer CK, Nat Med, 2023***	79*-95	*PRE:* *Bacteroides, Ruminococcus, Eubacterium and Akkermansia*	na
NHL(B-ALL)CART19	***Marcos-Kovandzic, L et al. Cancer Discov 2025***	58	*B. Uniformis B. intestinalis, A. muciniphilaR. Lactaris R. torques*	na

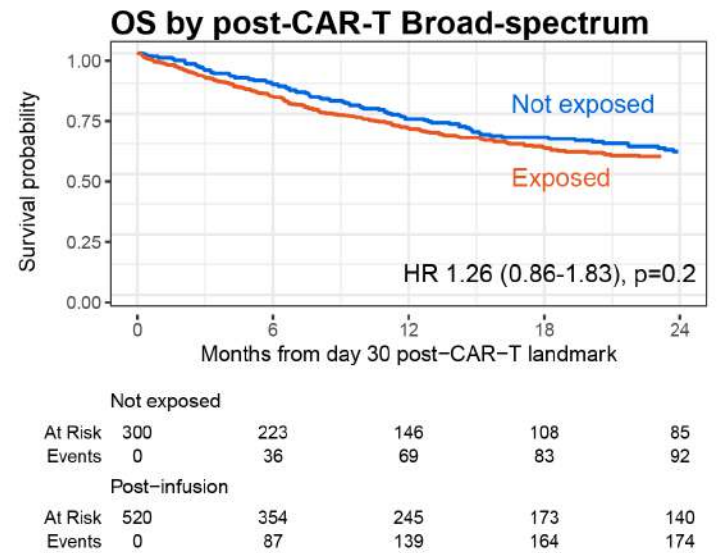
Also baseline a-diversity not associated with outcomes
*no exposure to PIM

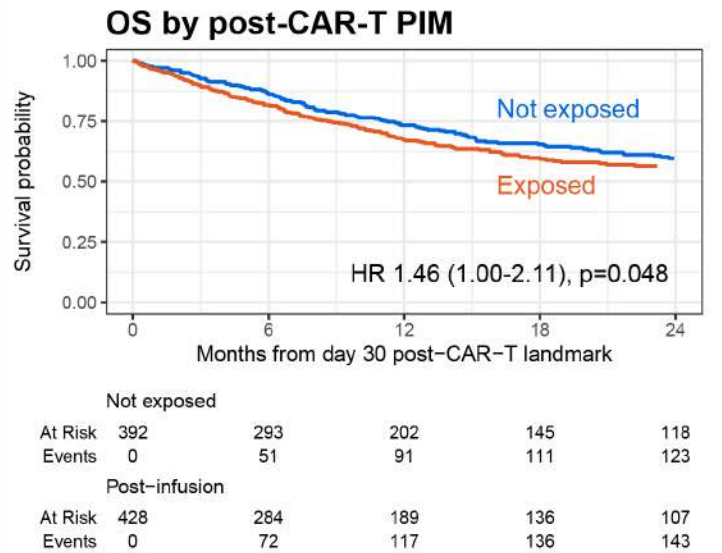
Larger Study on Antibiotic Exposure Pre and Post CART

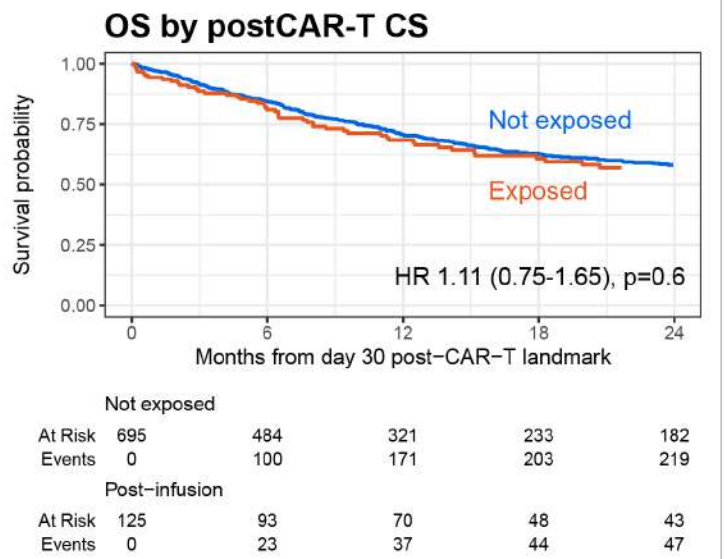












Landmarked hazard ratios (95%CI) for exposed vs. non-exposed derived from multivariable adjusted for age, bridging, baseline LDH, CAR-T product, and CRS

Shouval R., ASH, 2024

Heavy exposure to antibiotic: post-CART

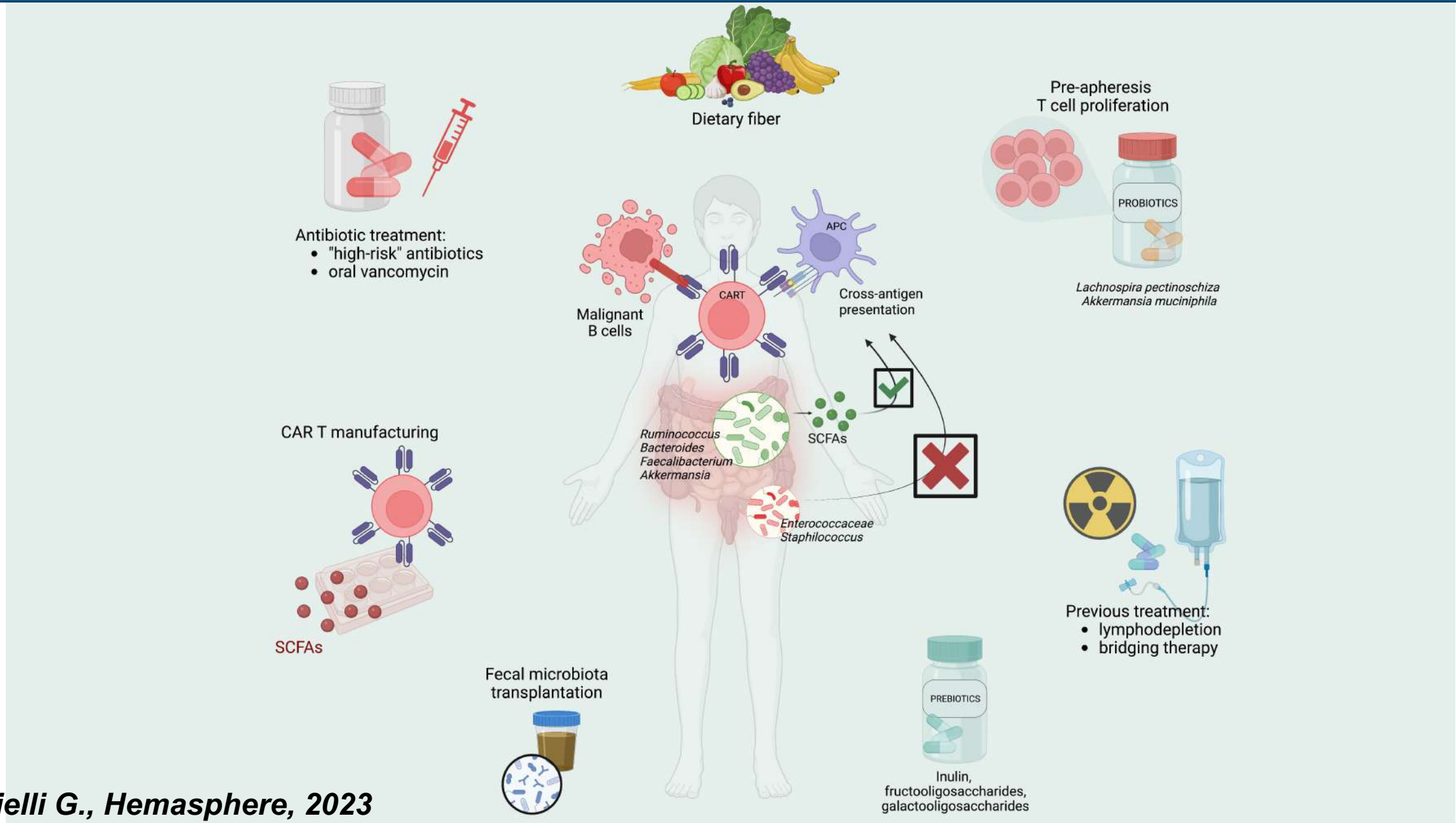
	Characteristic	Overall N = 956	MSKCC n = 319	UPENN n = 259	Sheba n = 237	LMU n = 88	Rambam n = 53
	Age[1]	63 (52, 70)	66 (56, 73)	62 (52, 70)	59 (45, 69)	64 (57, 68)	64 (55, 72)
	CAR-T Product						
	Axi-cel	379 (40%)	170 (53%)	58 (22%)	66 (28%)	47 (53%)	38 (72%)
	Tisa-cel	338 (35%)	73 (23%)	173 (67%)	44 (19%)	33 (38%)	15 (28%)
	Liso-cel	112 (12%)	76 (24%)	28 (11%)	0 (0%)	8 (9.1%)	0 (0%)
	Academic CD19-CAR-T	127 (13%)	0 (0%)	0 (0%)	127 (54%)	0 (0%)	0 (0%)
Pre-CAR	**CRS**	714 (75%)	235 (74%)	144 (56%)	208 (88%)	77 (88%)	50 (94%)
Pre-CAR	**PIM exposure < d0**	97 (10%)	45 (14%)	4 (1.5%)	10 (4.2%)	30 (34%)	8 (15%)
Pre-CAR	**CS^{4th} exposure < d0**	24 (2.5%)	8 (2.5%)	16 (6.2%)	0 (0%)	0 (0%)	0 (0%)
Post-CAR[2]	**PIM exposure ≥ d0**	536 (56%)	216 (68%)	27 (10%)	171 (72%)	79 (90%)	43 (81%)
Post-CAR[2]	**PIM median days of exposure**[1]	9 (6, 12)	12 (9, 16)	7 (5, 11)	7 (4, 9)	8 (7, 9)	10 (8, 12)
Post-CAR[2]	**CS^{4th} exposure ≥ d0**	151 (16%)	43 (13%)	105 (41%)	0 (0%)	3 (3.4%)	0 (0%)
Post-CAR[2]	**CS^{4th} days of exposure**[1]	5 (3, 8)	8 (4, 10)	4 (3, 6)	6 (3, 8)	-	-

1 - Median (IQR); 2. **67% of patients were exposed to broad-spectrum antibiotics post-CAR-T**
PIM - piperacillin-tazobactam, imipenem, and meropenem; CS - 4th generation cephalosporins

Shouval R., ASH, 2024

Memorial Sloan Kettering Cancer Center · Penn University of Pennsylvania · SHEBA Tel HaShomer · LMU Ludwig-Maximilians-Universität München · RAMBAM Health Care Campus

Modulation of the Gut Microbiota to improve CART



Gabrielli G., Hemasphere, 2023

Ongoing Trials

NCT06218602 Recruiting

Pilot Trial of Fecal **Microbiota** Transplantation for Lymphoma Patients Receiving Axicabtagene Ciloleucel Therapy.

Conditions

Lymphoma

Locations

Houston, Texas, United States

NCT07042438 Not yet recruiting

Fecal Microbiome Transplant to Remodel Intestinal **Microbiota** for Patients With Relapsed or Refractory Lymphoma With Exposure to High-Risk Antibiotics Who Are Receiving **Chimeric Antigen Receptor** T Cells

Conditions

Recurrent Diffuse Large B-Cell Lymphoma | Recurrent High Grade B-Cell Lymphoma With MYC and BCL2 or BCL6 Rearrangements | Recurrent Transformed Follicular Lymphoma to Diffuse Large B-Cell Lymphoma | Refractory Diffuse Large B-Cell Lymphoma

Show 2 more conditions

Locations

Duarte, California, United States

NCT06734624 Not yet recruiting New

The Microbiome in Blood Cancer and HLH

Conditions

Diffuse Large B Cell Lymphoma (DLBCL) | Follicular Lymphoma (FL) | Haemophagocytic Lymphohistiocytosis

Locations

Nottingham, Nottinghamshire, United Kingdom

NCT04203459 Unknown status *

The Mechanism of Enhancing the Anti-tumor Effects of CAR-T on PC by Gut **Microbiota** Regulation

Conditions

CAR-T | Gut Microbiota | Pancreatic Cancer

Locations

Harbin, Heilongjiang, China

NCT06041815 Recruiting

Correlation Between Gut **Microbiota** and Clinical Response to CAR-T Treatment for Hematological Malignancies

Conditions

Chimeric Antigen Receptor T-cell Therapy | Hematological Malignancies

Locations

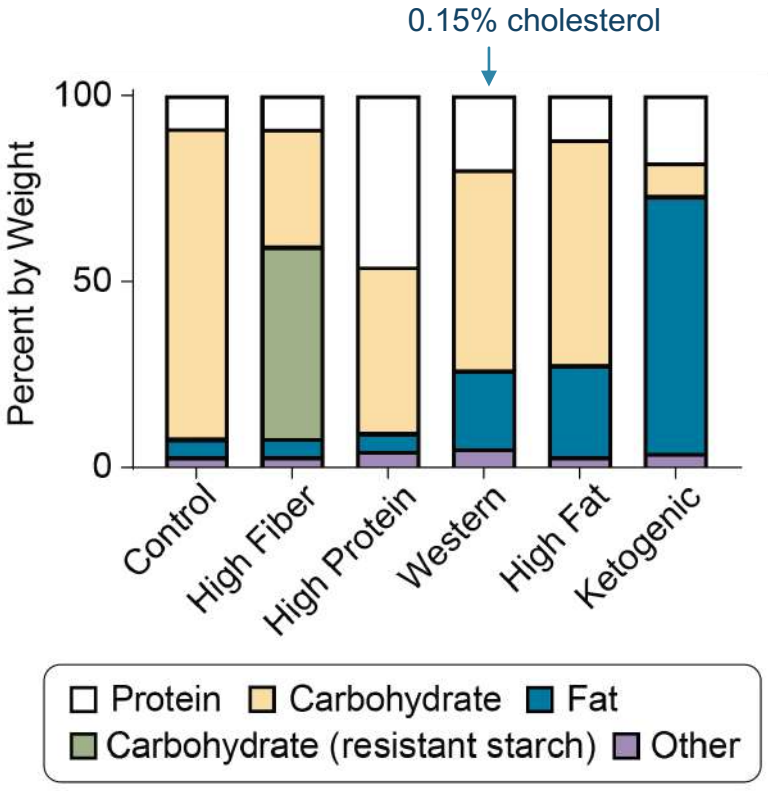
Suzhou, Jiangsu, China

Effect of Diet on CART Immunotherapy

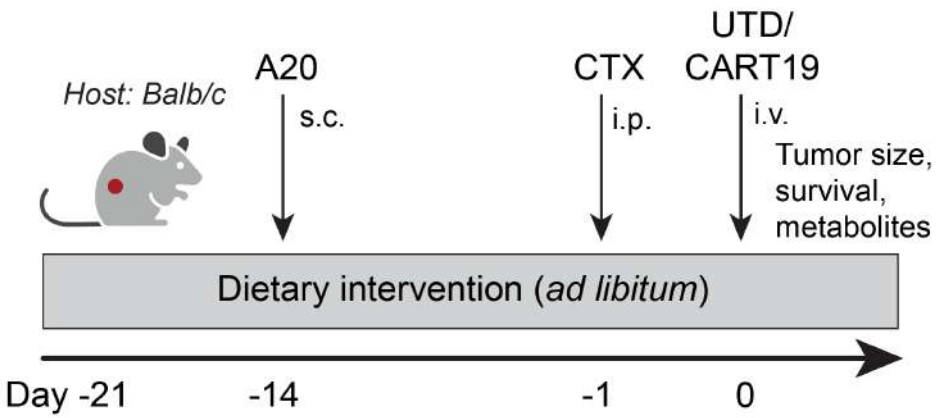
Shan Liu, PhD

Puneeth Guruprasad, PhD

Diet composition

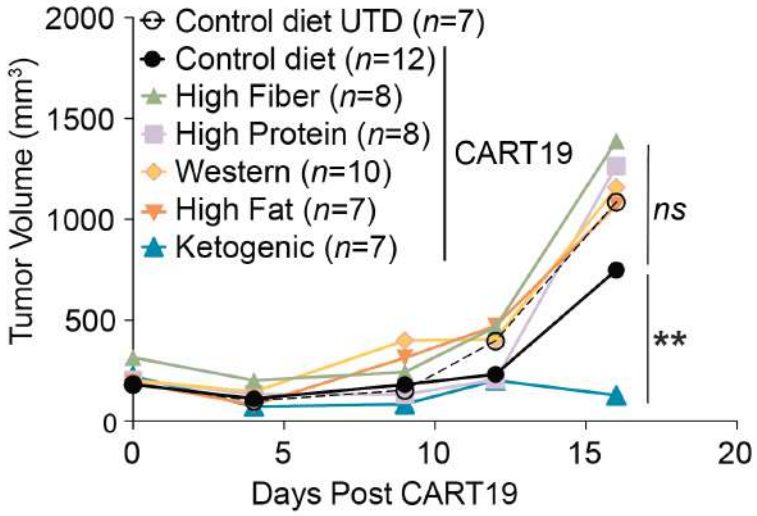


Diet and CART screening



UTD: untransduced T cells
CTX: cyclophosphamide

Tumor burden

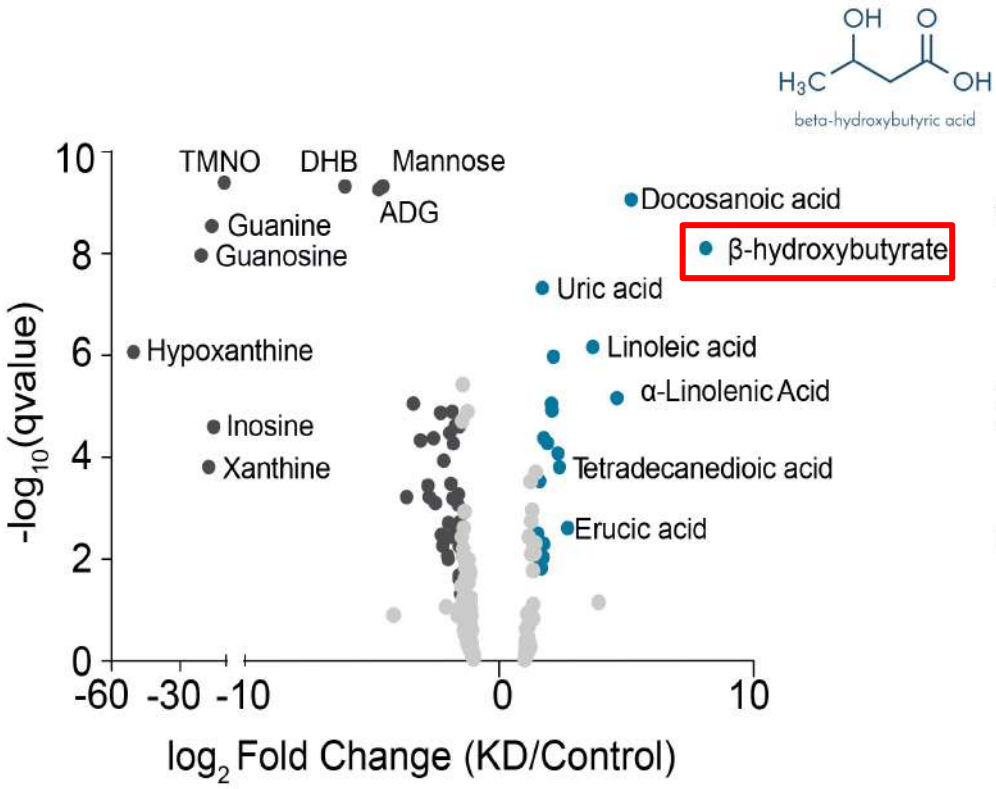


ASH Plenary Talk #4, 2024

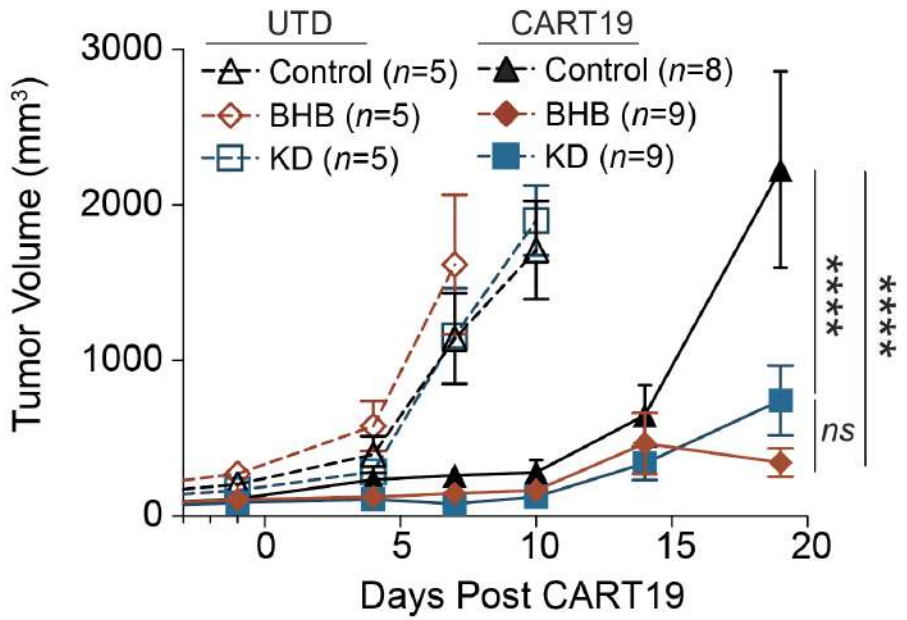
Liu S., Guruprasad P., Cell, in press

Ketogenic diet enhances CART proliferation via β-hydroxybutyrate (BHB)

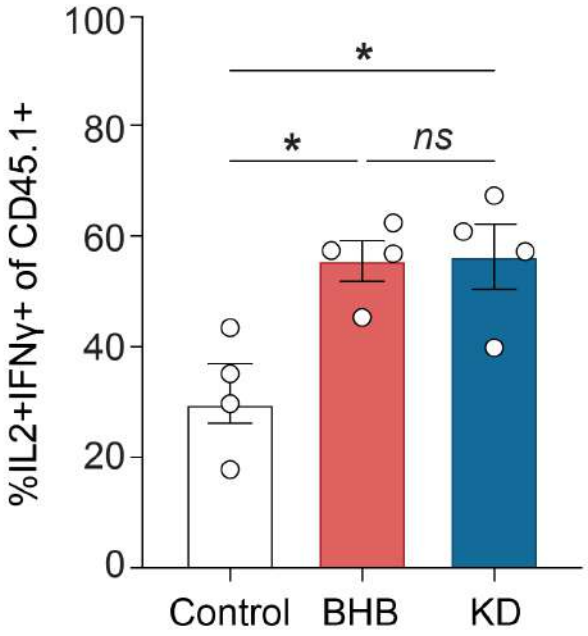
KD-enriched metabolites



Tumor burden



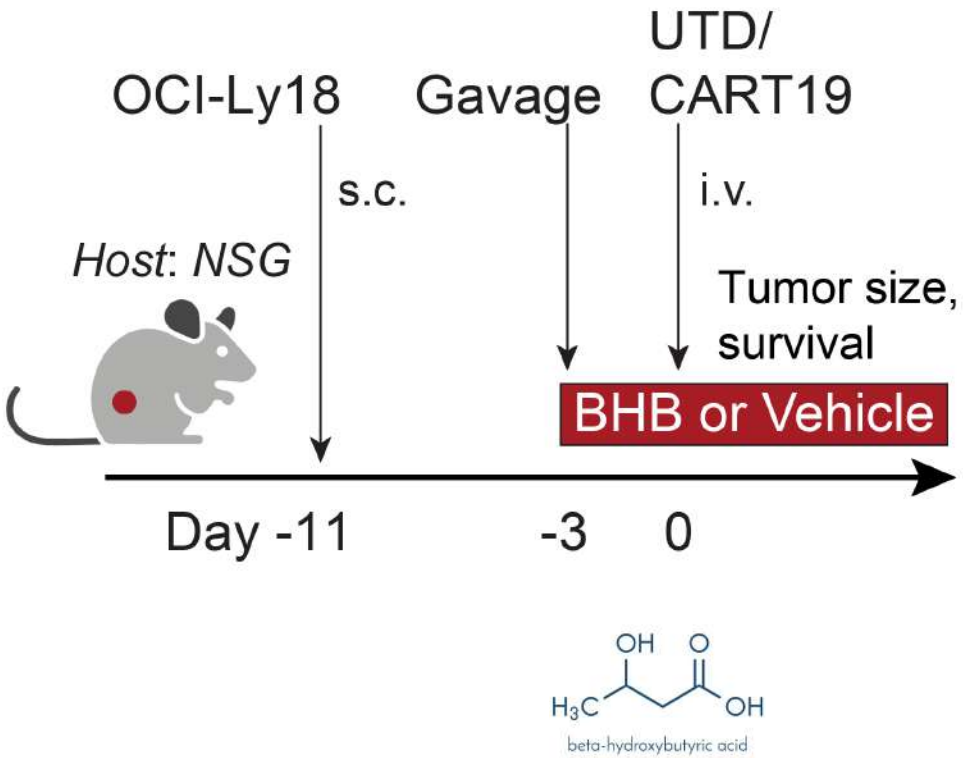
IL2⁺IFNγ⁺ tumor infiltrating CAR T cells (day 7)



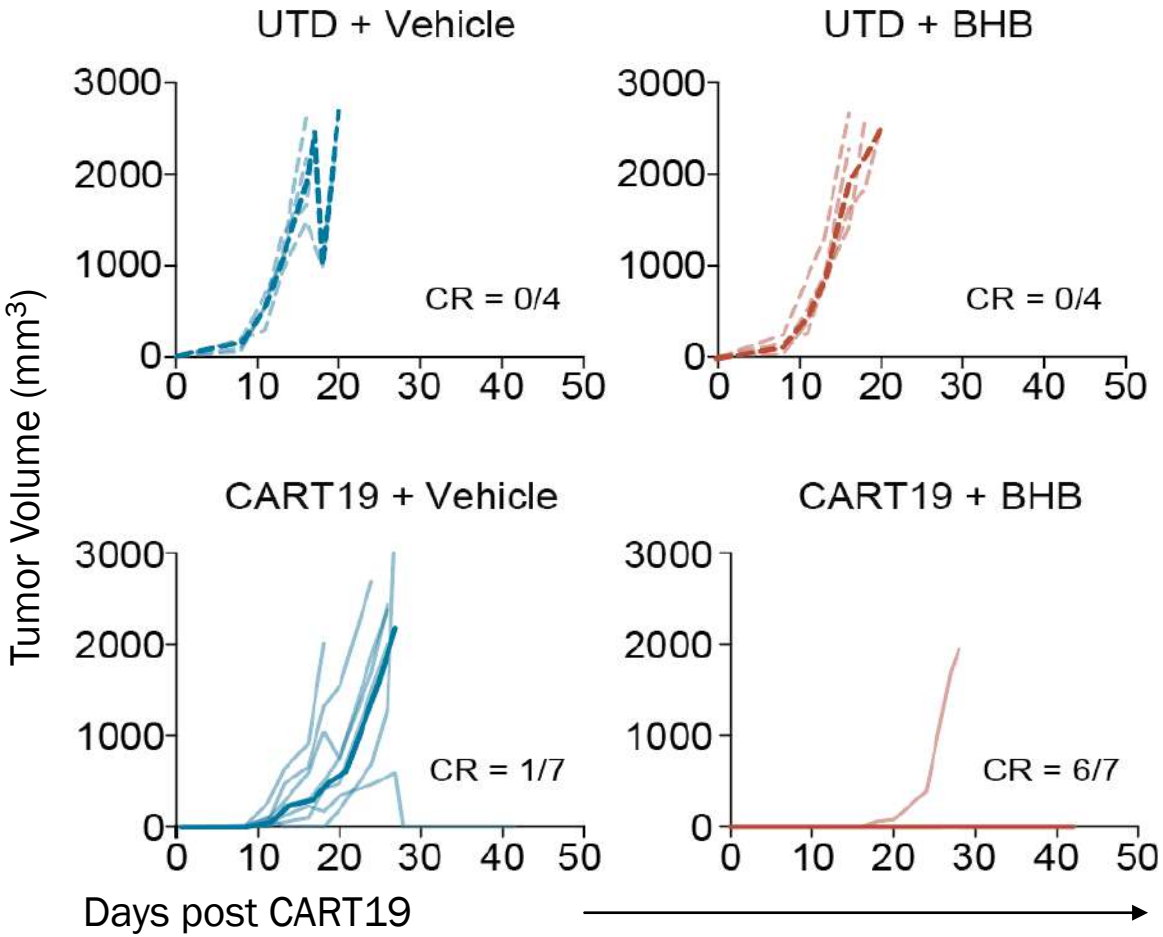
Liu S., Guruprasad P., Cell, in press

BHB improves human CART function in a human xenograft model of lymphoma

Human lymphoma (DLBCL) model



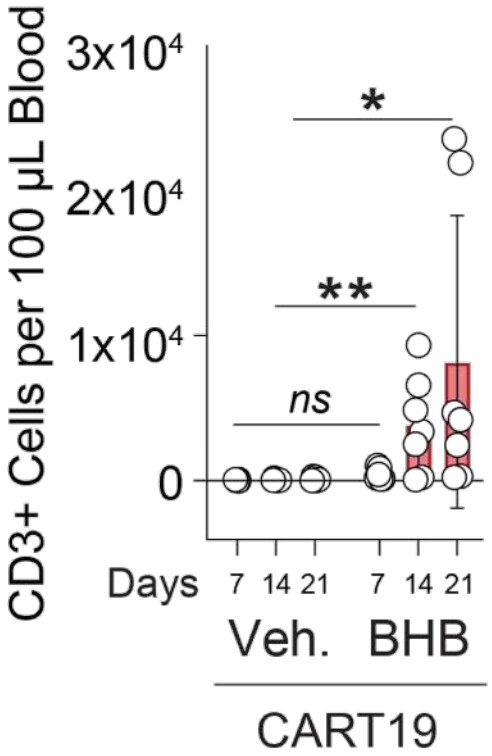
Tumor burden



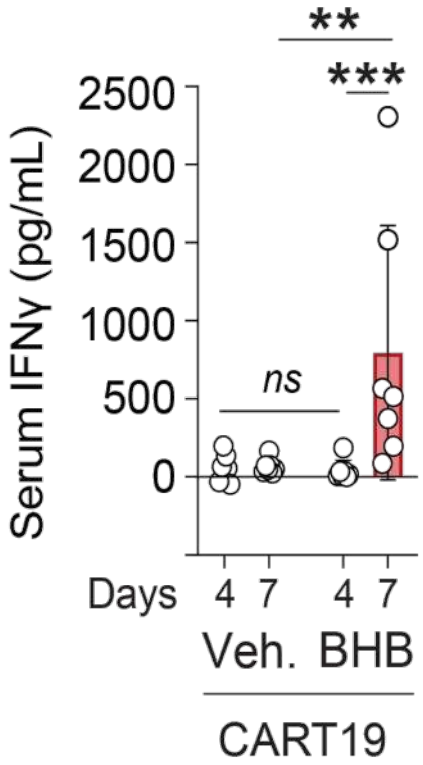
Liu S., Guruprasad P., Cell, in press

BHB increases peripheral T cell expansion and IFNγ release

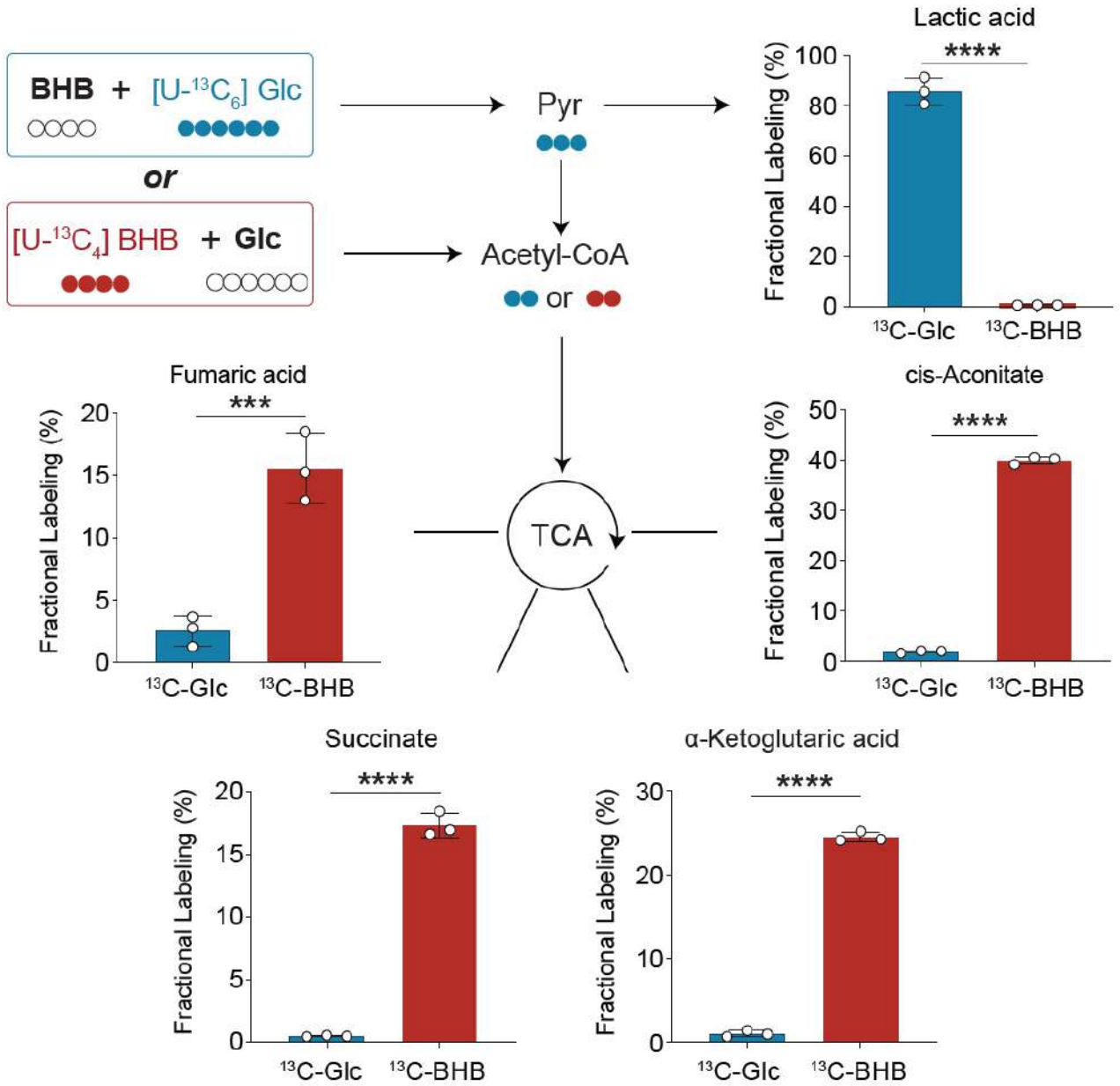
T cell *in vivo* expansion



In vivo IFNγ release

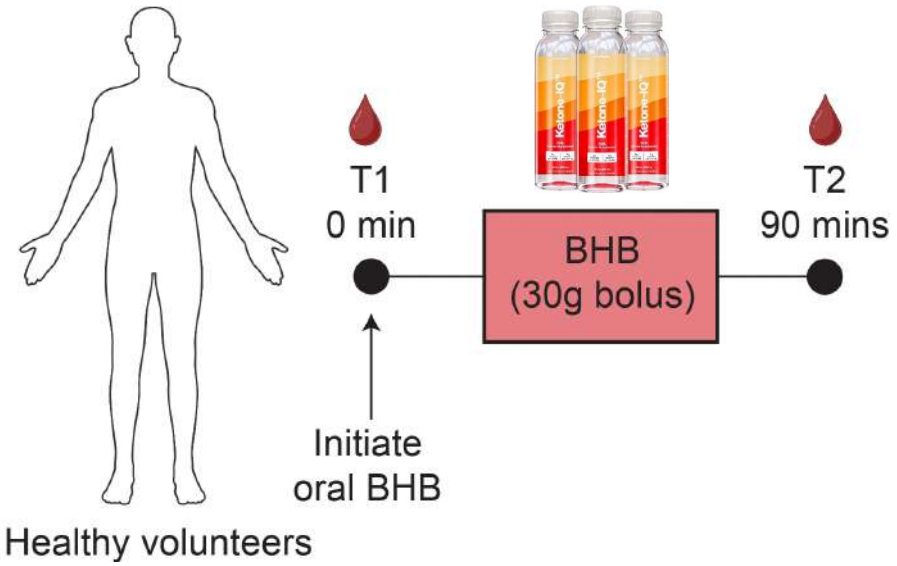


BHB enhances the TCA cycle and OxPhos

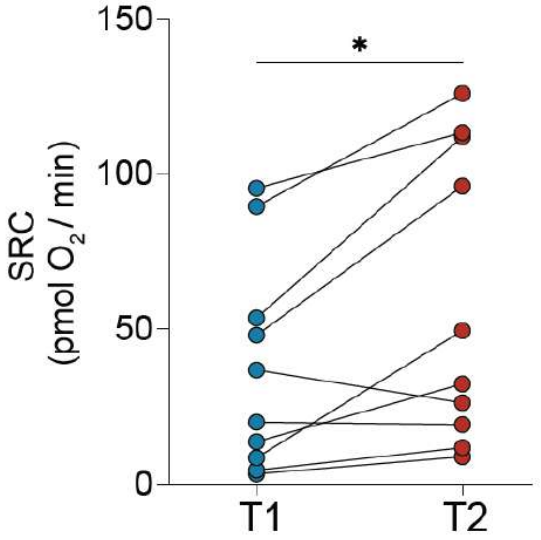


BHB enhances OXPHOS in peripheral T cells of healthy volunteers

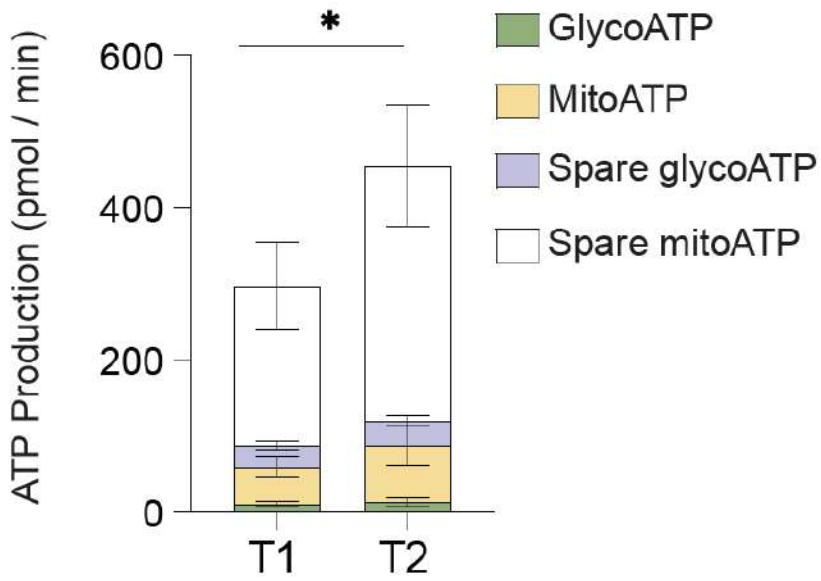
BHB administration to volunteers (n=10)



Maximum O_2 consumption



Total bioenergetic capacity



Roddy O'Connor
Andre Kelly

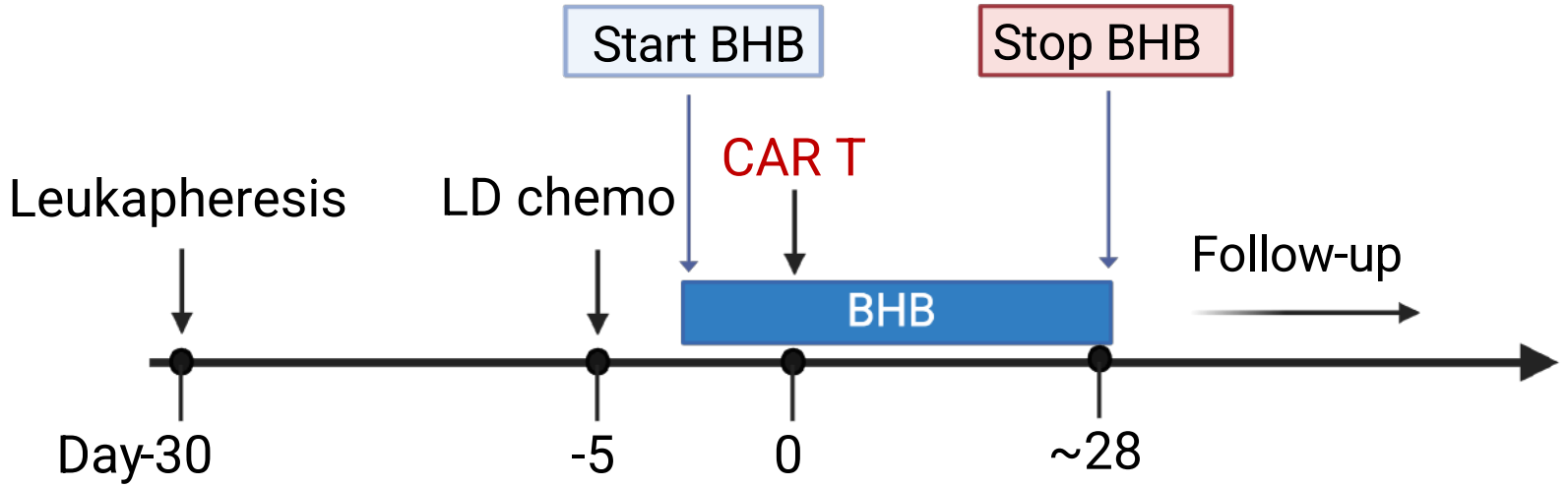
Liu S., Guruprasad P., Cell, in press

BHB supplementation during CART19 therapy for Lymphoma

PI: Elise Chong, MD

NCT06610344
(Proof-of-concept trial)

r/r NHL
Commercial
CART19



Key correlative studies:

- Serum metabolites
- CART expansion and phenotype
- Microbiome

CONCLUSIONS AND PERSPECTIVES

- We demonstrated associations *between fecal microbiome composition and clinical outcomes* of patients treated with CD19 CAR T cell therapy. Other studies have now confirmed and added to our study.
- Exposure to specific broad-spectrum *(P-I-M)* antibiotics prior to cell infusion is associated with *worse survival and increased toxicity*.
- ALL and NHL patients have gut microbiota dysbiosis before treatment with CAR T cells.
- Taxonomic analyses of the fecal microbiome revealed *interesting candidate taxa* (Ruminococcus, Faecalibacterium) to study in preclinical models of CAR T cell function.
- In preclinical models, the *modulation of the gut microbiota with vancomycin* enhances CART activity via antigen-presentation

- ***Ketogenic diet*** enhances CART function in multiple murine and human cancer models → *Effect in solid cancers and complex TME? Direct effect on cancer cells?*
- ***Beta-Hydroxy Butyrate*** is the main mediator for this effect and works as a single agent in multiple cancer models to boost CART
- BHB drives increased ***TCA cycle and OxPhos*** of CART cells within the tumor microenvironment → *Additional mechanisms, such as microbiota, epigenetics*
- Safety under evaluation in a ***pilot clinical trial***

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