MINERVA BIOTECHNOLOGIES

Novel MUC1 Therapies for Solid Tumors*

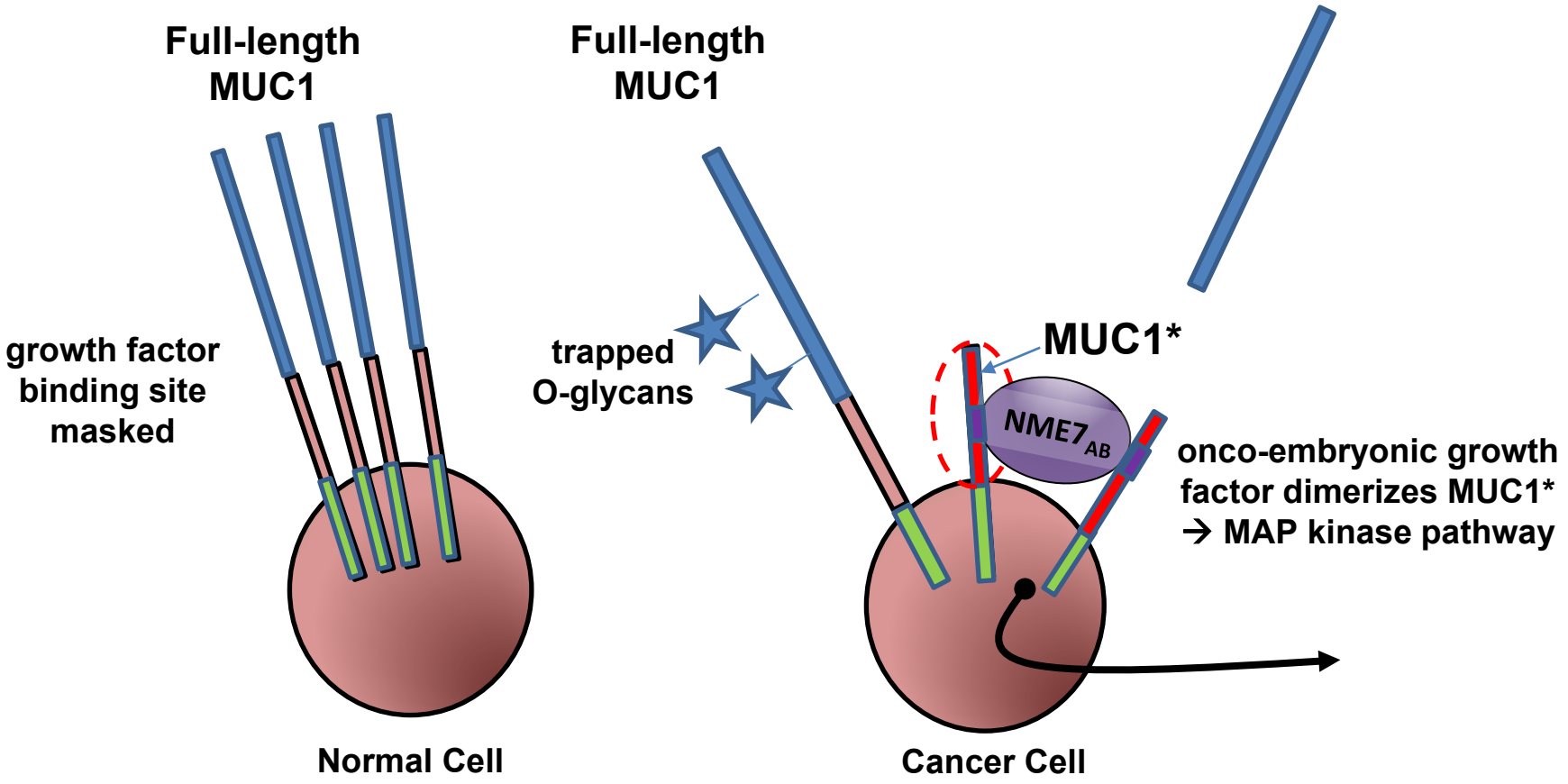
Universal Assay to Measure CAR T Cell Potency

Contact Us:

Dr. Cynthia Bamdad, CEO	cbamdad@minervabio.com	617-821-8773
Ron Axelrod, COO	raxelrod@minervabio.com	617-785-9491
Michael Crowther, CBO	mcrowther@minervabio.com	908-540-1751

1st-in-human clinical trial for metastatic breast cancers NCT04020575

Autologous CAR T cells that target MUC1* - the growth factor receptor form



MINERVA BIOTECHNOLOGIES

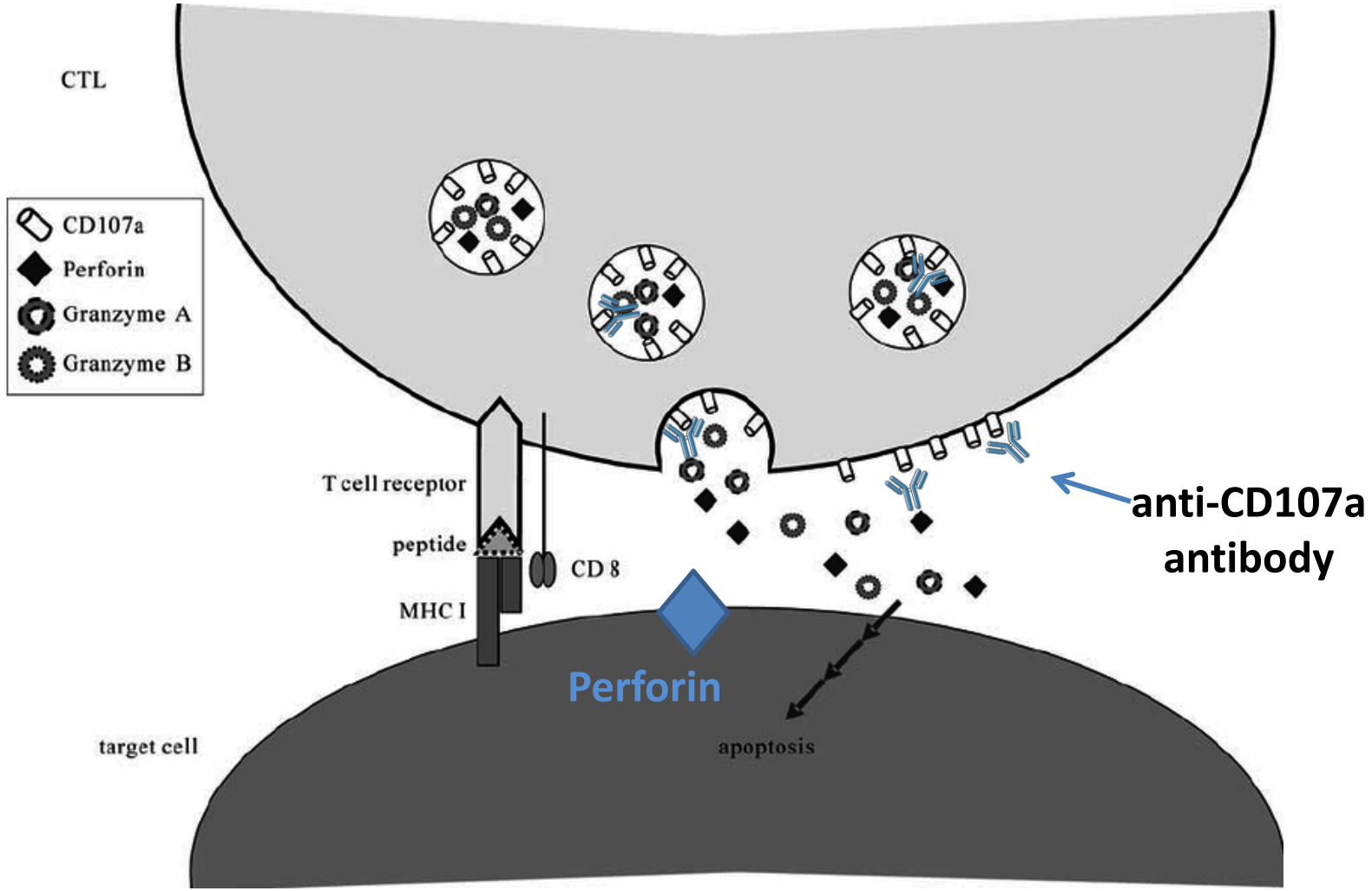
Characterization of the Cellular Product

CAR T Cell Potency

^{51}CR Release		xCELLigence	
PROS	**CONS**	**PROS**	**CONS**
Recognized killing assay	Radioactive	No Radioactivity	New physics-based assay
Directly measures killing of target cells	Often banned by clinical sites or performed in remote buildings	Directly measures killing of target cells	$$ Specialized equipment
Short 3-4 hr results practical for clinical setting	Significant variability in results between samples	Reproducible results between samples	Long 24-48 hr results impractical for clinical setting
	Significant variability in results between patients	Some variability in results between patients	

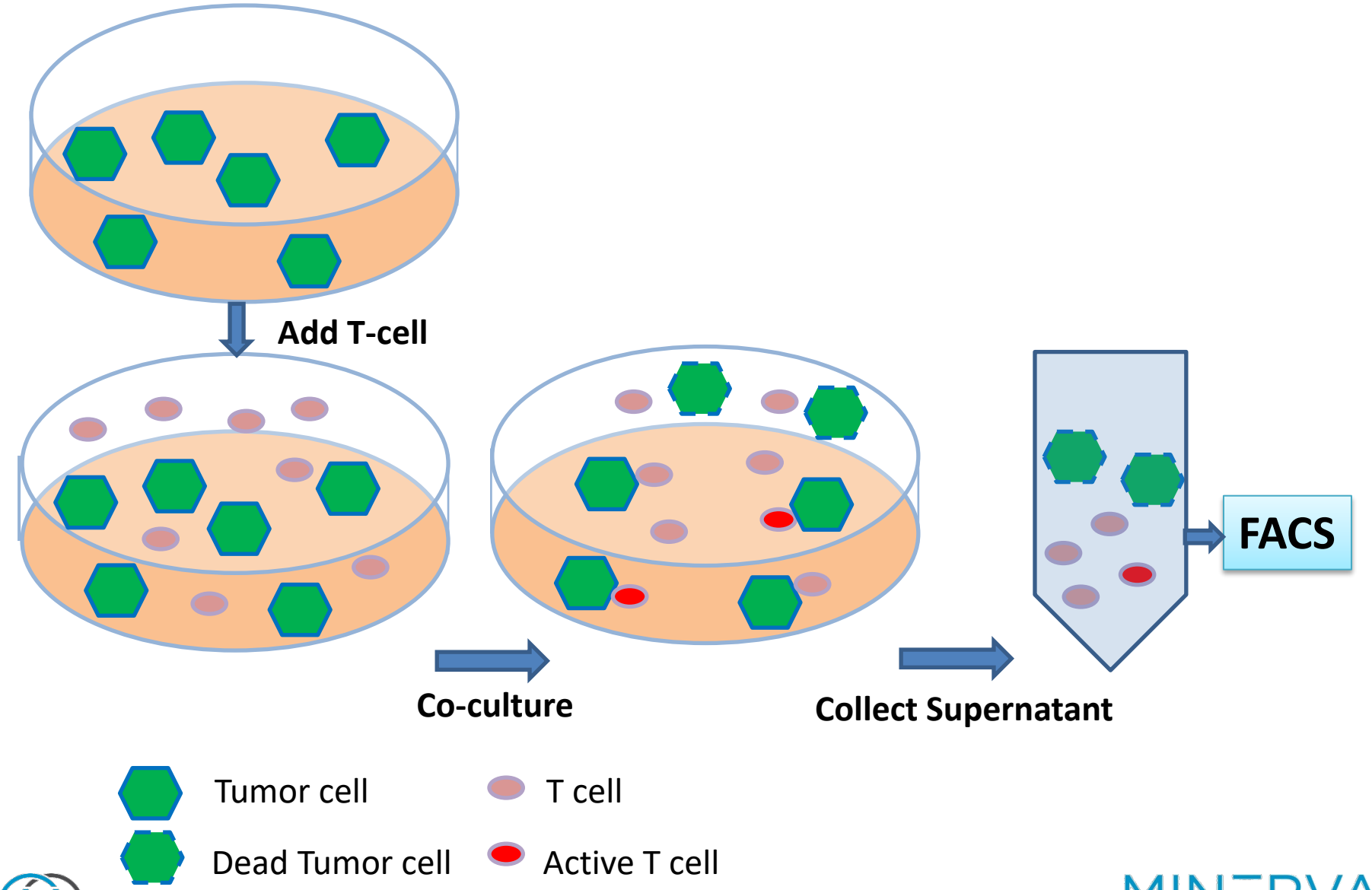
NEW: CD107a Degranulation Assay

Measures changes on CAR T cell that indicate killing mechanism has been initiated



MINERVA BIOTECHNOLOGIES

Workflow: Co-Culture CAR Ts & Target Cells → FACS CD8+/CD107a+



MINERVA BIOTECHNOLOGIES

Scientific articles describe CD107a degranulation assays

Use different Timing, Effector:Target Ratios, Reagents and Methods

Ex vivo identification, isolation and analysis of tumor-cytolytic T cells

Valerie Rubio[1], Tor B Stuge[1], Naileshni Singh[1], Michael R Betts[2], Jeffrey S Weber[3], Mario Roederer[4] & Peter P Lee[1]

NATURE MEDICINE VOLUME 9 | NUMBER 11 | NOVEMBER 2003

Published in final edited form as:
Clin Cancer Res. 2018 January 01; 24(1): 95–105. doi:10.1158/1078-0432.CCR-17-2041.

Regional Delivery of Chimeric Antigen Receptor–Engineered T Cells Effectively Targets HER2+ Breast Cancer Metastasis to the Brain

Saul J. Priceman[1,2], Dileshni Tilakawardane[1,2], Brook Jeang[1,2], Brenda Aguilar[1,2], John P. Murad[1,2], Anthony K. Park[1,2], Wen-Chung Chang[1,2], Julie R. Ostberg[1,2], Josh Neman[3], Rahul Jandial[4], Jana Portnow[5], Stephen J. Forman[1,2], Christine E. Brown[1,2]

ONCOIMMUNOLOGY
2018, VOL. 7, NO. 2, e1380764 (13 pages)
https://doi.org/10.1080/2162402X.2017.1380764

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

OPEN ACCESS Check for updates

Co-stimulatory signaling determines tumor antigen sensitivity and persistence of CAR T cells targeting PSCA+ metastatic prostate cancer

Saul J. Priceman[a,b], Ethan A. Gerdts[a], Dileshni Tilakawardane[a], Kelly T. Kennewick[a], John P. Murad[a], Anthony K. Park[a], Brook Jeang[a], Yukiko Yamaguchi[a], Xin Yang[a], Ryan Urak[a], Lihong Weng[a], Wen-Chung Chang[a], Sarah Wright[a], Sumanta Pal[c], Robert E. Reiter[d], Anna M. Wu[e], Christine E. Brown[a,b], and Stephen J. Forman[a,b]

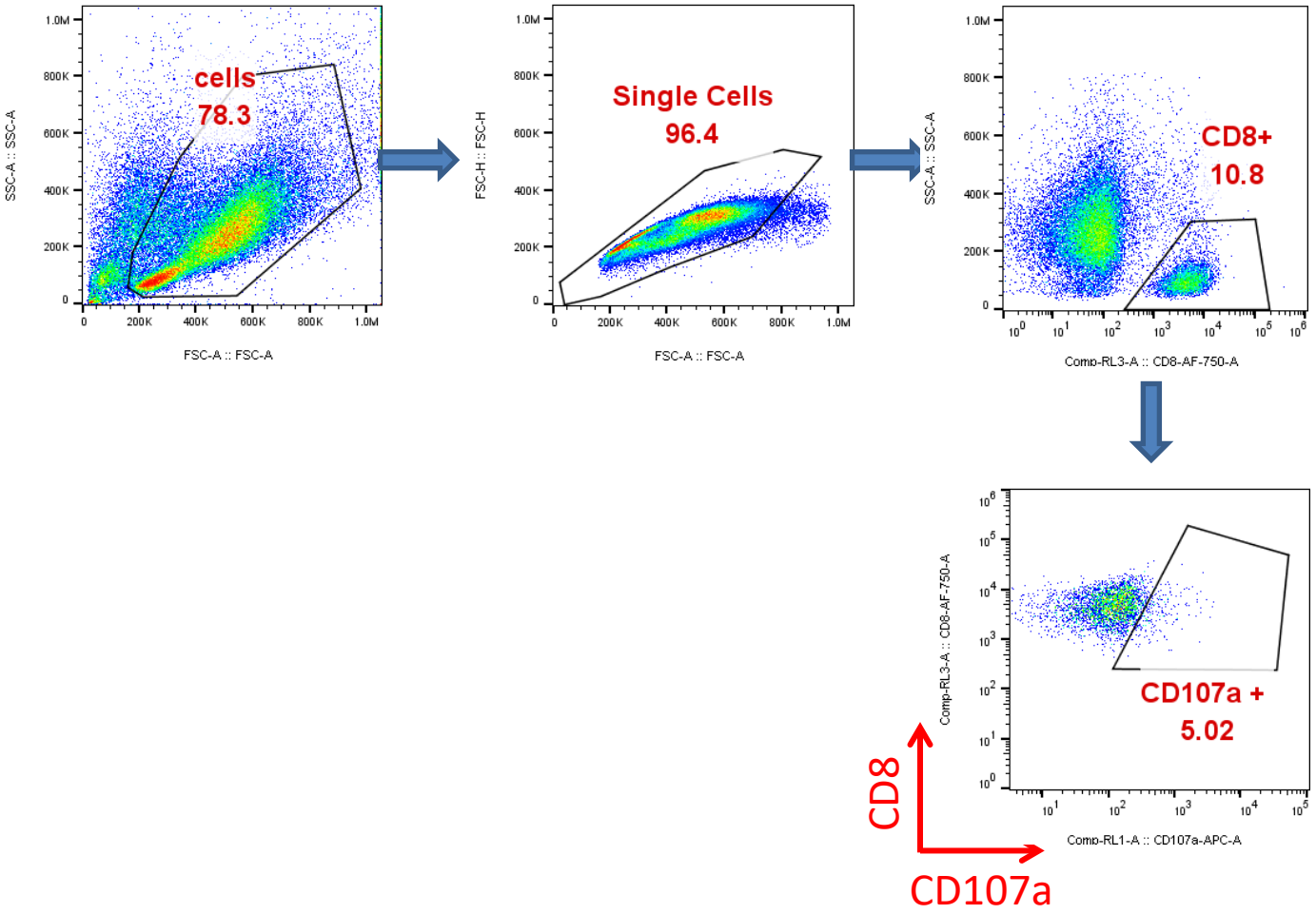
[a]Department of Hematology and Hematopoietic Cell Transplantation, City of Hope, Duarte, CA, USA; [b]T Cell Therapeutics Research Laboratory, City of Hope, Duarte, CA, USA; [c]Department of Medical Oncology & Therapeutics Research, City of Hope, Duarte, CA, USA; [d]Department of Urology, University of California, Los Angeles, Los Angeles, CA, USA; [e]Department of Molecular and Medical Pharmacology, University of California, Los Angeles, Los Angeles, CA, USA

MINERVA BIOTECHNOLOGIES

Optimize Protocol: Flow Cytometry Gating Strategy

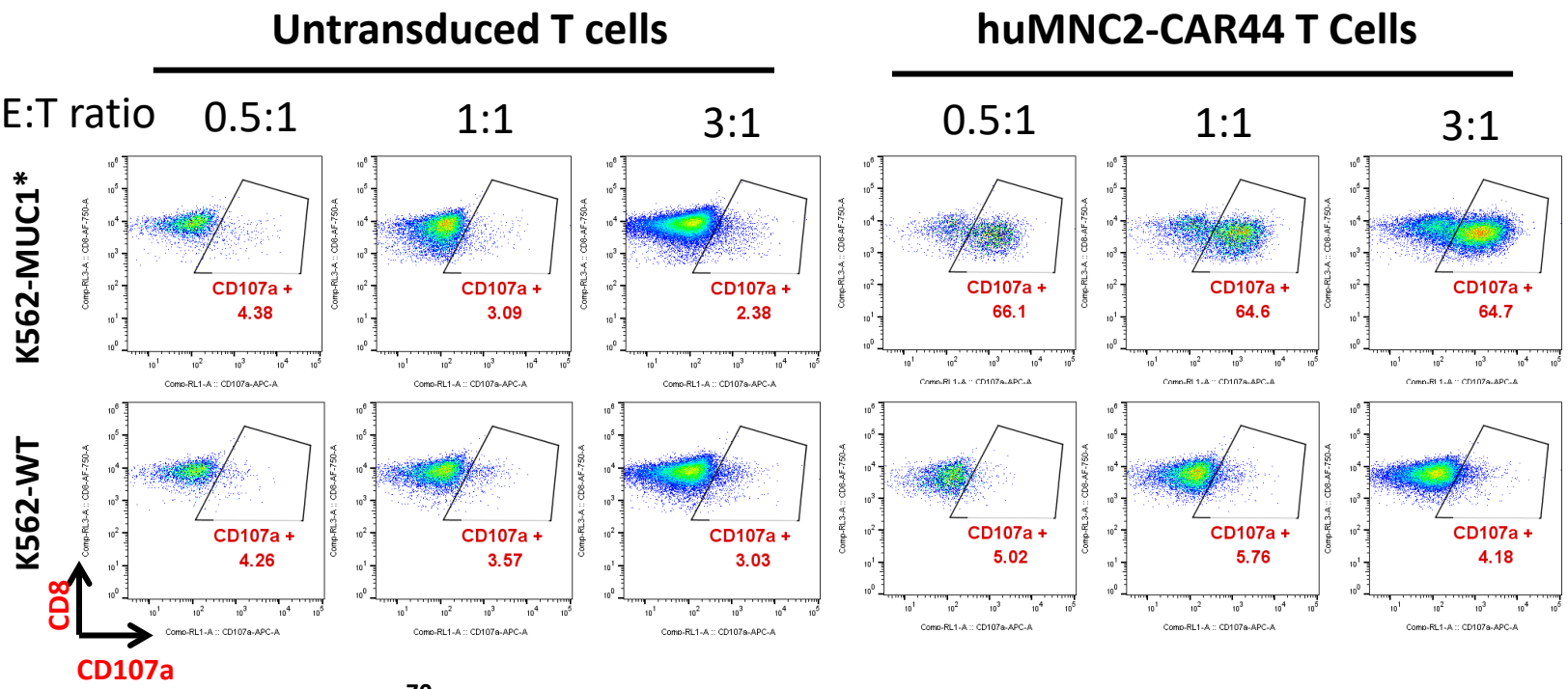
Live cells

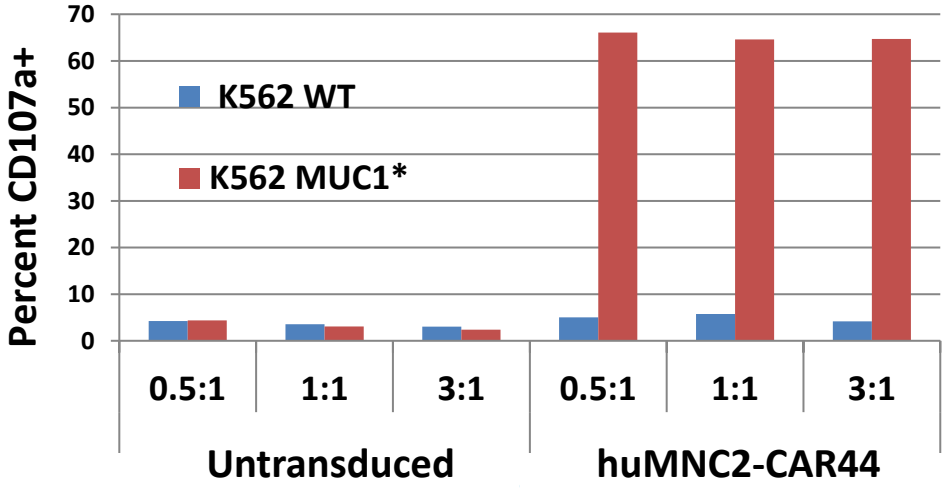
Pregated on FSC/SSC, singlet, CD8+ cells



MINERVA BIOTECHNOLOGIES

Target cells: K562 cells +/- target antigen; time 4 hrs; E:T = 3:1





MINERVA BIOTECHNOLOGIES

CD107a Degranulation Assay, Optimized

CD107a assay yields more consistent, reproducible results, including between different sites

^{51}CR direct lysis			CD107a implied lysis			
Fred Hutch			City of Hope		Minerva	
FH-ENG1 (2018)	FH-ENG2 (2019)	FH-ENG3 (2020)	COH ENG-1 (2021)	COH ENG-2 (2021)	COH ENG-1 (2021)	COH ENG-2 (2021)
72% 30:1 E:T	**43%** 30:1 E:T	**17%** 30:1 E:T				
			68% 3:1 E:T	**65%** 3:1 E:T	**59%** 3:1 E:T	**63%** 3:1 E:T

MINERVA BIOTECHNOLOGIES

PMA/Ionomycin bypasses TCR complex → max T cell activation

PMA + Ionomycin activate several intracellular signaling pathways → T cell activation and production of many cytokines.

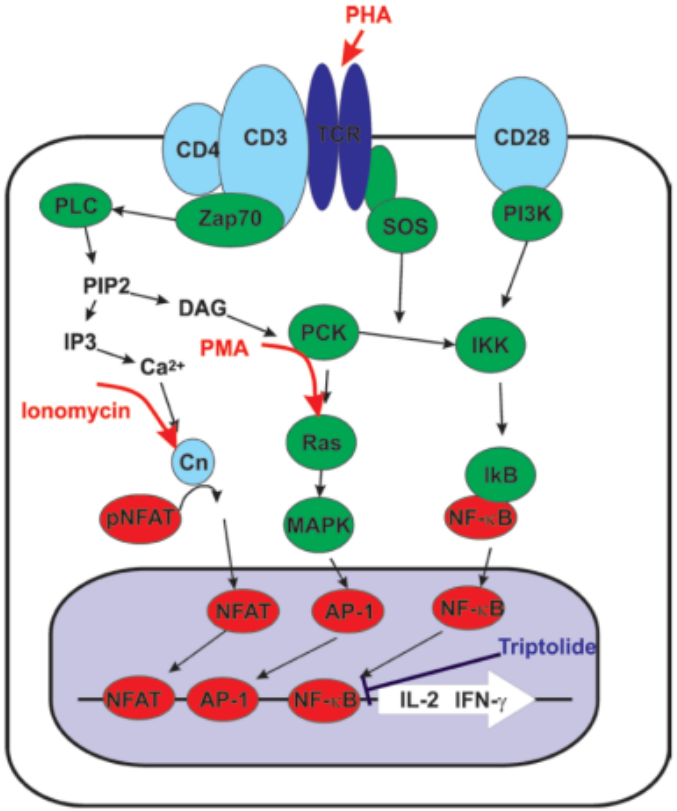


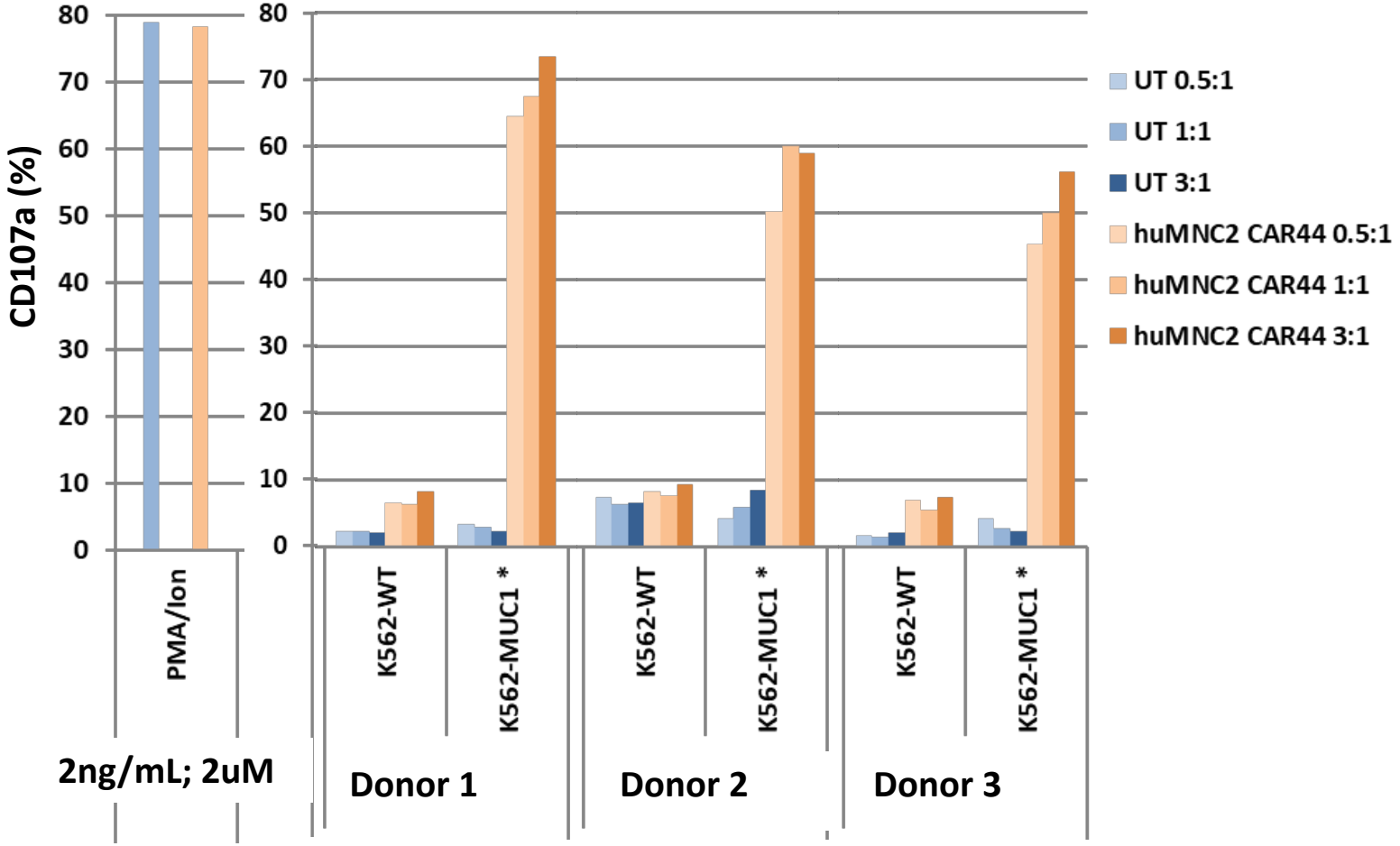
Figure 1. Schematic of signal cascade for stimulation of IL-2 and INF-γ secretion.

Stimulation of Human Peripheral Blood Mononuclear Cells Using the Cytation 7 Cell Imaging Multi-Mode Reader to Image and Analyze ELISpot Assays, Paul Held, Ph.D., Laboratory Manager, Applications Department, BioTek Instruments, Inc., Winooski, VT

MINERVA BIOTECHNOLOGIES

Killing potential varies among CAR T cells from different donors

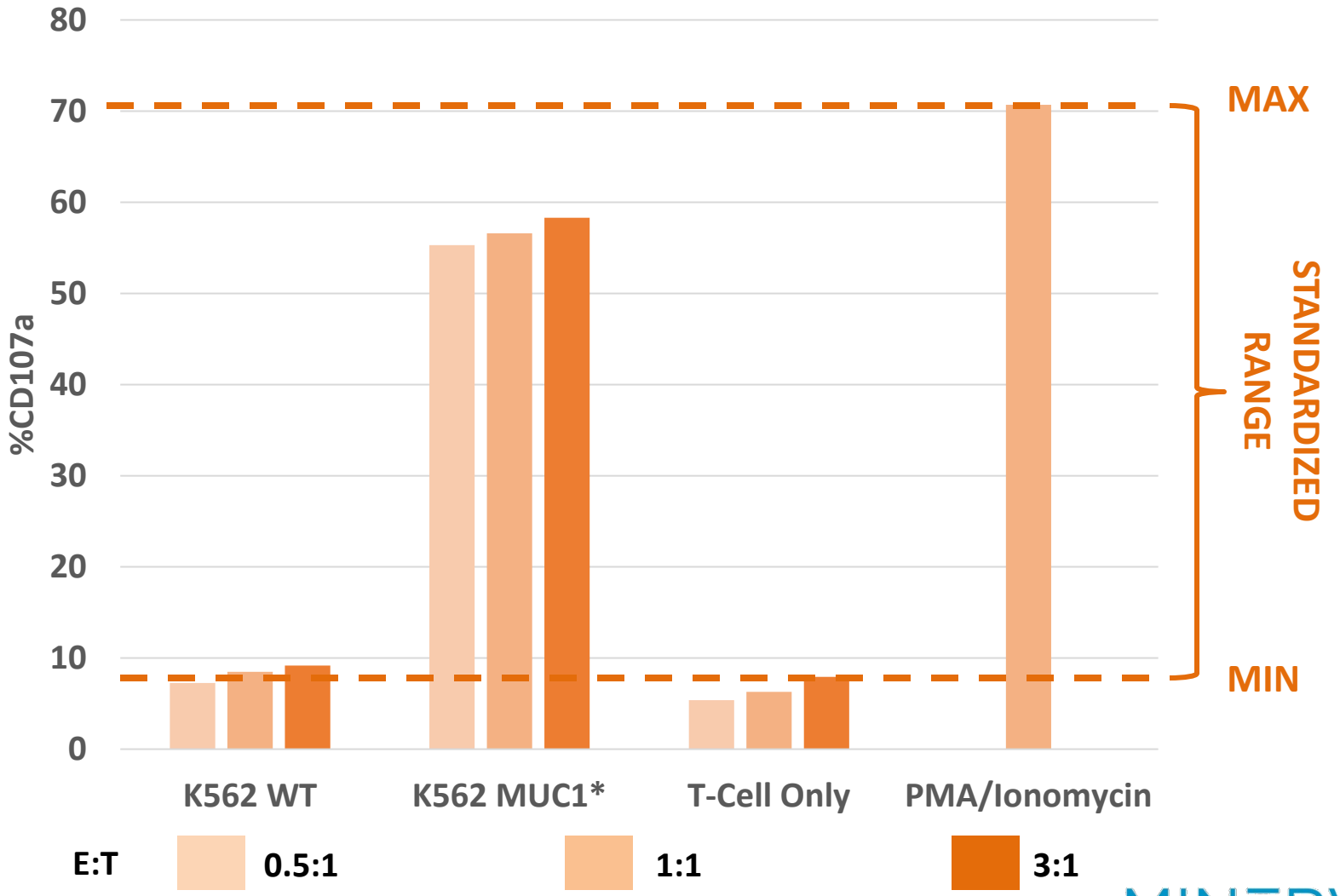
Induced activation by PMA/Ionomycin yields maximum activation for each donor CAR T cell; CAR T potency is normalized to max killing for each donor



MINERVA BIOTECHNOLOGIES

CAR T killing potency reported as percent of that person's max killing potential

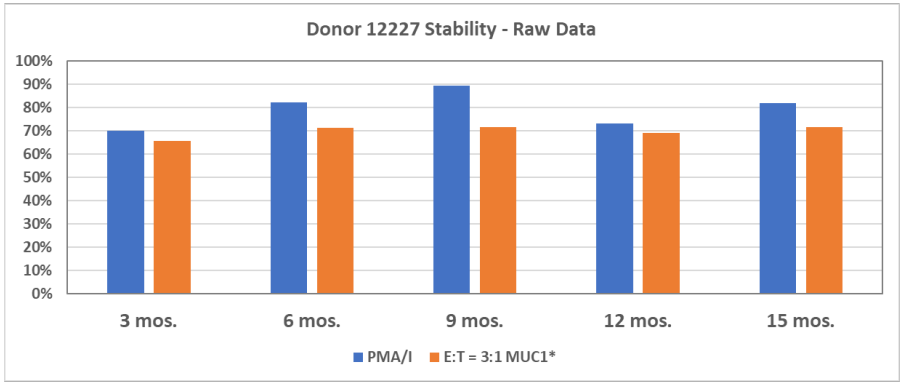
Standardized measure of potency; independent of donor or target antigen

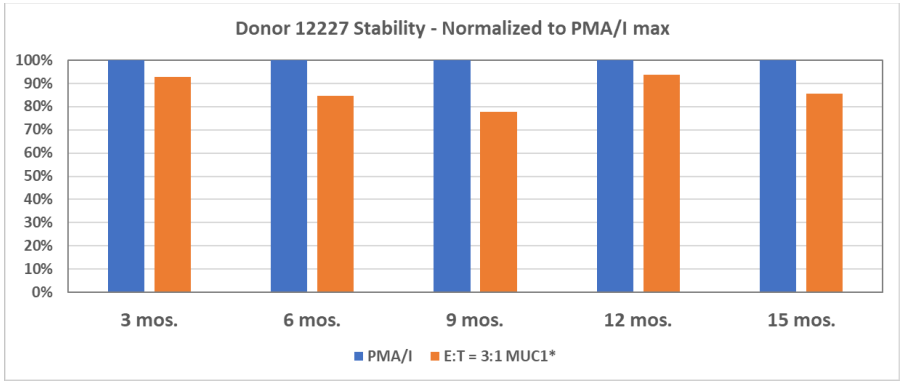


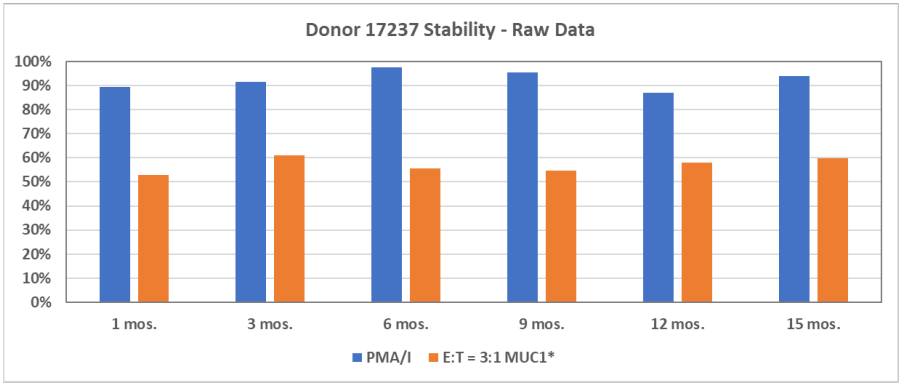
MINERVA BIOTECHNOLOGIES

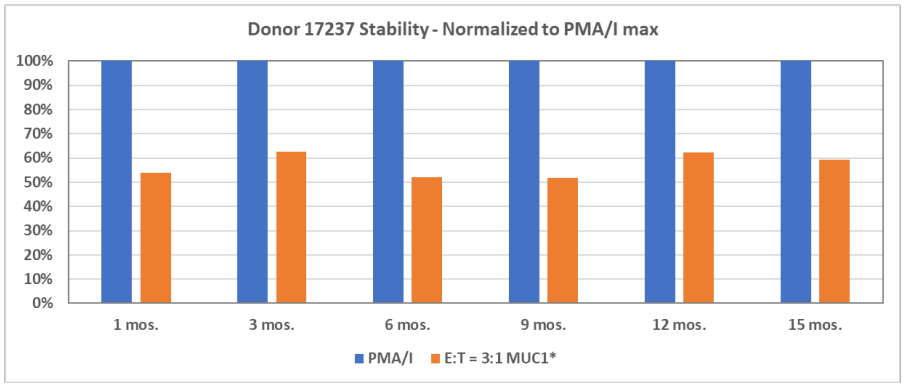
Between donors, PMA/Ionomycin induced activation is more consistent than target cell induced activation

Percent of MIN – MAX activation range is more accurate measure of CAR T potency





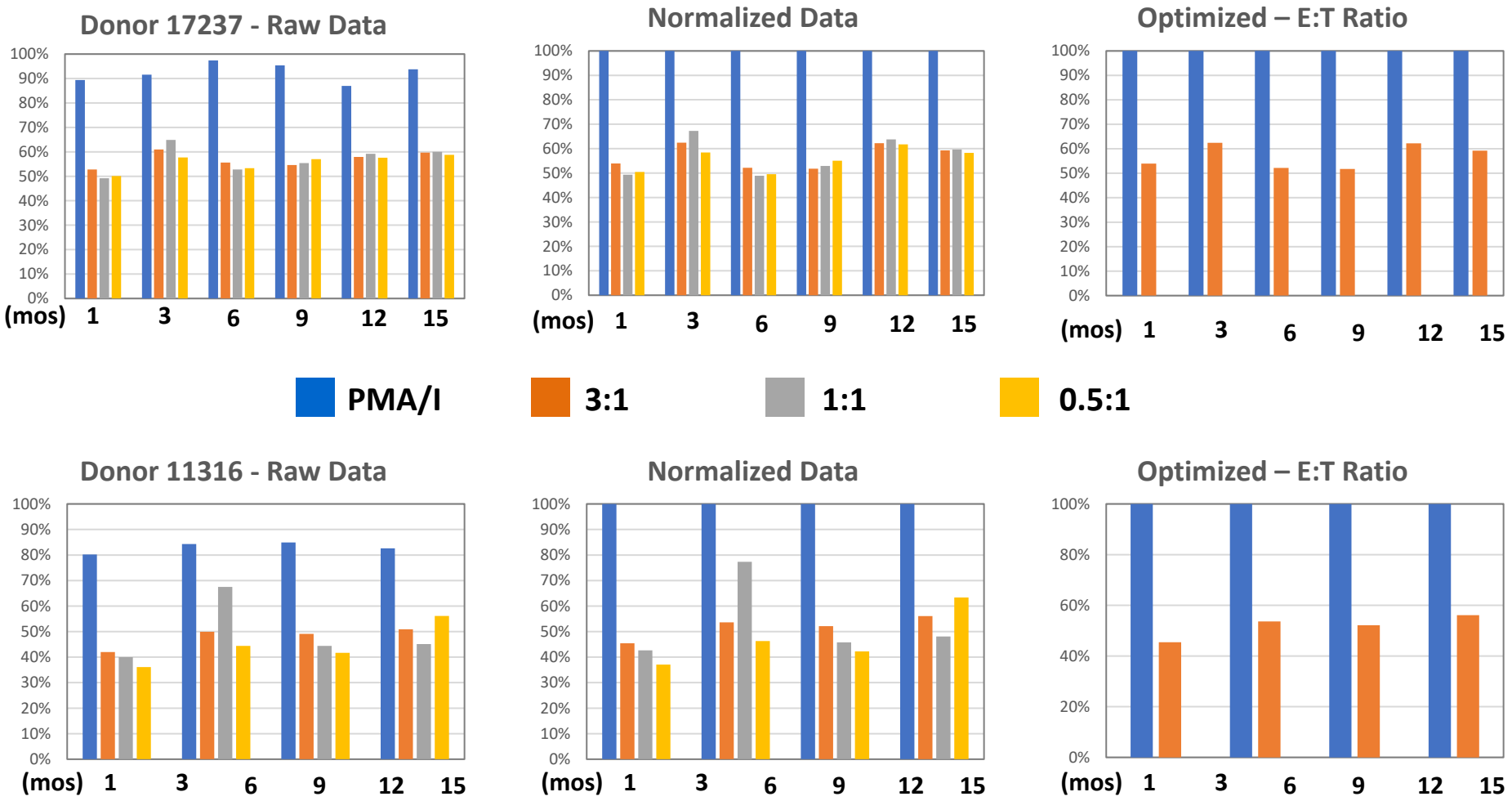




MINERVA BIOTECHNOLOGIES

Standardization important for CAR T stability studies

PMA/Ionomycin activation provides an internal control for long term stability studies of Patient frozen product



*Frozen cells require overnight culture before measurement

MINERVA BIOTECHNOLOGIES

Minerva Advisors and Collaborators

Minerva Scientific Advisory Board

Michel Sadelain, MD, PhD

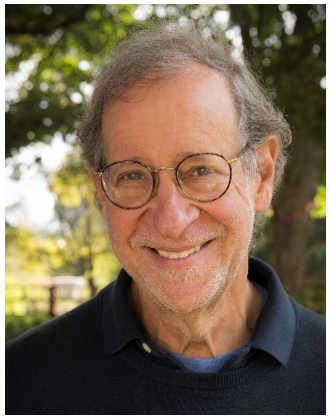
Memorial Sloan Kettering Cancer Center

JUNO THERAPEUTICS

Stan Frankel, MD

Celgene

Bristol Myers Squibb™



Stephen J. Forman, MD

City of Hope

Joanne Mortimer, MD

City of Hope

Mark Fleming, MD D Phil

Boston Children's

Stan Riddell, MD

FRED HUTCH CURES START HERE™

Mitch Finer, PhD

MPM

MINERVA BIOTECHNOLOGIES

Minerva's Scientific Teams

Minerva Scientists operate in a team-based environment; scientists are on multiple teams. All Scientists have key areas of expertise but all can operate cross-platform

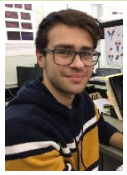
CAR-T immunotherapy team

- In vitro studies


Dr Benoit Smagghe


Dr Mark Carter

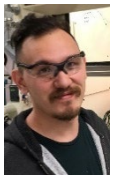

Danica Page


Michael Nash


Dr Trevor Grant


Jac-Leen Nash

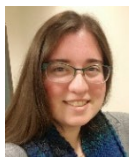

Dr Scott Moe


Thomas Jeon

CAR-T immunotherapy team

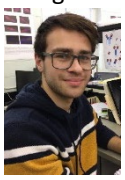
- In vivo studies


Danica Page


Laura Reale

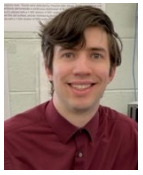

Dr Trevor Grant


Jac-Leen Nash


Michael Nash


Kevin Yi

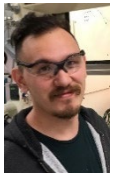

Victoria Bemis

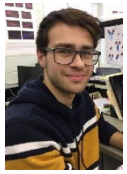

Gregory Riley

Oncology small molecule team

- In vitro studies


Dr Scott Moe


Thomas Jeon

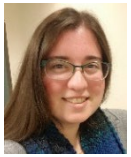

Michael Nash

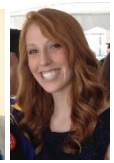

Dr Kyle Mills

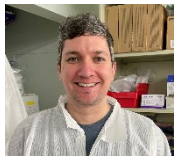
Oncology small molecule team

- In vivo studies


Kevin Yi


Laura Reale


Danica Page


Dr Trevor Grant

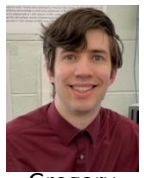

Andrew Stewart, D Phil
Project Manager

Oncology MUC1*/NME7 Antibody Development team


Dr Benoit Smagghe


Dr Mark Carter


Dr Trevor Grant


Gregory Riley

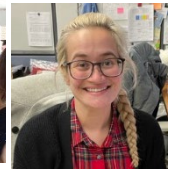

Jac-Leen Nash

Stem Cell Team


Dr Mark Carter


Kevin Yi


Danica Page


Jac-Leen Nash

Thank You

MINERVA BIOTECHNOLOGIES