Cord Blood derived Virus Specific T cells

Cath Bollard, MD

THE GEORGE WASHINGTON UNIVERSITY

WASHINGTON, DC

Children's National Health System

Disclosures

Catherine Bollard, M.D., FRACP, FRCPA.

Advisory Board: Cellectis, BMS

Co-Founder: Mana Therapeutics and Catamaran Bio

Board of Directors: Cabaletta Bio

Stock/ownership: Repertoire Immune Medicines and Neximmune Therapeutics

Limitations of Current Antiviral Therapy

Antiviral drugs not 100% effective

Cost $$$

Toxicities – neutropenia, renal toxicity

Vaccines ineffective in immunodeficiency and can’t be applied to CB donors

Rationale for Antiviral T cell Therapy After SCT

T cell immunity is the guardian against reactivation

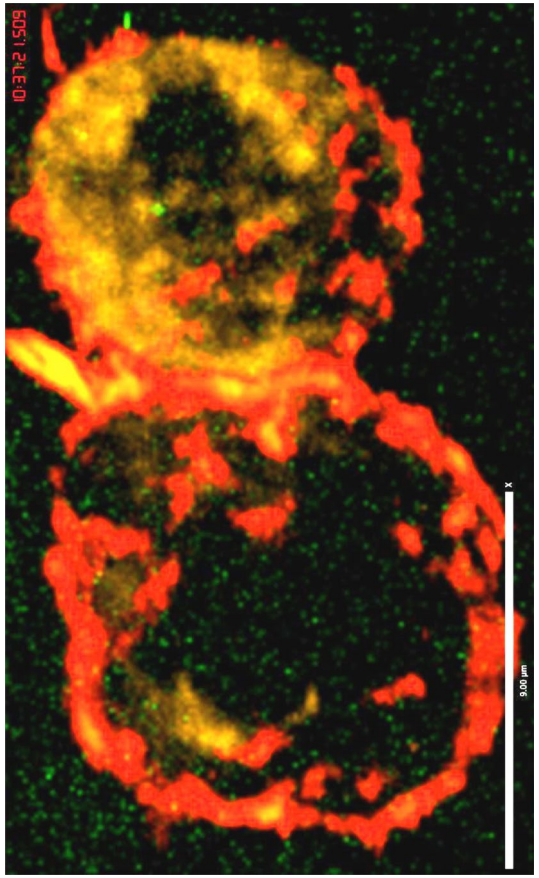
Virus specific T cells frequency ⬇ Viremia ⬆

Adoptive transfer of VST have proven efficacy in the seropositive setting

Improved technology increases access to VSTs beyond boutique centers

Adoptive Transfer of VSTs

- Utilizes selection or *ex vivo* expansion
- Highly successful in post-HSCT period
  - >400 patients treated internationally
  - Minimal risk of GVHD



Heslop and Bollard, Blood 2016
Keller and Bollard, Blood 2020

Alternative Donor Stem Cell Transplantation and Virus Infection

- High incidence of viral infections (not just single viruses EBV or CMV) post alternate donor transplant

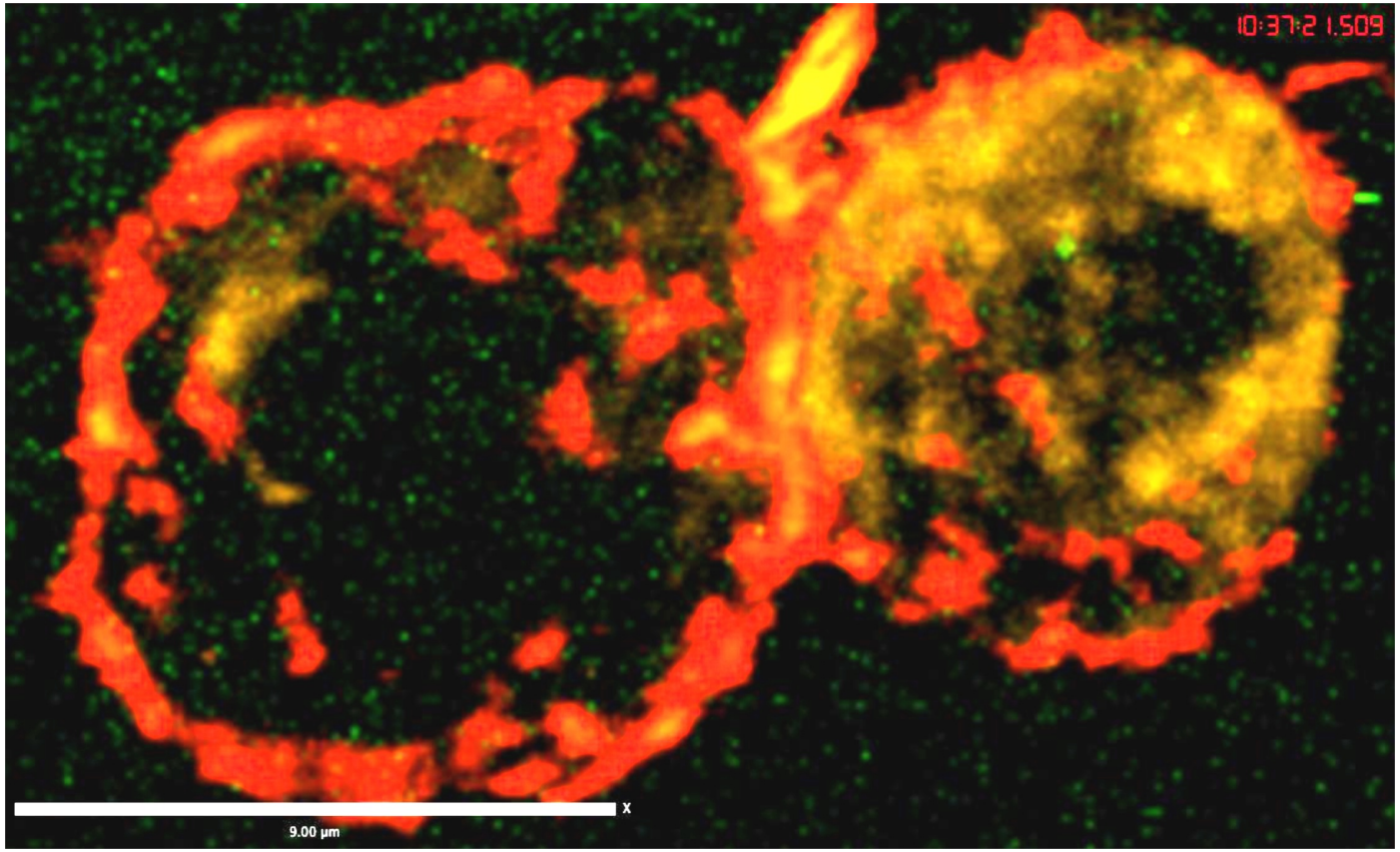
- **Highest incidence when donor seronegative (i.e. cord blood)**

Successful CB engraftment may be associated with viral morbidity

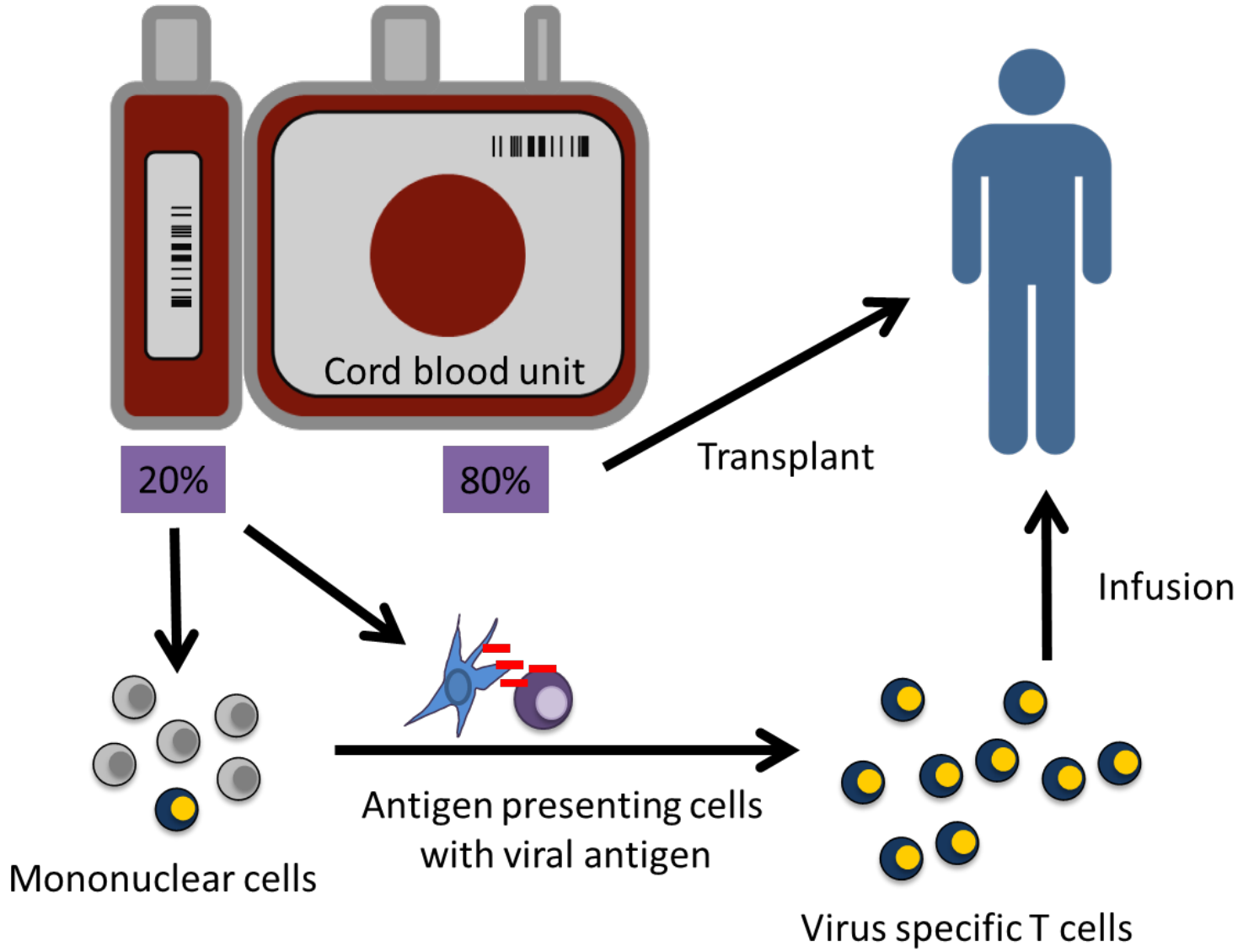
- Lymphocyte depletion by an effective conditioning regimen
  - Improved engraftment from ~50 to 80% in unrelated CB transplant for sickle cell disease
  - Led to immunodeficiency
  - 78% of had viral infections (CMV, EBV, RSV, Adeno)

Abraham et al, *Biol Blood and Marrow Transplantation 2017*

Moving CB-derived Virus-specific T cells to the Clinic

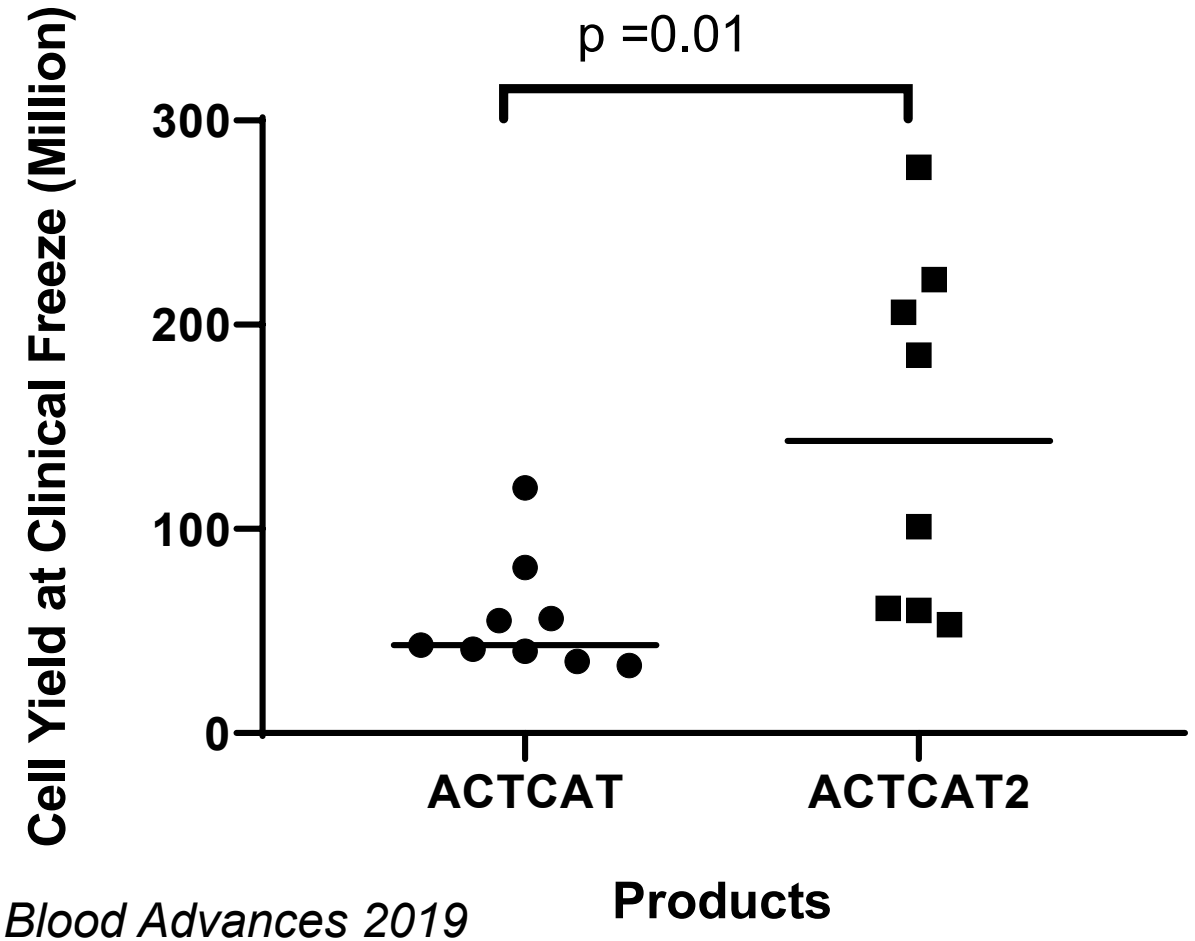


Manufacture of CB-Derived VSTs for Clinical Use



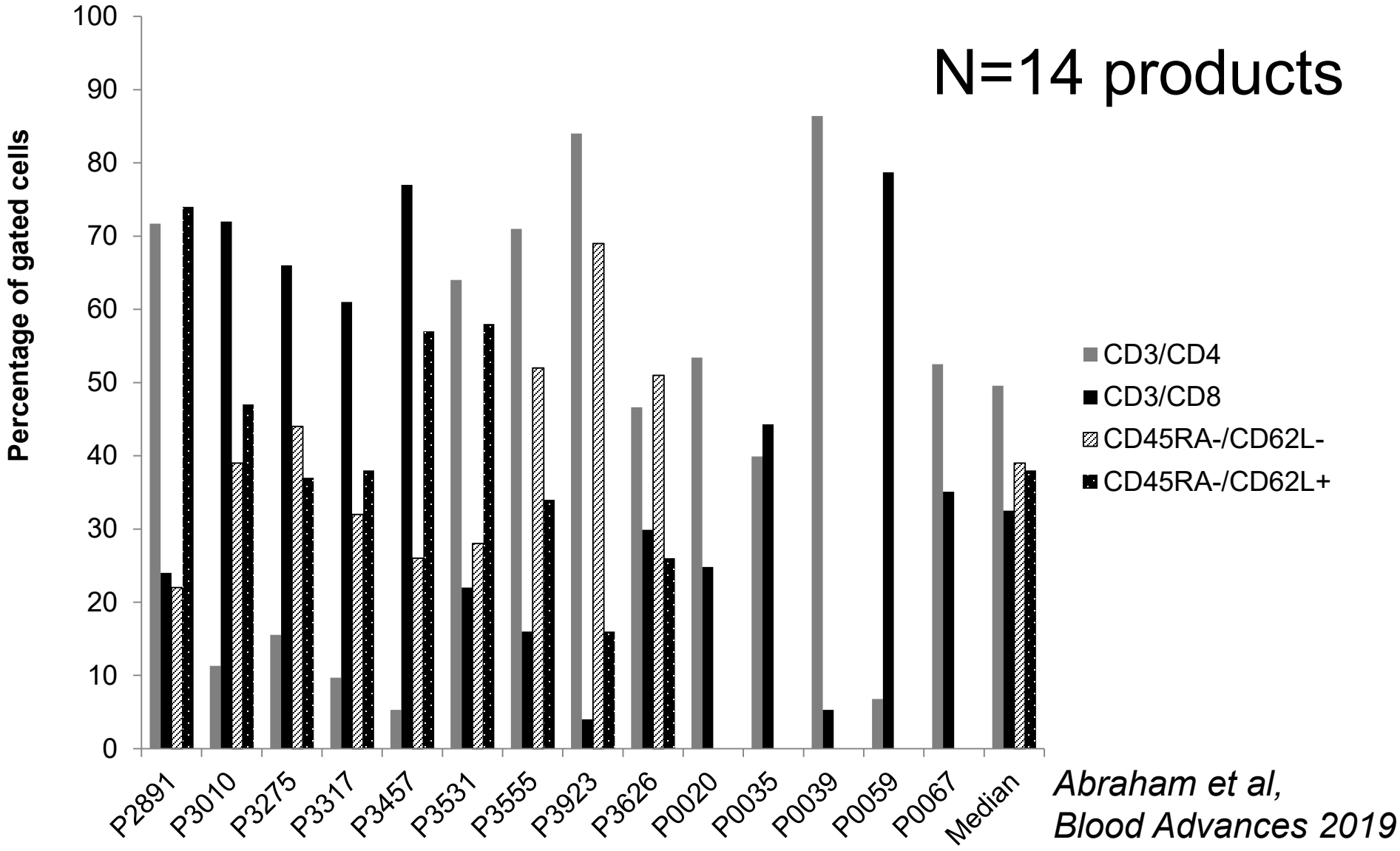
Abraham et al, Blood Advances 2019

Expansion of CB-derived VSTs Enhanced when using Pepmix Pulsed APC?



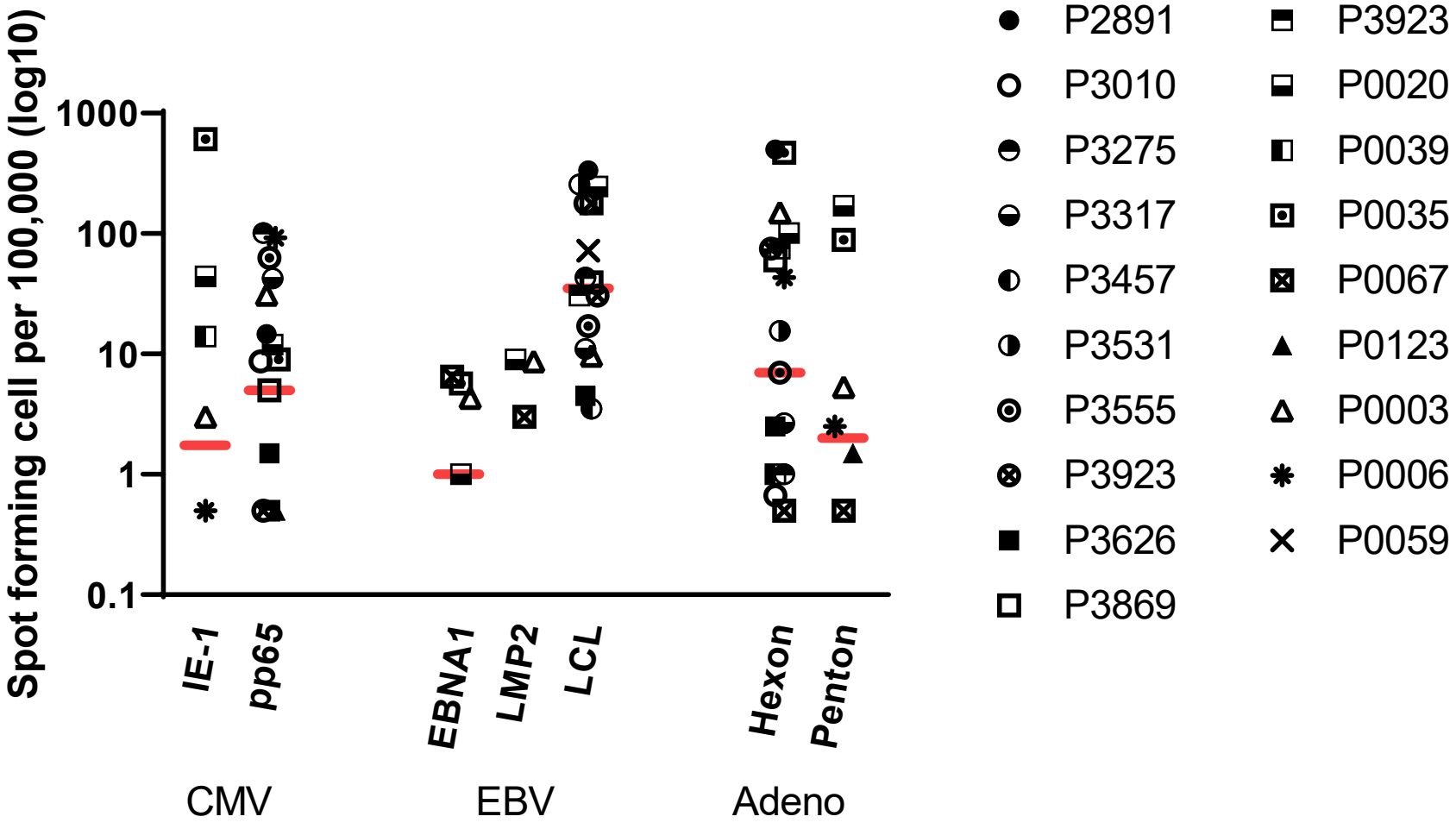
Abraham et al, Blood Advances 2019

CB-derived VSTs Are Polyclonal with Effector and Central Memory Phenotypes

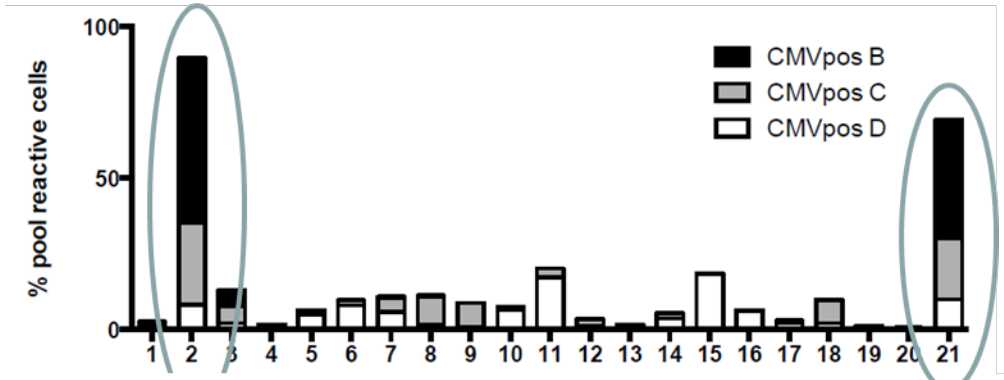


Abraham et al, Blood Advances 2019

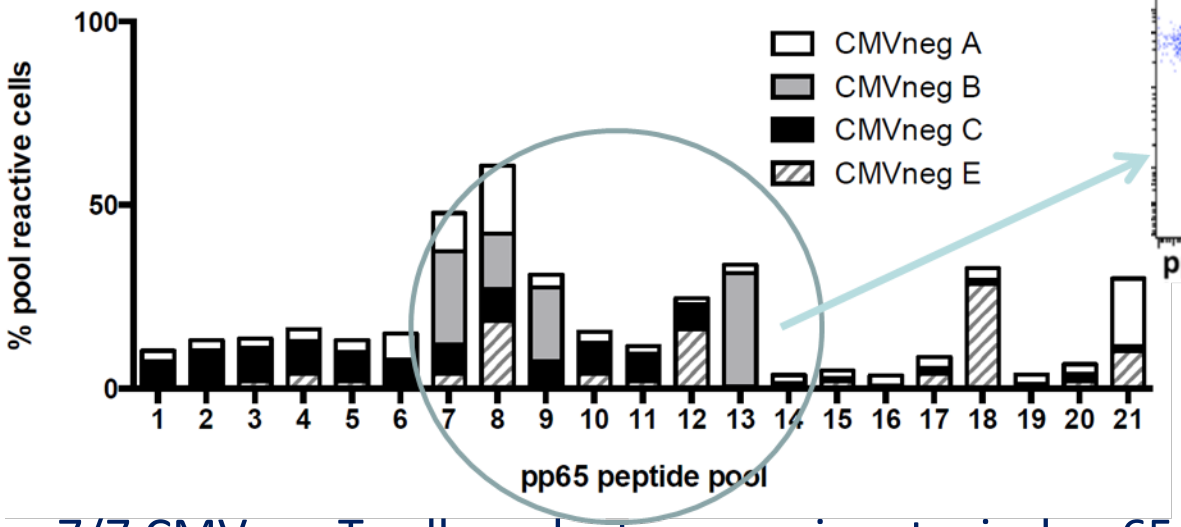
CB-derived VSTs have Broad Virus specific Activity in vitro

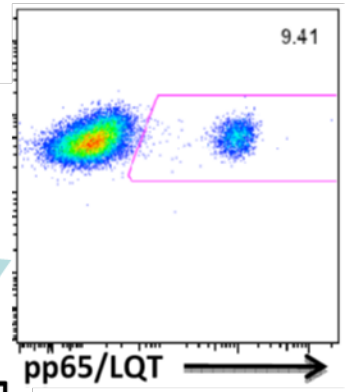


pp65-specific T cells From CMVneg donors Recognize Atypical Epitopes of CMVpp65



CMV-Seropositive





CMV-Seronegative

-7/7 CMVneg T cell products recognize atypical pp65 epitopes

Hanley et all Science TM 2015

- >30/30 cord blood T cell products recognize atypical pp65 epitopes

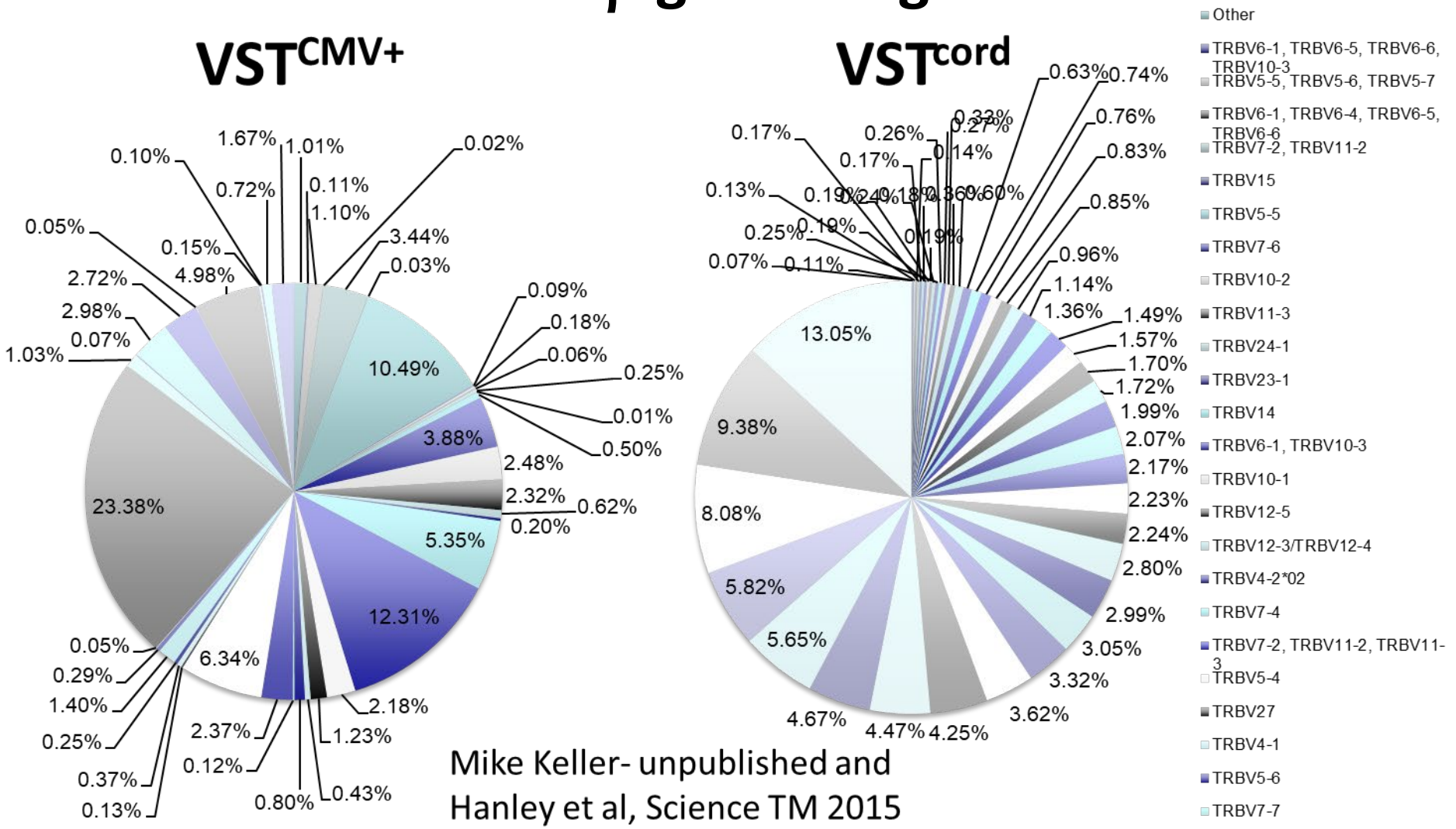
Hanley et al, Blood 2009

-11/11 CMVpos T cell products recognize typical pp65 epitopes

Hanley et al, Cytotherapy 2011

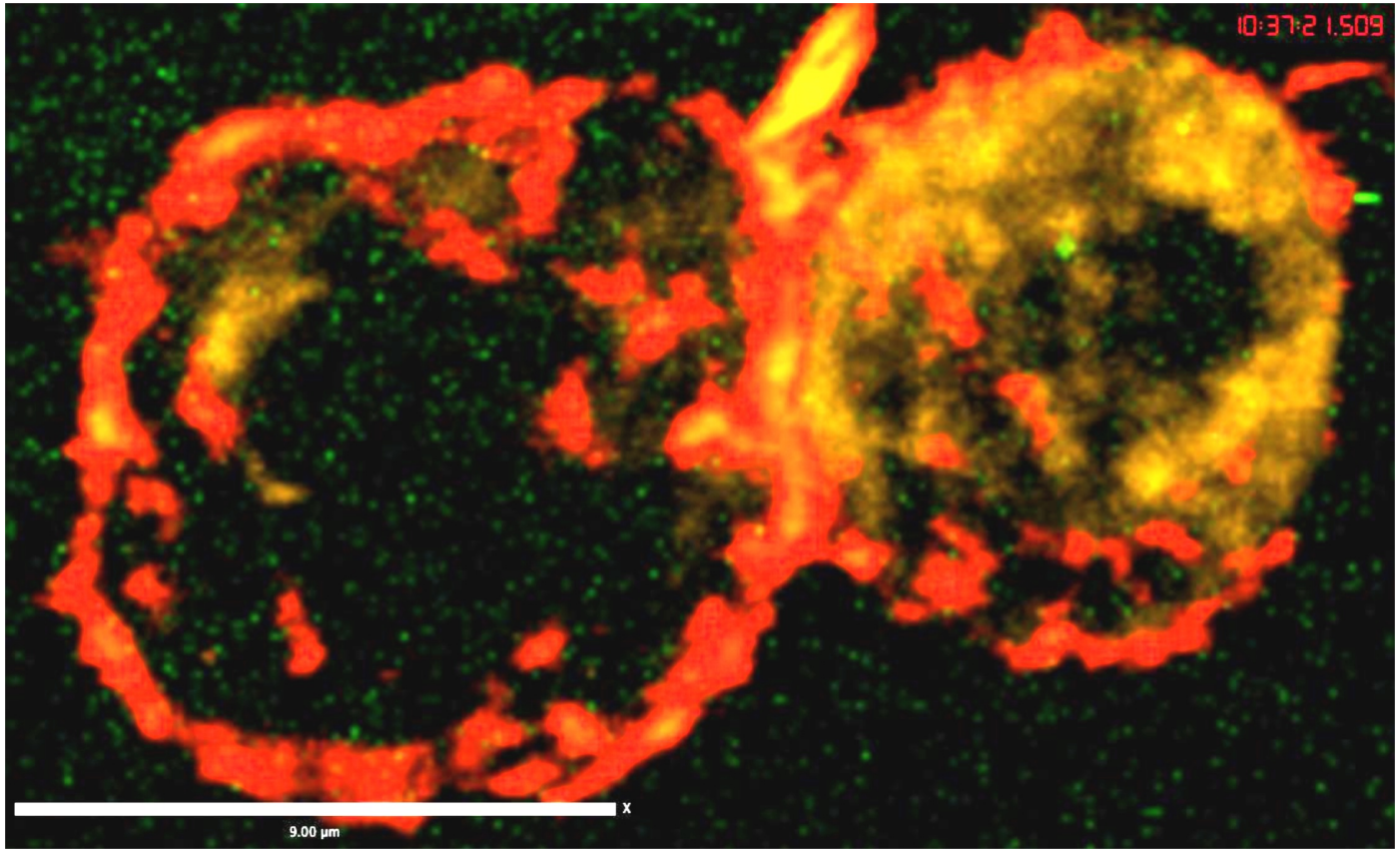
VST^{cord} more genetically diverse than VST^{CMV+}

TCR-Vβ gene usage



Mike Keller- unpublished and
Hanley et al, Science TM 2015

Are CB-derived Virus-specific T cells efficacious?



Demographics of patients receiving cord blood derived VST infusions

P#	Diagnosis	Age (yrs)	Sex	Weight (kg)	Conditioning Regimen	HLA Match (out of 6)	Immunosuppression prior to VST	Recipient CMV serostatus
ACT-CAT								
P2891	Fanconi Anemia	5	M	13.0	Flu/Cy/TBI	5	CSA,MMF, steroids	Positive
P3010	ALL	2	M	11.0	Flu/Cy/TBI	5	CSA, MMF	Positive
P3275	SCID	1	M	5.2	Bu/Cy/Flu	6	CSA, MMF, steroids	Positive
P3317	Myelofibrosis	2	M	9.7	Bu/Cy/Flu	5	CSA, MMF, steroids	Positive
P3457	JMML	2	M	12.3	Bu/Cy/Flu	5	CSA, MMF	Positive
P3531	SCID	1	M	6.4	Bu/Cy/Flu	5	CSA, MMF, steroids	Positive
P3555	SCID	1	M	8.0	Bu/Cy/Flu	6	CSA, MMF, steroids	Positive
P3923	AML	1	F	7.0	Bu/Cy/Flu	5	CSA, MMF, steroids	Positive
P3626	JMML	1	M	9.0	Bu/Cy/Flu	5	CSA, MMF	Positive
ACT-CAT2								
P0020	AML	3	F	16.7	Bu/Cy	5	CSA, MMF, steroids, ECP	Positive
P0035	Hurler's syndrome	1.1	F	8.8	Bu/Flu/ATG/ Rituximab	4	CSA, steroids	Positive
P0039	Infant ALL	1.9	M	12.2	Flu/Cy/TBI	6	CSA, MMF, steroids	Positive
P0067	Sickle cell disease	8	F	19.8	Flu/Mel/Thio/ Alem/HU	6	CSA, MMF, Infliximab, steroids	Positive
P0123	Sickle cell disease	4	M	16.6	Flu/Mel/Thio/ Alem/HU	5	CSA, Tacro, MMF, steroids	Negative

Patient Infection/reactivation status and outcome

Patient ID	Indication	Prior therapy	Viral course post VST	Status within 1st year
P0123	Adv	Cidofovir	CR (with brincidofovir)	Alive. GVHD resolved at 1 yr
P0067	CMV	Foscarnet/Cidofovir/Ganciclovir	CR→ relapse CMV retinitis	Alive, CMV retinitis ->CR with VST+ GCV, GVHD resolved at 1 yr
P0039	CMV	Foscarnet/Cidofovir/Ganciclovir	CR	Leukemia relapse after 90days, in remission following CAR-T therapy
P0035	CMV	Foscarnet	CR (with valganciclovir)	Alive. Quiescent GVHD on cyclosporine at 1 yr
P2891	CMV	Foscarnet, CMV Immunoglobulin	CR (3x CB-VST + ganciclovir)	Alive. GVHD resolved at 1 yr
P3626	EBV	Cidofovir for prior adenoviremia	PR→CR	Alive. MDS + monosomy 7 at <1 yr resolved at 2 yrs . No GVHD off immunosuppression at 1 yr.
P3457	EBV viremia	None	SD	*Died after second transplant for relapsed leukemia*
P3010	Prophylaxis	Ganciclovir prophylaxis	No reactivation requiring therapy, no disease	Alive. No GVHD off immunosuppression at 1 yr.
P3275	Prophylaxis	None	No reactivation requiring therapy, no disease	Alive. No GVHD off immunosuppression at 1 yr.
P3317	Prophylaxis	Ganciclovir prophylaxis	No reactivation requiring therapy, no disease	*Relapse/AML progression < 1 yr post HCT, deceased D+284.*
P3531	Prophylaxis	Valganciclovir prophylaxis	No reactivation requiring therapy, no disease	Alive. No GVHD off immunosuppression at 1 yr.
P3555	Prophylaxis	None	No reactivation requiring therapy, no disease	Alive. Resolved GVHD at 1 yr
P3923	Prophylaxis	None	No reactivation requiring therapy, no disease	Alive. No GVHD off immunosuppression at 1 yr.
P0020	Prophylaxis/ PHx of Adv	Cidofovir	Adv reactivation 3 months post VSTs →CR with CB-VST+Cidofovir	Alive. Quiescent GVHD on cyclosporine at 1 yr

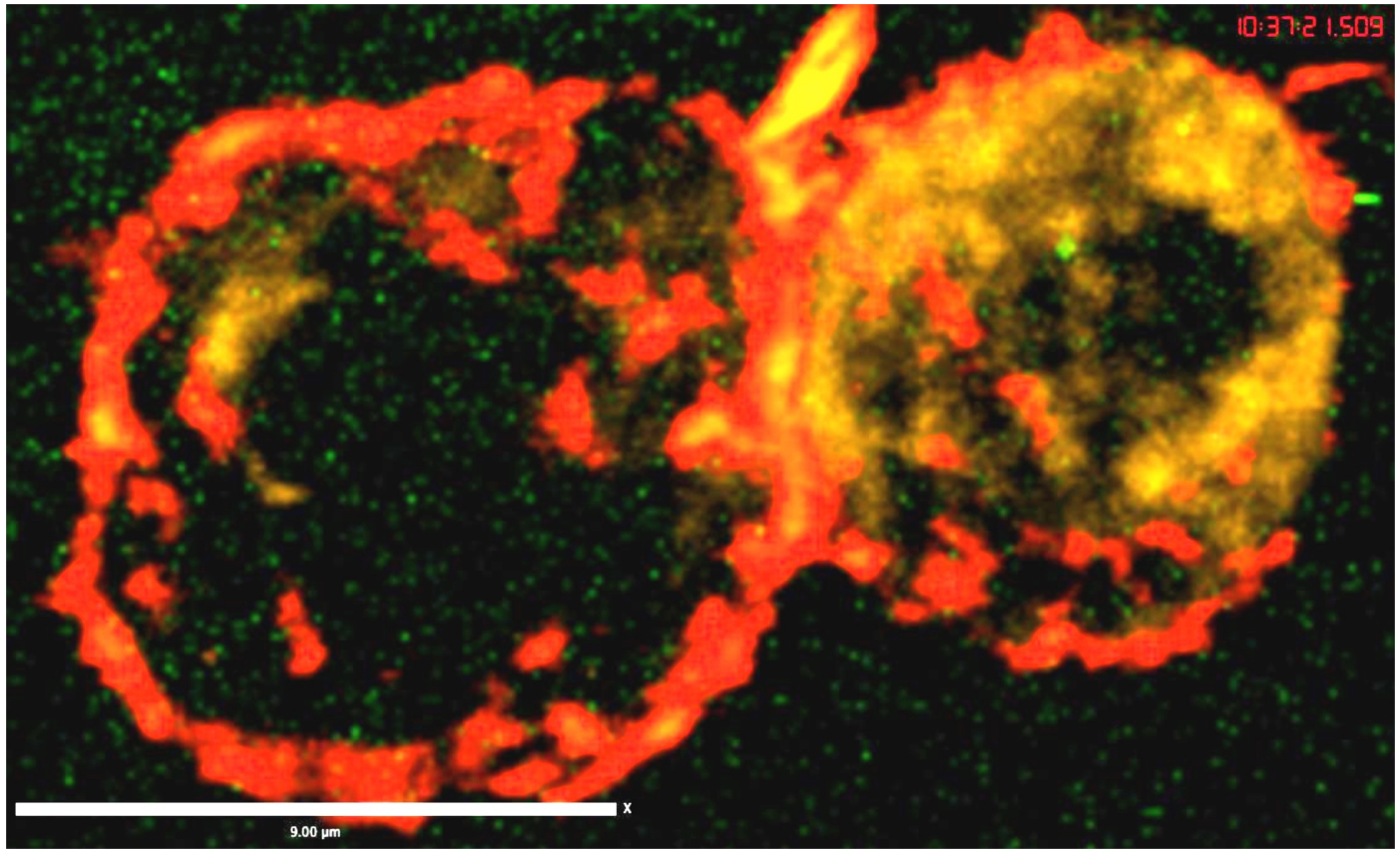
Minimal Related Toxicity – No GVHD

Study	Donor/Recipient Matching	# of Patients	Acute GvHD
Peripheral Blood VST	Haplo	14	None
	MUD	6	1 Grade I
	MRD	10	1 Grade I
Cord Blood VST	CBT: 5/6	6	None
	CBT: 6/6	4	None

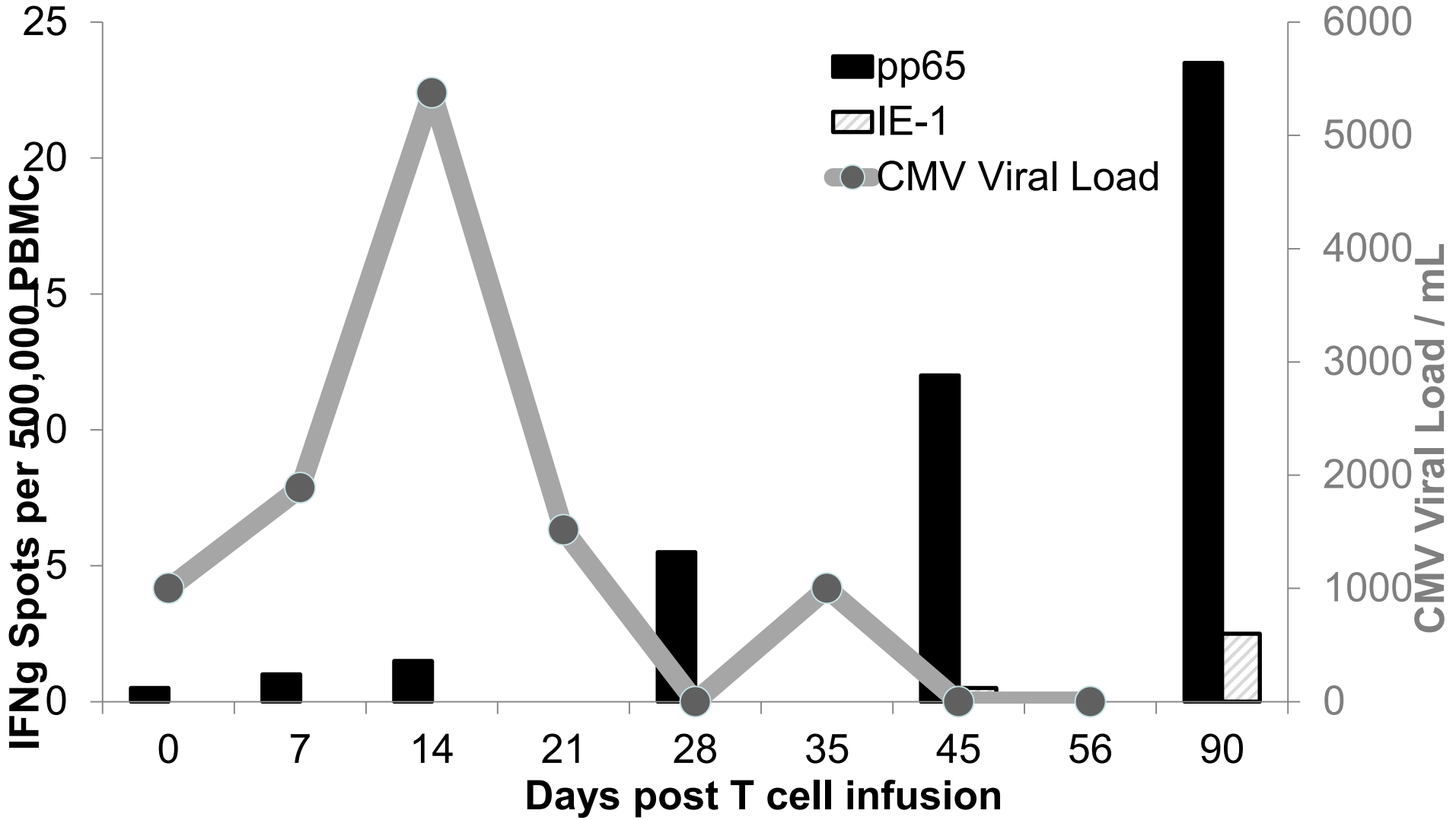
Melenhorst, Castillo et al, Mol Ther 2015

Melenhorst et al, Blood 2010

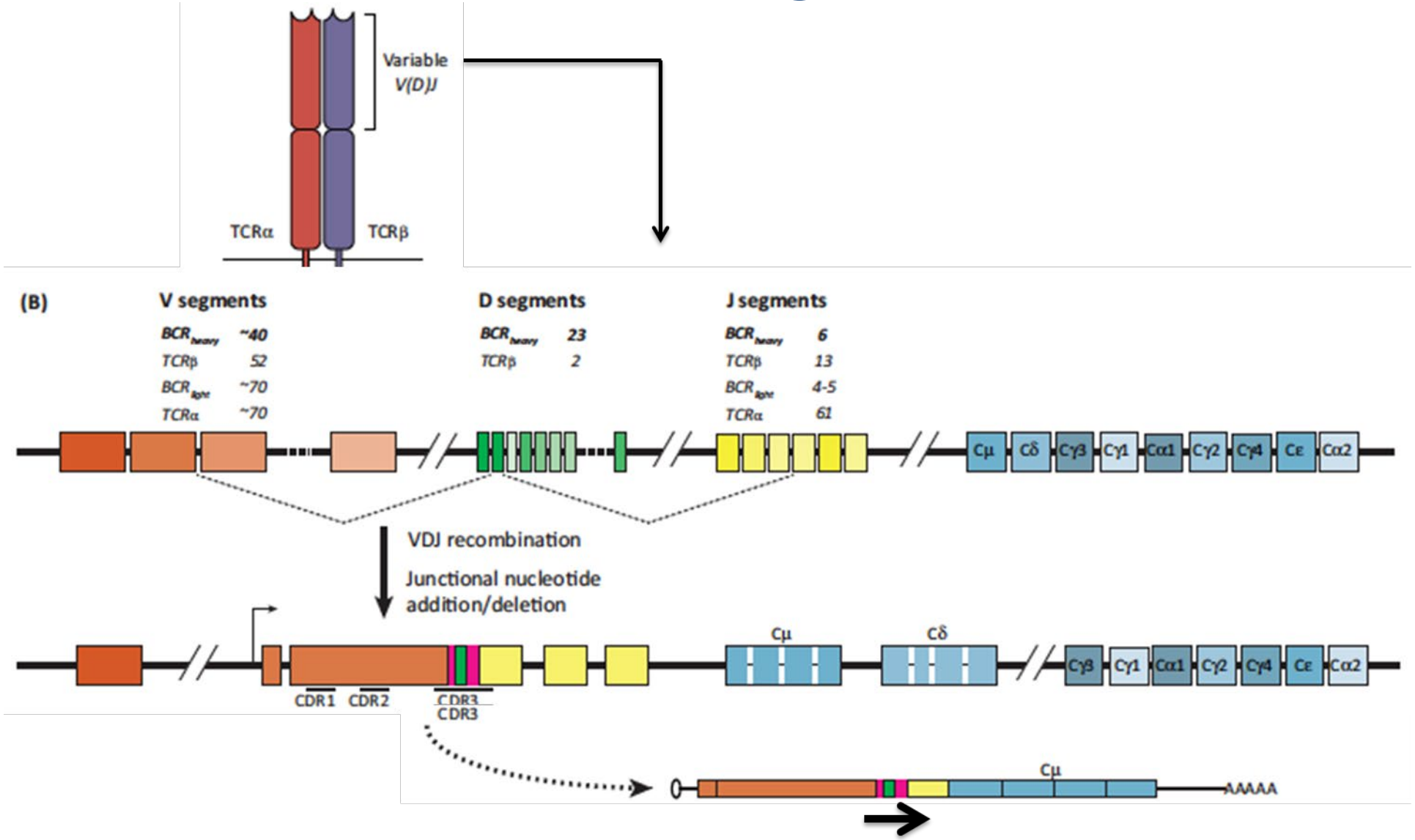
Do Virus-specific T cells persist?



Clearance of CMV Viral Load Corresponds to rise in CMV specific T cells *in vivo*

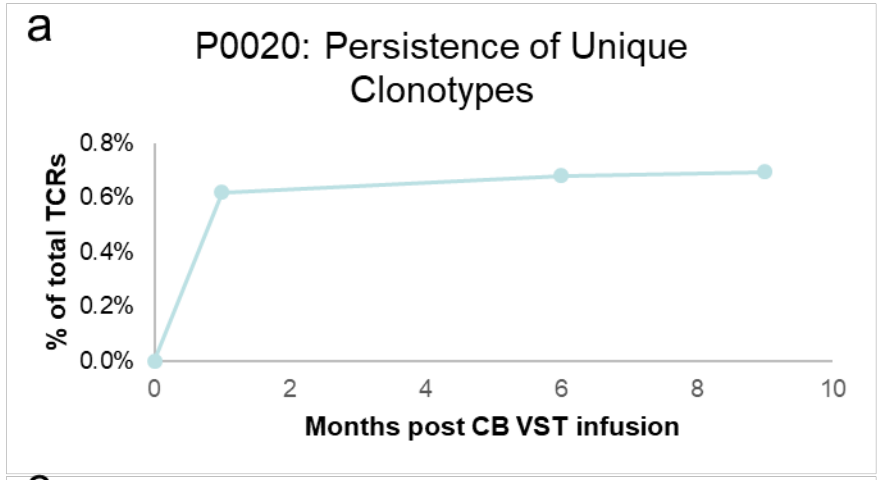


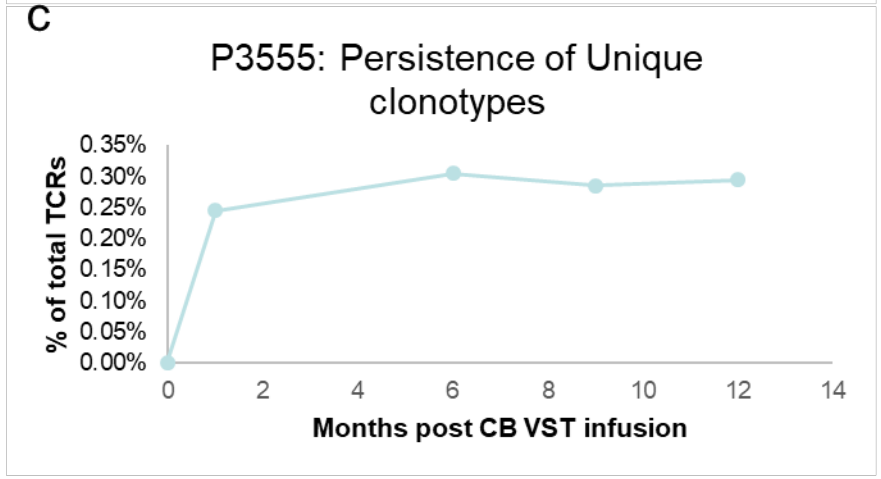
TCRβ sequencing allows high-resolution T-cell tracking *in vivo*

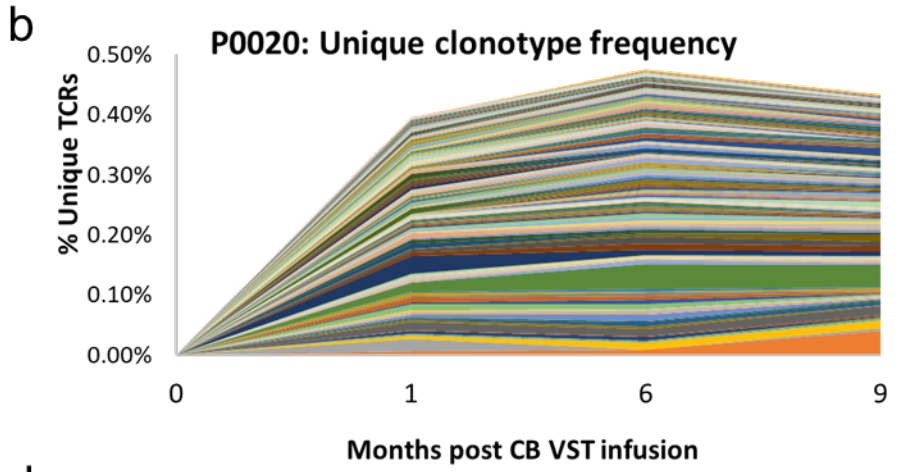


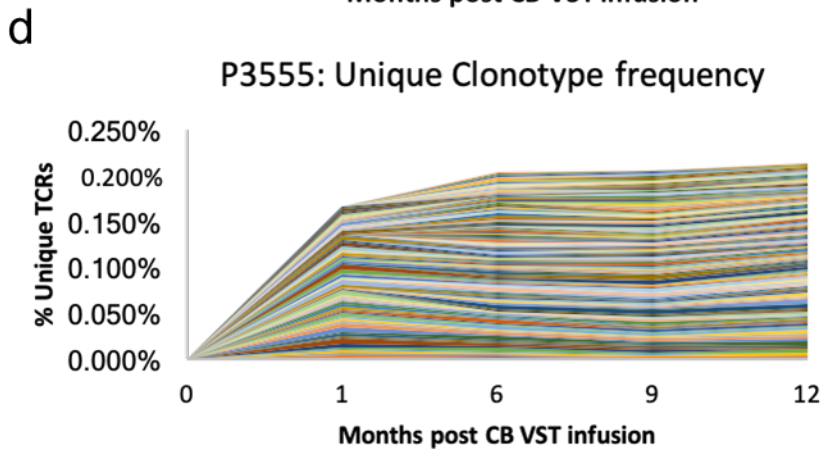
Ultradeep sequencing

Infused CB-derived VST Clones Persist and Expand *in vivo*



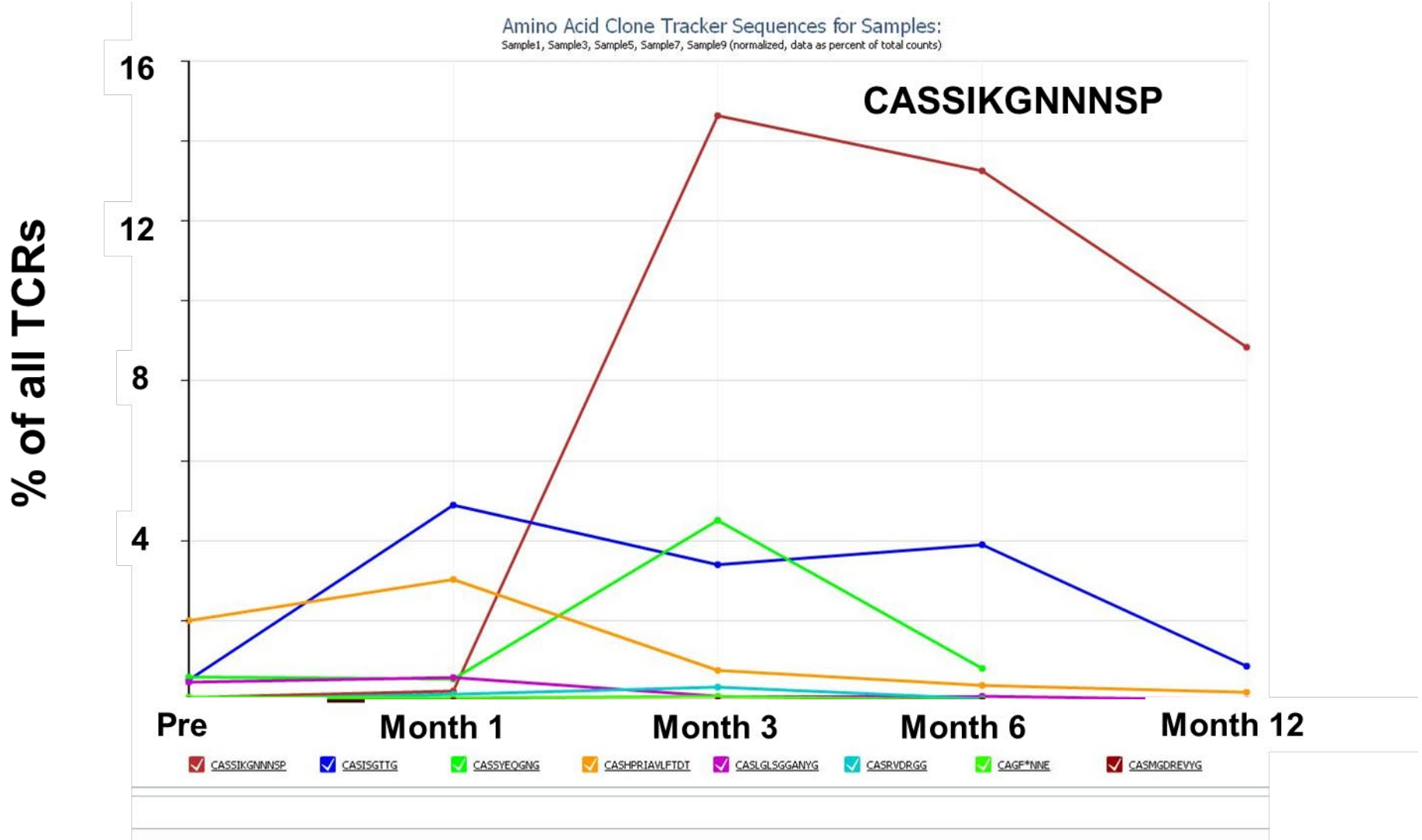






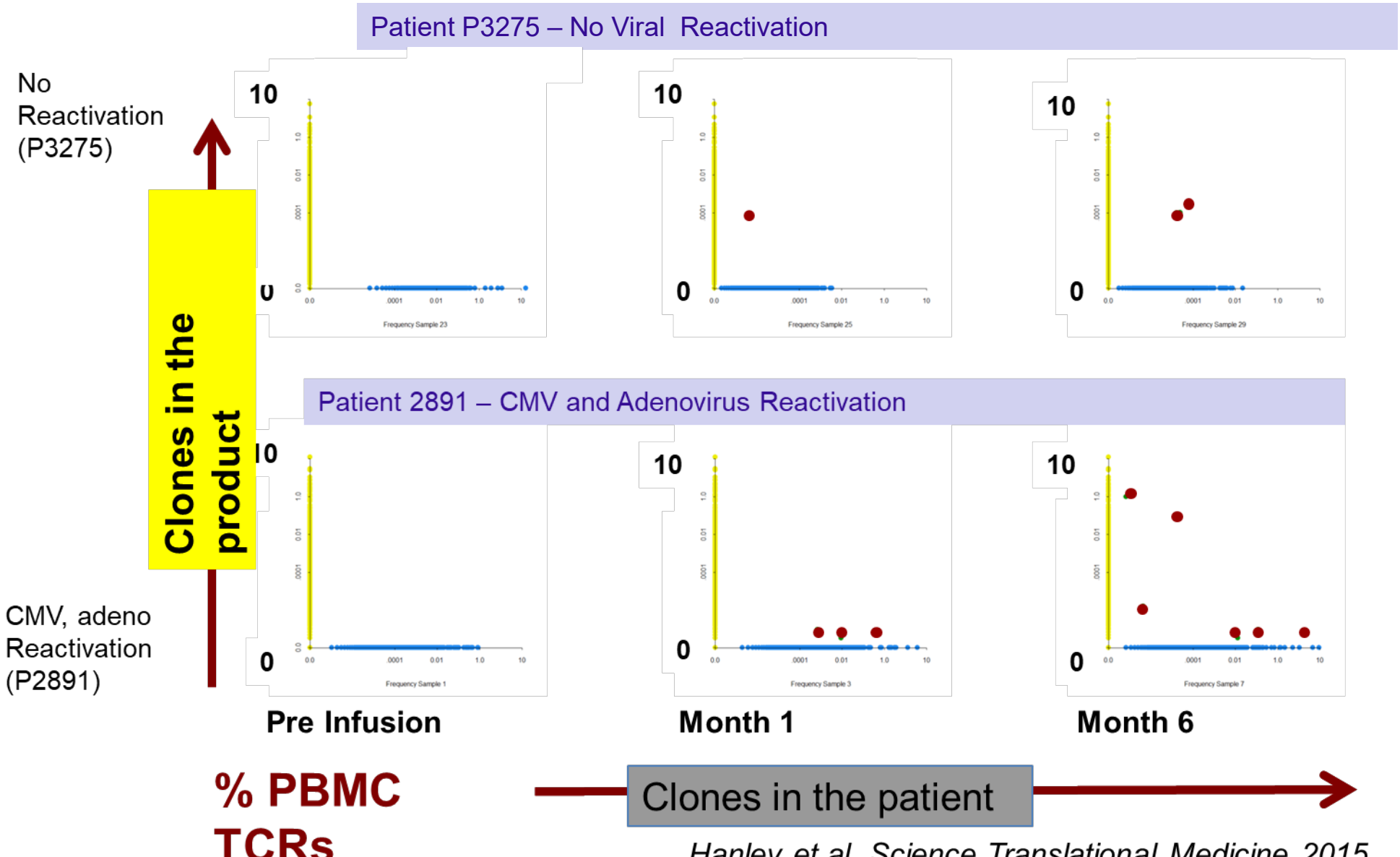
Abraham et al, Blood Advances 2019

Infused VST Clones Persist and Expand *in vivo*



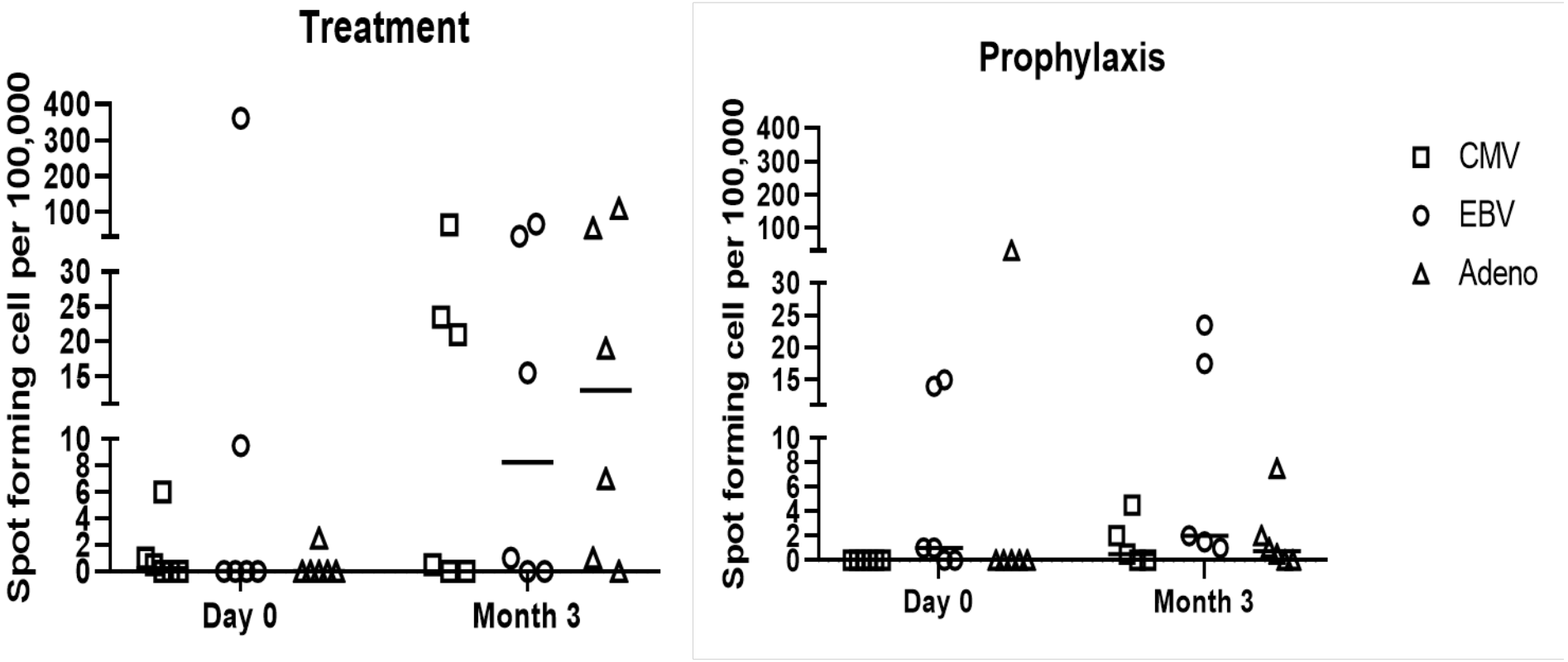
Hanley et al, Science Translational Medicine 2015

Duration of CB-derived T cell Persistence Depends on Viral Reactivation



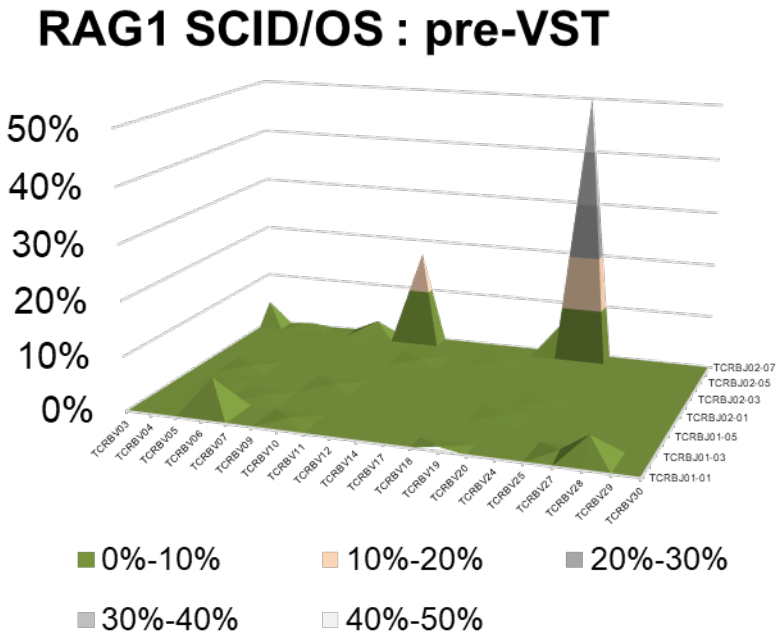
Hanley et al. Science Translational Medicine, 2015

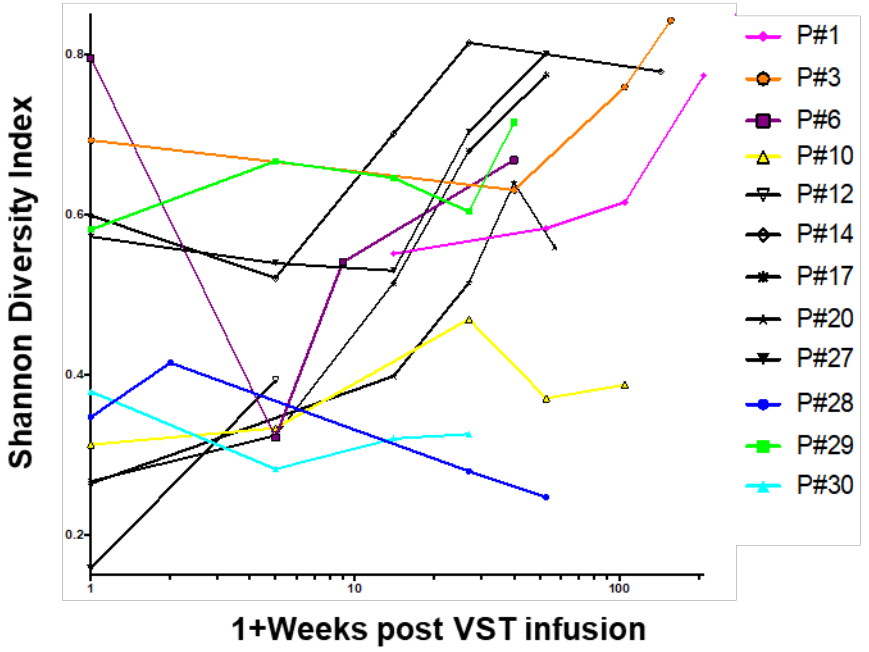
Detecting the Expansion of VSTs in vivo Depends on Viral Reactivation

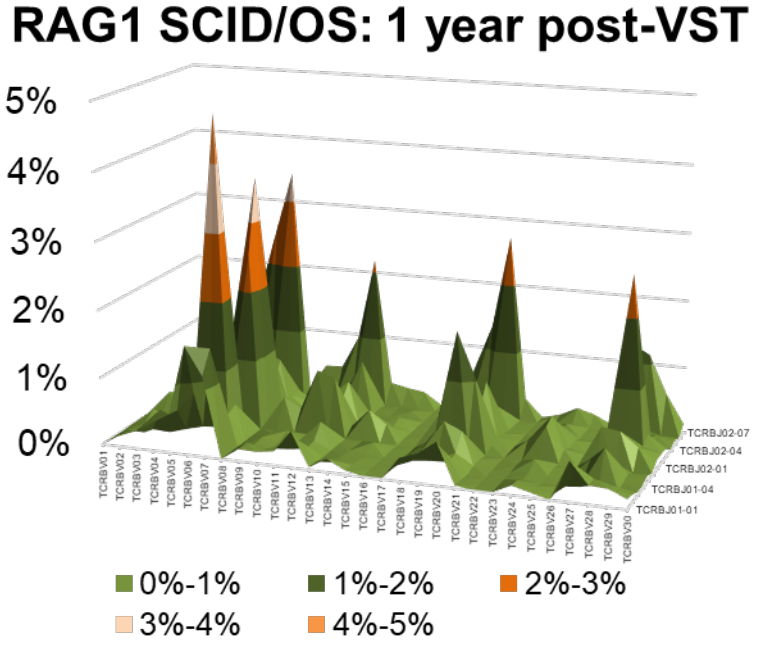


Abraham et al, Blood Advances 2019

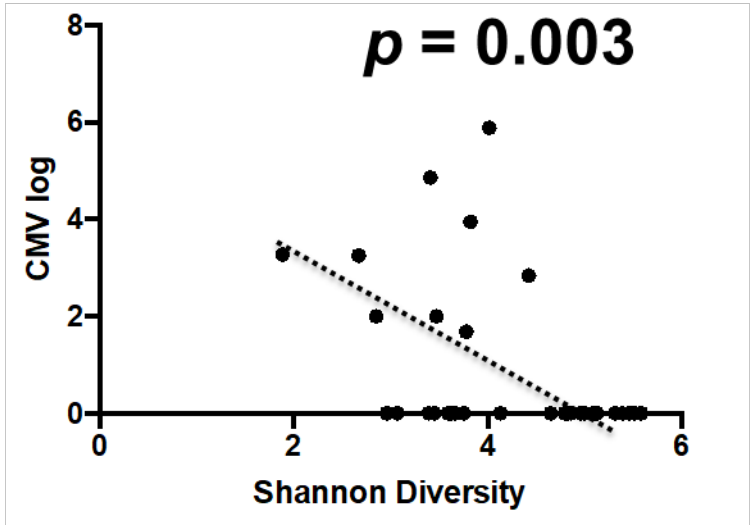
TCRβ repertoire correlates with CMV control







Keller et al BJH 2019



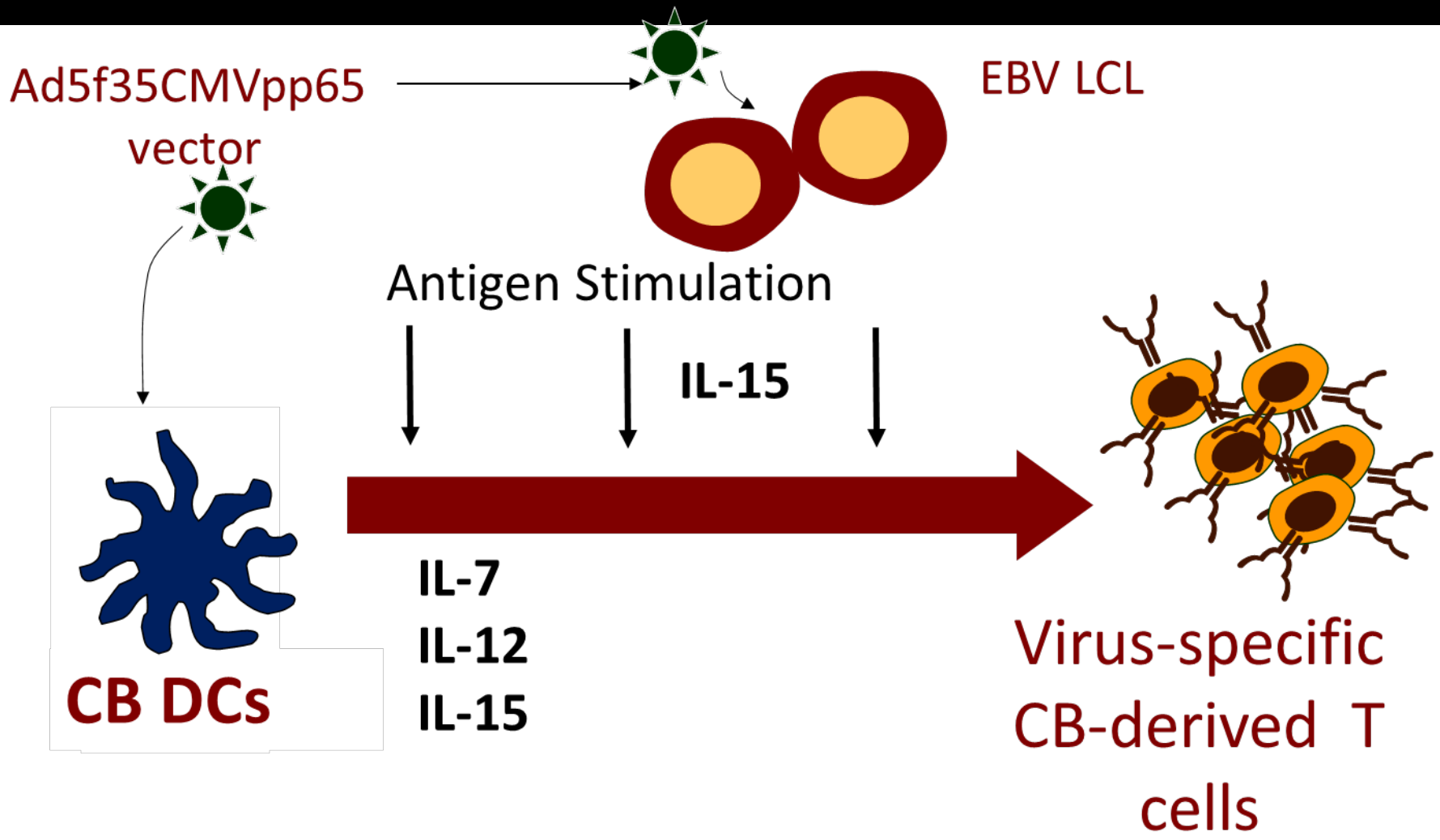
Summary: CB Multivirus-specific T cells are Safe and Protective in vivo

- CB-derived VSTs can be reliably expanded for clinical use
- Safe to infuse to patients (minimal toxicity)
- Long term (12mths) persistence of CB-VSTs especially in presence of antigen
- CB-derived virus-specific T cells appear protective in vivo

Can we manufacture faster and infuse earlier and Broaden to Other Viruses?

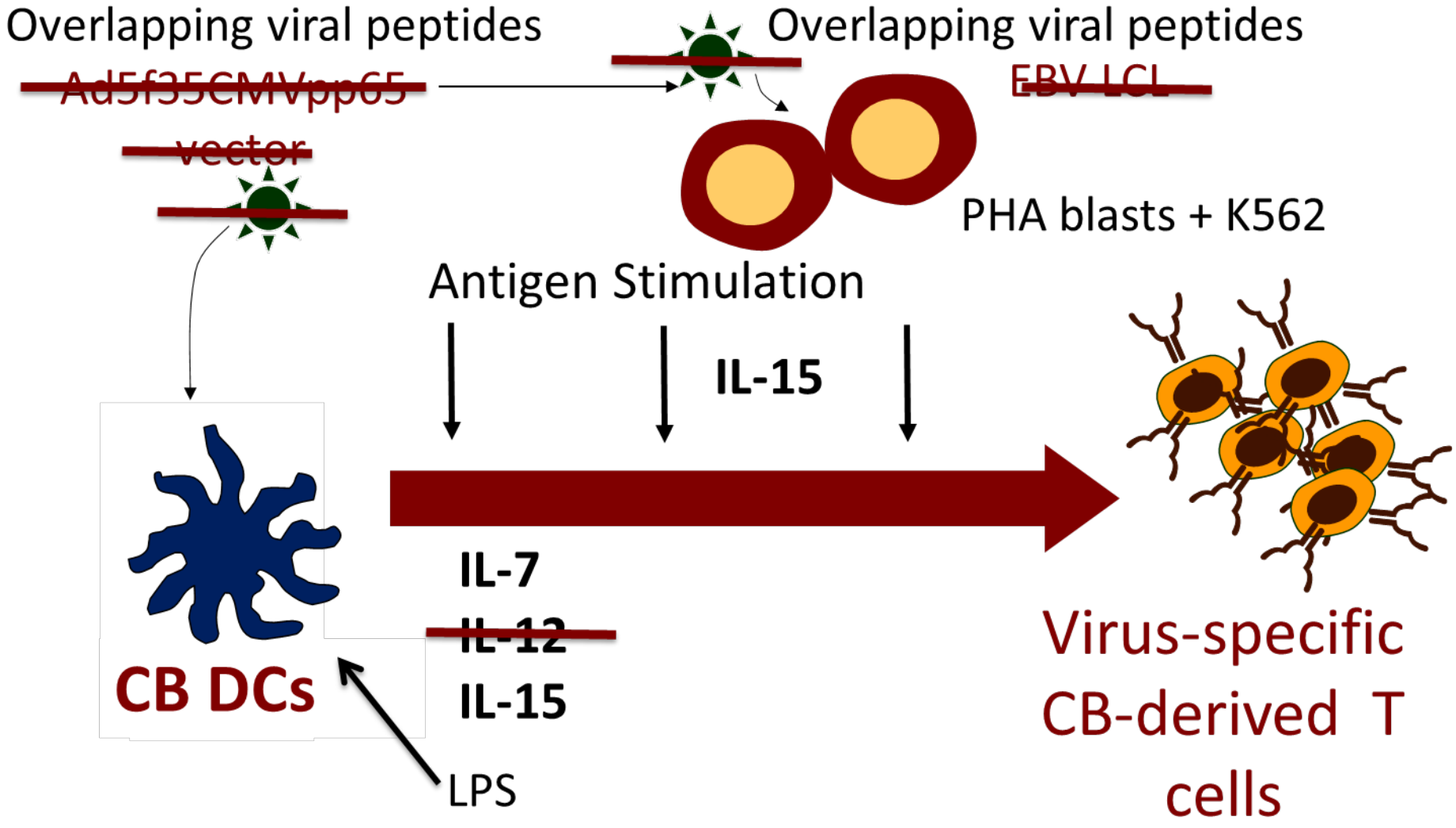
- manufacture time up to 70+ days
- median time-to-infusion: 92 days

Original Manufacturing Method using LCL



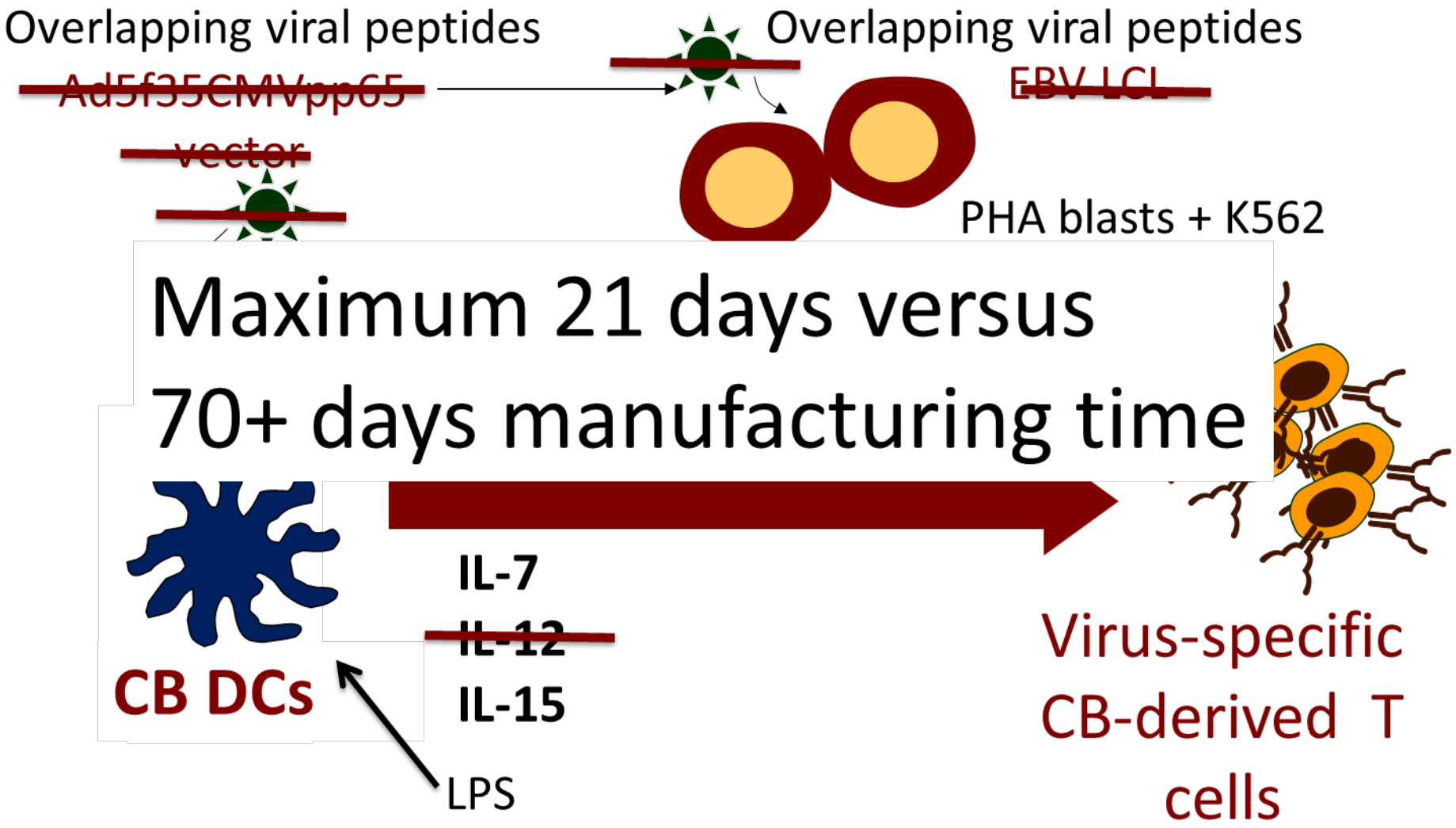
Dave et al, Mol Ther Methods Clin Dev. 2017

Optimized Manufacturing Method – Replacing LCL with PHA blasts + GM K562



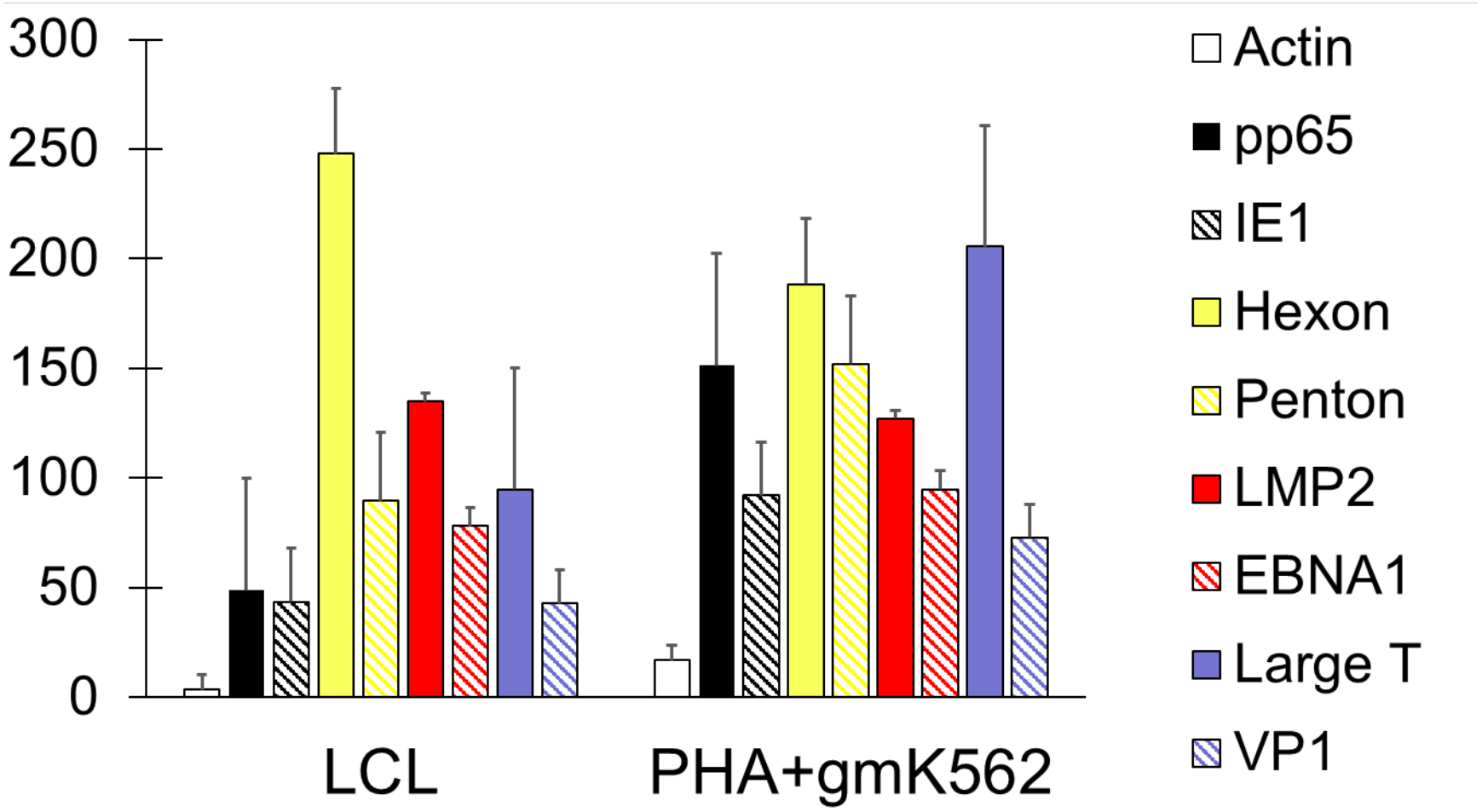
Dave et al, Mol Ther Methods Clin Dev. 2017

Optimized Manufacturing Method – Replacing LCL with PHA blasts + GM K562



Dave et al, Mol Ther Methods Clin Dev. 2017

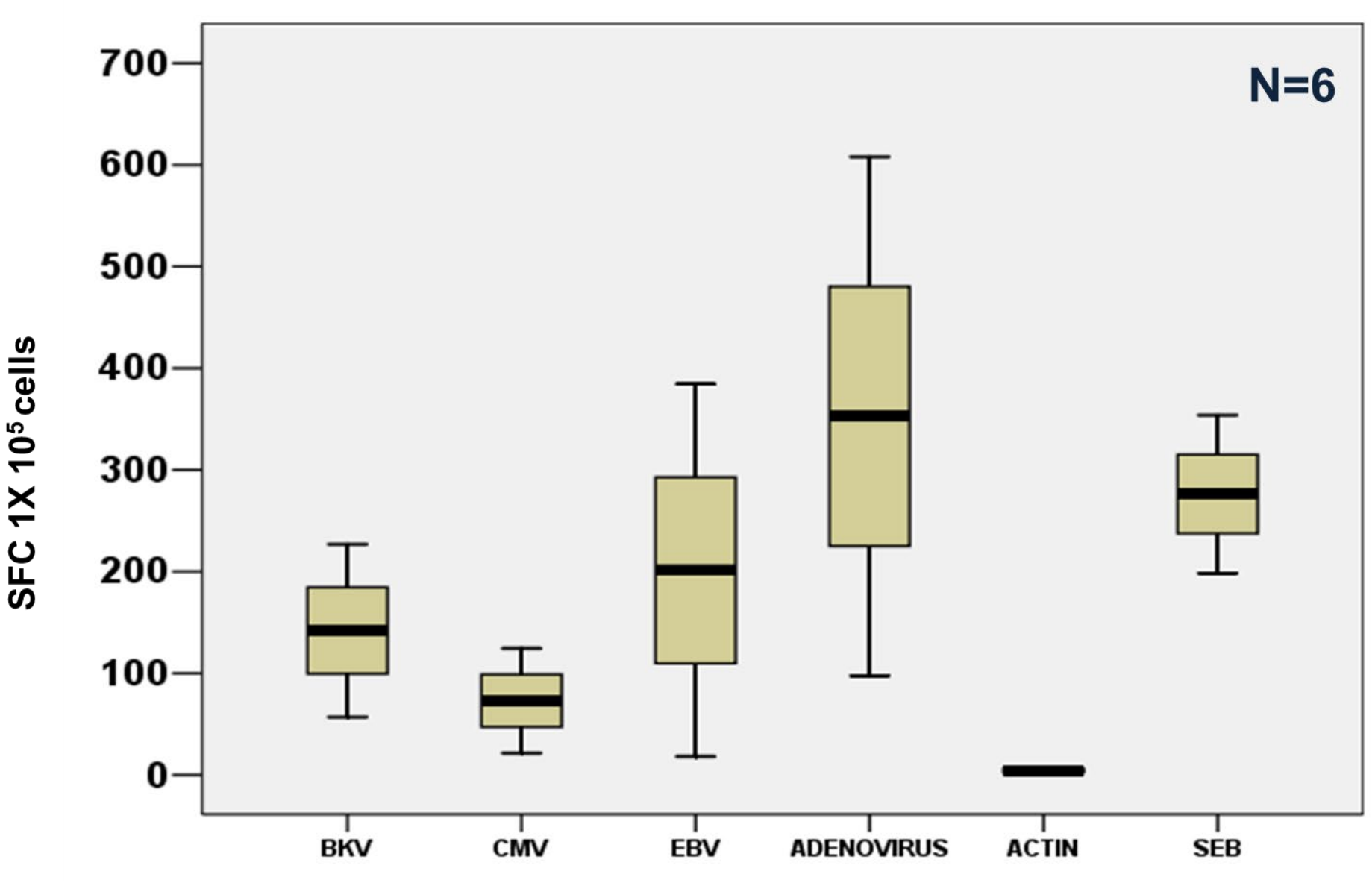
Rapidly expanded VSTs Have Equal Specificity including BKV



Dave et al, Mol Ther Methods Clin Dev. 2017

Can we manufacture faster and infuse earlier and Broaden to Other Viruses?

Rapid Manufacture CB-derived multi-virus T cells Specific for BKV, CMV, EBV, Ad



Dave et al, Mol Ther Methods Clin Dev. 2017

CB-derived VST Trial: CHEERs

Adoptive Cord Blood Immunotherapy using Expanded Cord Blood T cells for BK virus, EBV, CMV, BKV and Adenovirus Reactivation/Infection or Prophylaxis (IND16909). NCT03594981

Children's National (contact) PI Allistair Abraham
MDACC PIs: Amanda Olsen/ EJ Shpall
Sponsor : Cath Bollard

1 patient treated with 4-virus specific CB-T for CMV viremia
→no GVHD, weaned off IV antivirals, no viral disease

Where to now with CB-derived VSTs?

- Potential Third party donor source (Haque 2007, Barker 2010, Doubrovina 2012, Leen 2103,Tzannou 2017, Withers 2017, Tzannou 2019, Prockop 2020)

→Consortium/Cooperative Group and Pharma trials

- Expanding to other viruses

VSTs Can Target Multiple (? Any) Viruses/ Pathogens after CBT, BMT and Beyond!

- **HIV** *(Patel et al, Mol Ther 2018, Mol Ther Methods Clinical Devel 2019, Sung et al,Mol Ther 2018, Patel et al, BBMT 2016)*
- **EBV+ Lymphoma**

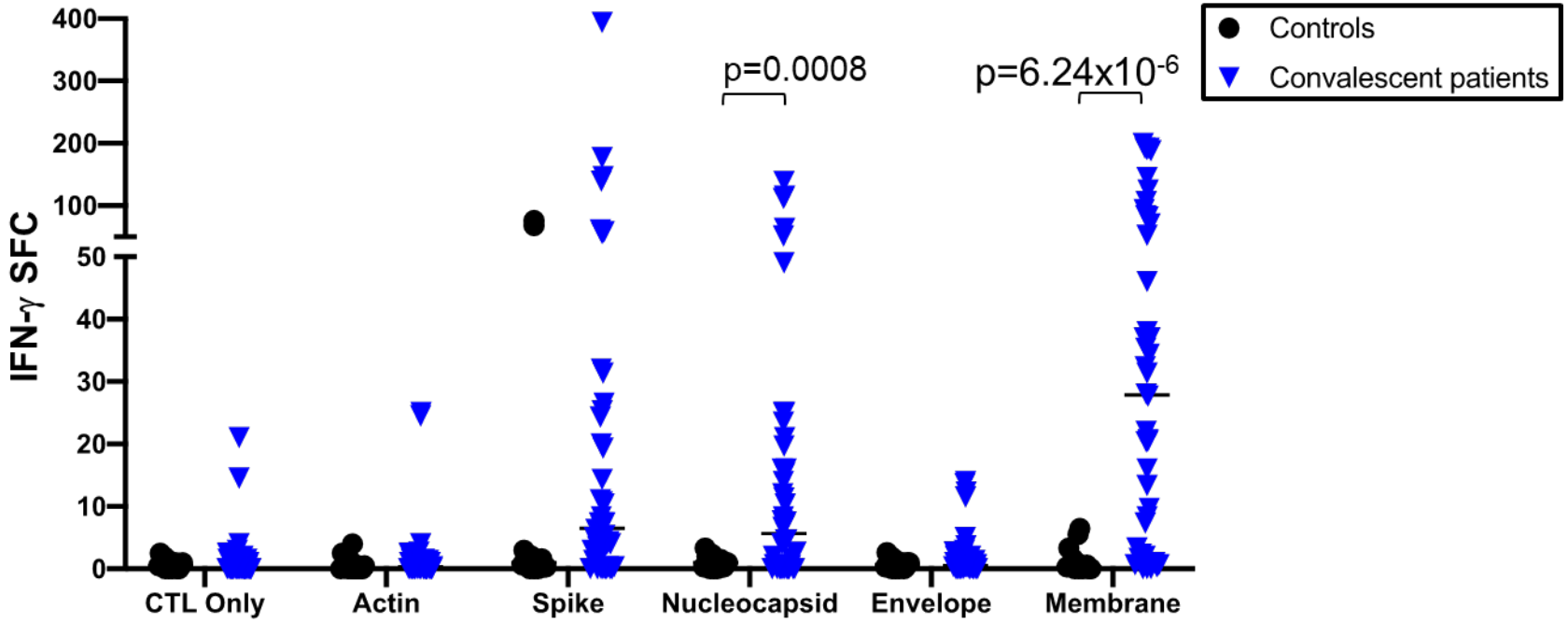
(McLaughlin et al, Blood 2018, Bollard et al, JCO 2018)

- **Pre-clinical targets**
- **Parainfluenza**(McLauglin et al Cytotherapy, 2018, Harris et al, Frontiers Immunol 2020 in press)
  - **Norovirus** *(Hanajiri et al, JID 2019)- IND approved*
  - **Zika Virus** *(Hanajiri et al, Cytotherapy, 2019)*
  - **Mycobacteria** *(Patel et al, Frontiers Immunology, 2019)*
  - **Fungal** (*Castillo et al, Molecular Therapy - Methods & Clinical Development. 2018)*
  - **HPV** *(McCormack et al Cytotherapy 2018)*

Broadening to SARS-CoV2

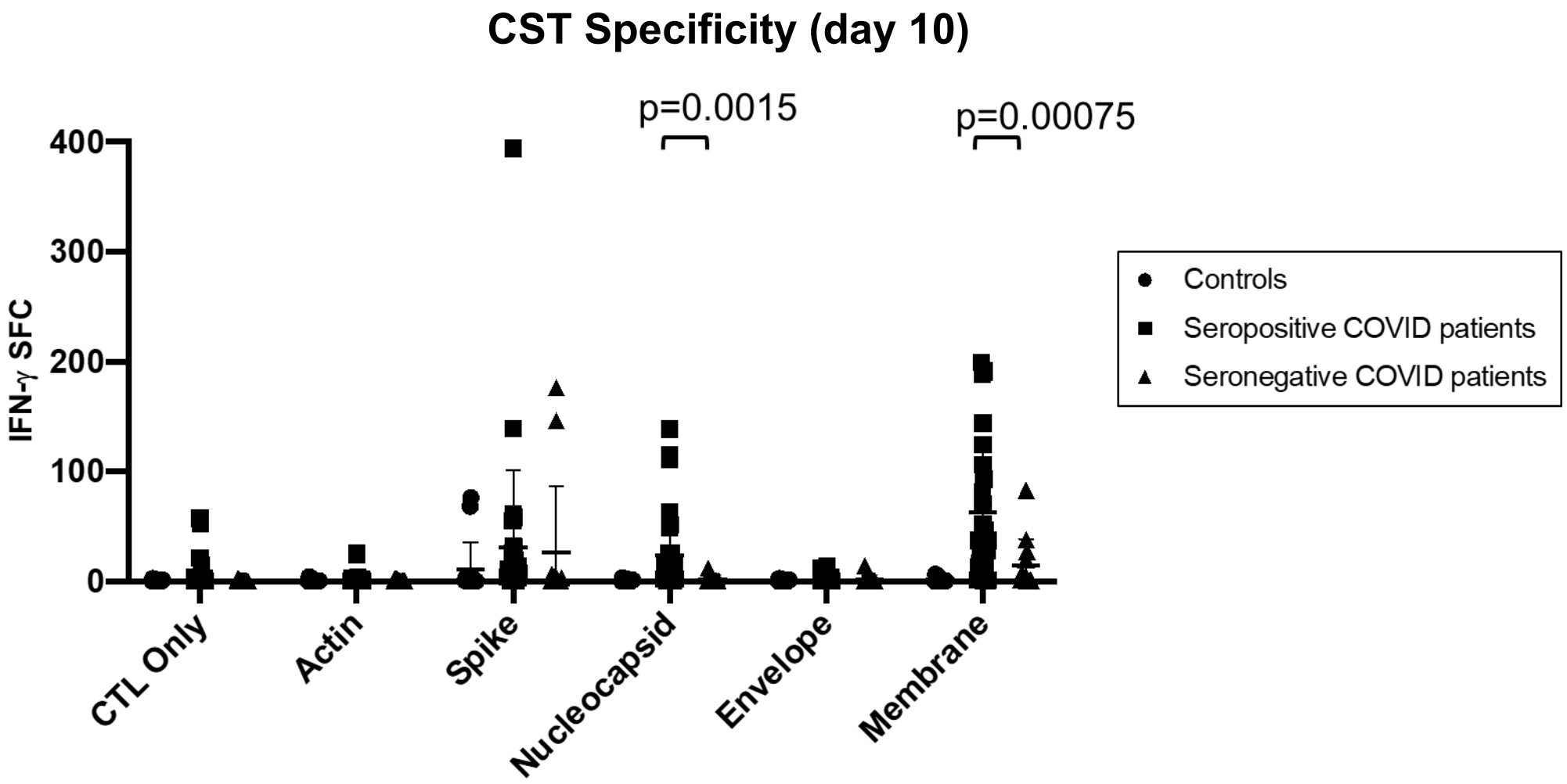
SARS-CoV2 specific T cells Generated from Convalescent Donors Recognize Multiple SARS-CoV-2 Structural Proteins

T-cell Specificity by ELISpot (day 10 post expansion)

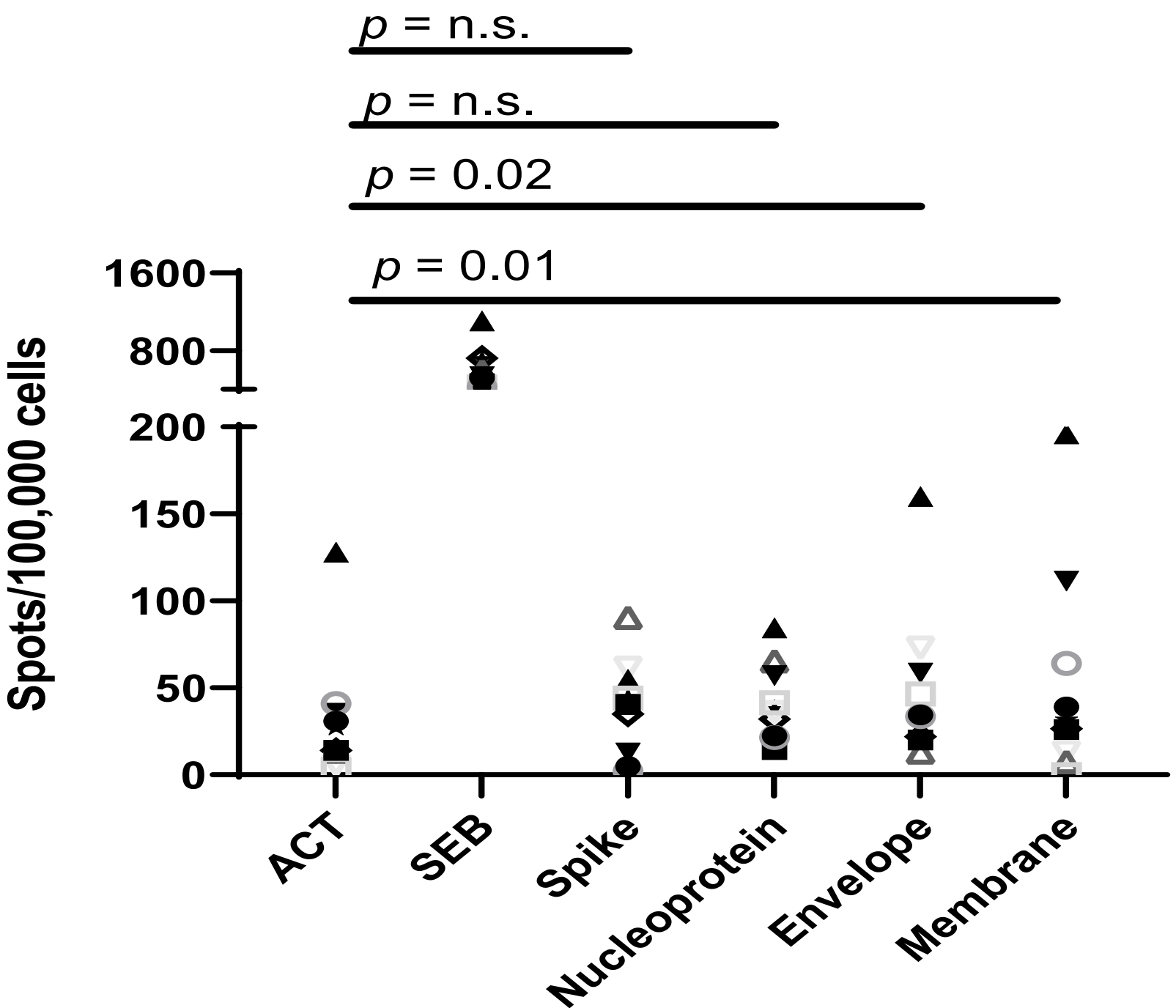


Keller et al, in revision

Seropositive Donors Recognize a Broader Range of Viral Proteins



Keller et al, in revision



SARS-CoV-2 specific T cells can be generated from CB

Abraham, Hanley, *unpublished*

Overall Conclusions- Utilizing CB as Source of VSTs

- VSTs (?for any virus?) can be expanded from naive cord blood T cells
- CB-derived VSTs can prevent and safely treat viral infection after single CBT
- Need to determine if rapidly expanded CB-derived VSTs can protect and treat virus infections in patients after double CBT
- Application for third party setting (HIV, ZIKV, SARS-CoV2)
- Platform for gene modification and gene editing strategies…

Acknowledgements

CETI

Dr. Cath Bollard
Dr. Patrick Hanley
Dr. Russell Cruz
Dr. Michael Keller
Dr. Fahmida Hoq
Dr. Lauren Jerkins
Dr. Hema Dave
Haili Lang
Dr. Shabnum Patel
Allison Powell
Dr. Melanie Grant
Dr. Amy Hont
Dr. Katie Harris
Morgan Galligan
Dr. Maja Stanojevic
Dr. Ryo Hanajiri
Dr. Adora Lin
Dr. Ping Hsien Lee
Dr. Blachy Davila
Dr. David Jacobsoh

CNMC

Dr. Jeff Dome
Dr. Mark Batshaw
Dr. Vittorio Gallo

Collaborators

Dr. Eric Yvon (GWU)
Dr. Brad Jones (Cornell
Dr. Kieron Dunleavy (GWU)
Dr. David Margolis (UNC)
Dr. EJ Shpall (MDACC)
Dr. Katy Rezvani (MDACC)
Dr. Jeff Molldrem (MDACC)
Dr. Helen Heslop (BCM)
Dr. Clio Rooney (BCM)
Dr. Rick Jones (JHH)
Dr. Rich Ambinder (JHH)
Dr. Danny Douek (NIH)
Dr. Jeff Cohen (NIAID
Dr. Kim Green (NIAID)
Dr. Alison Han (NIAID)