Development Of T-Cell Therapies For EBV

Texas Children's Hospital

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CENTER FOR
CELL AND GENE THERAPY

Baylor College of Medicine

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Houston Methodist
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Cliona Rooney

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Convention Center

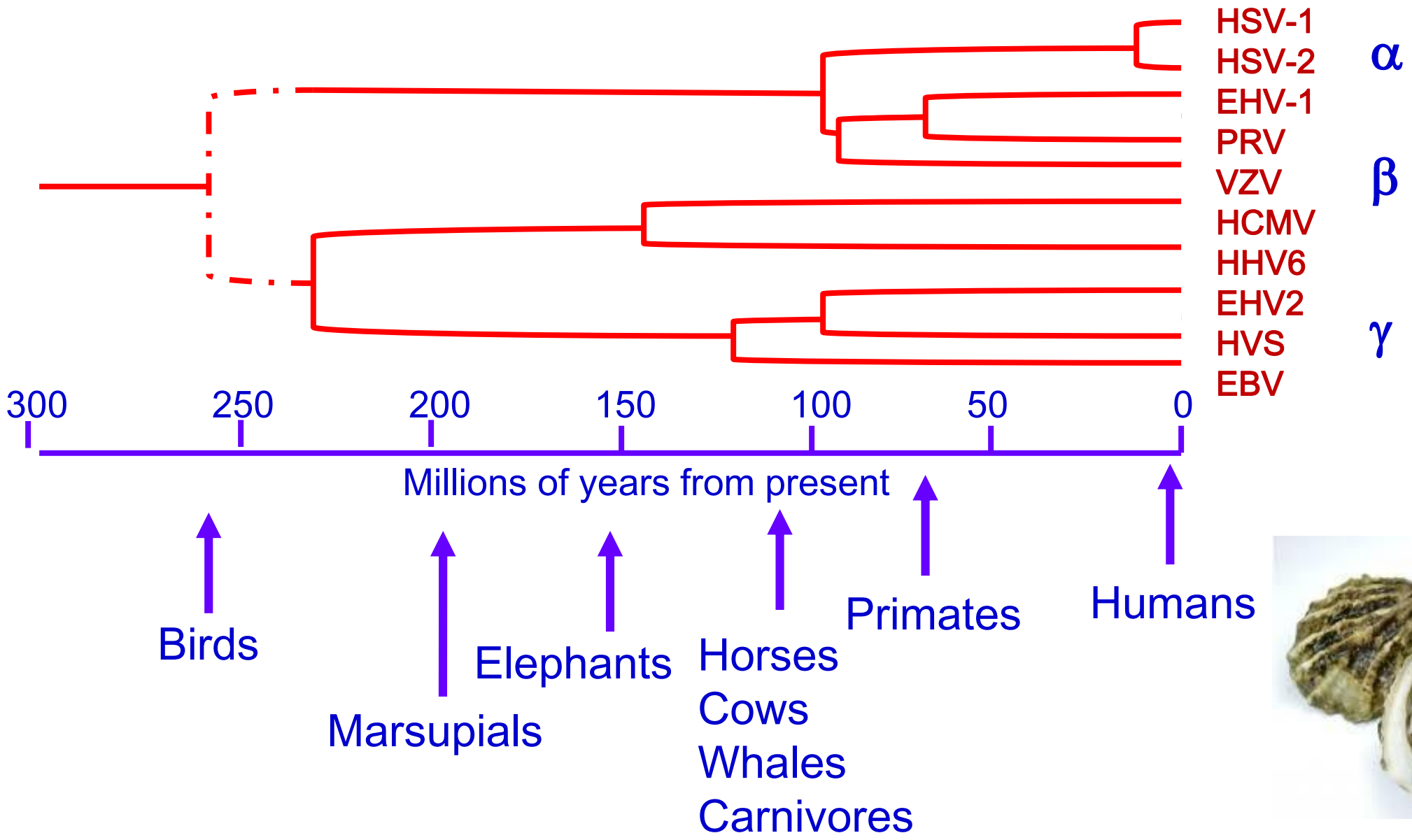
Disclosures

- Founder member of Allovir and Marker Therapeutics
- SAB CellGenix
- Sponsored research and consultancy with Tessa Therapeutics

Take away points

- EBV associated with a range of malignancies
- Different patterns of protein expression
  - Require specific targeting strategies
    - Post transplant lymphoproliferative disease PTLD
    - Lymphoma outside the HSCT setting
- Importance of manufacturing strategies
- Role of allogeneic EBVST banks

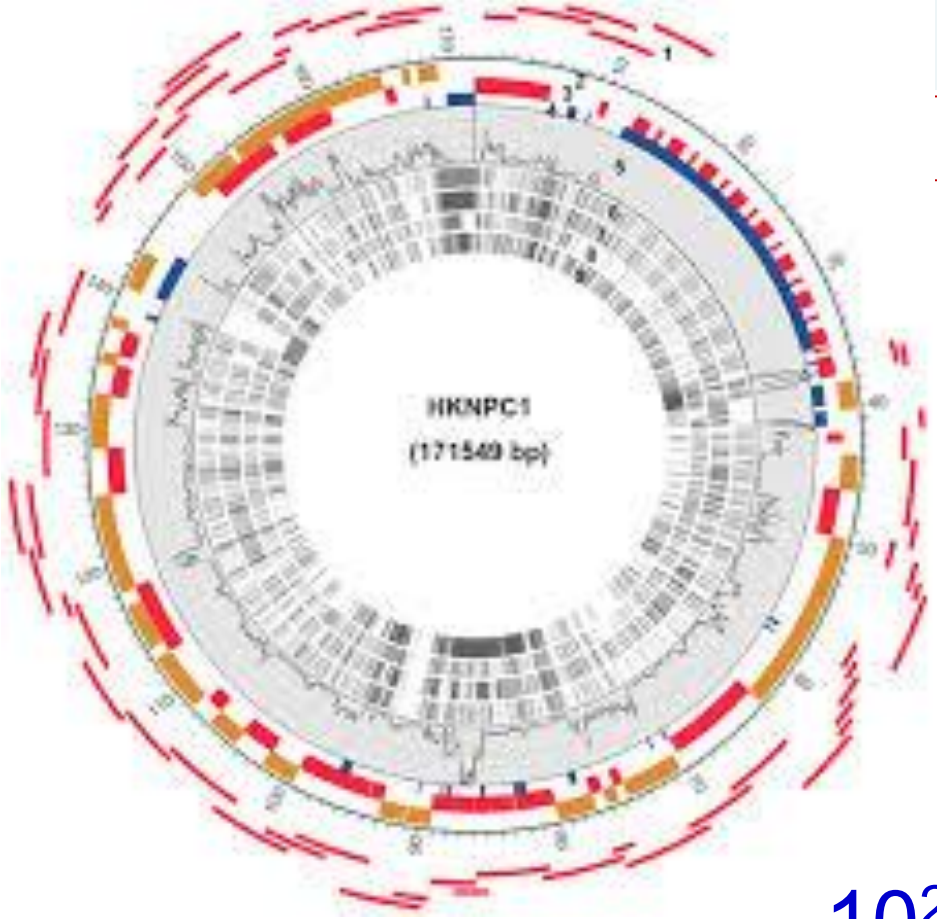
Evolution of herpesviruses relative to species



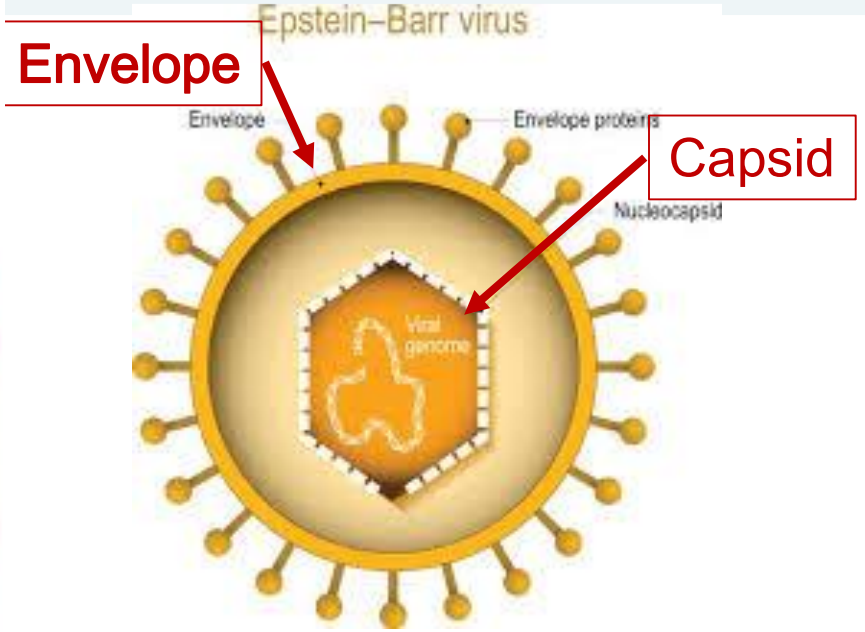
EBV persists for life

- In B lymphocytes
  - Up to 9 “latent” cycle proteins
  - Low level production of infectious virus in oropharynx
- In oropharyngeal epithelial cells
  - ~80 “lytic cycle proteins
    - involved in production of infectious virus particles
  - High levels of virus production

EBV Transmission



90 proteins

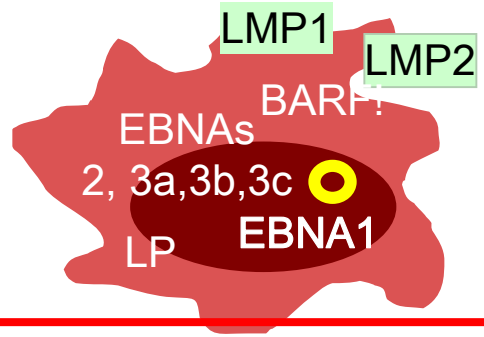
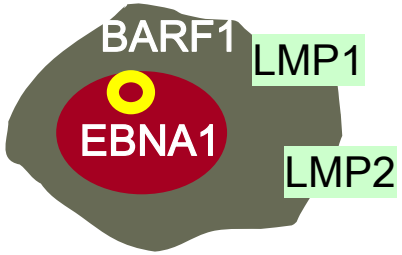


10^2 to 10^8 EBV particles per mL saliva

2×10^4 to 7.2×10^{10} per day

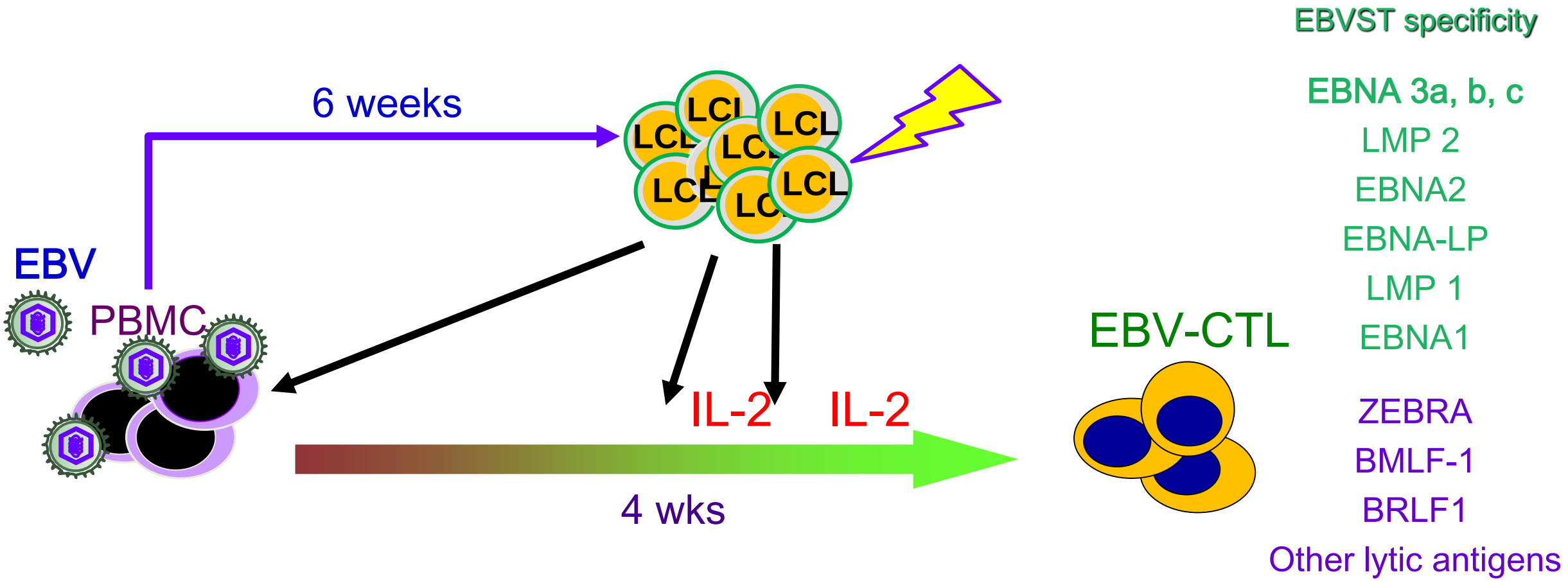
David Thorley-Lawson

EBV protein expression in EBV-malignancies

Latent Gene Expression	Normal B-cell	Latency/Malignancy	Immunogenicity
	Newly infected Tonsillar B-cell EBV-LCL (in vitro)	Type 3 Immunodeficiency-associated lymphoma	
	Germinal Center	Type 2 Hodgkin's lymphoma NHL Nasopharyngeal carcinoma Gastric adenocarcinoma	
	Circulating, memory	Type 1 Burkitt's lymphoma	

Generation of LCL-activated EBV-specific T-cells (EBVSTs)

Wallace, Rickinson and Moss



SJCRH ~1990-1998

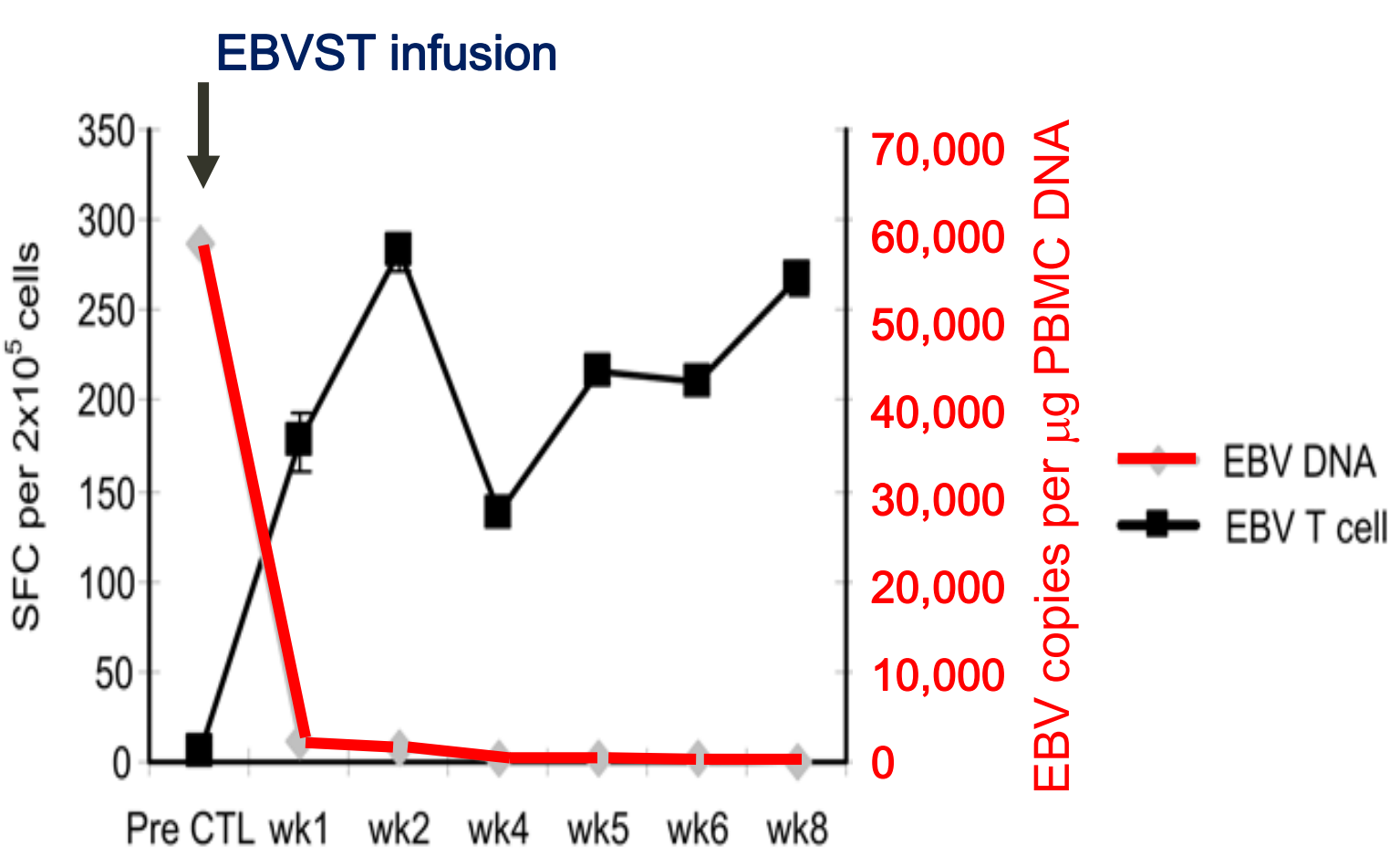
Helen Heslop
Malcolm Brenner

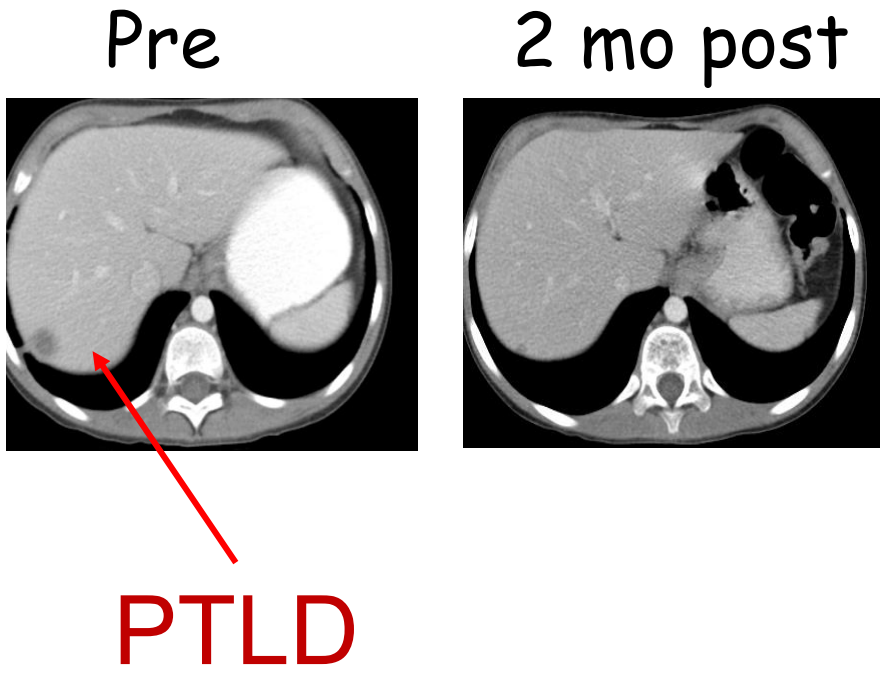
- Incidence of PTLD in HSCT recipients was ~15%
- Associated with T cell depletion

- Donor leukocyte infusions benefitted some patients
  - Severe GVHD

- Could selectively expanded EBVSTs provide benefit w/o GVHD?

Resolution of EBV load and PTLD following EBVST infusion

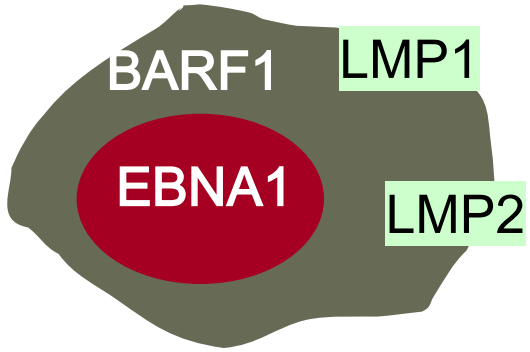




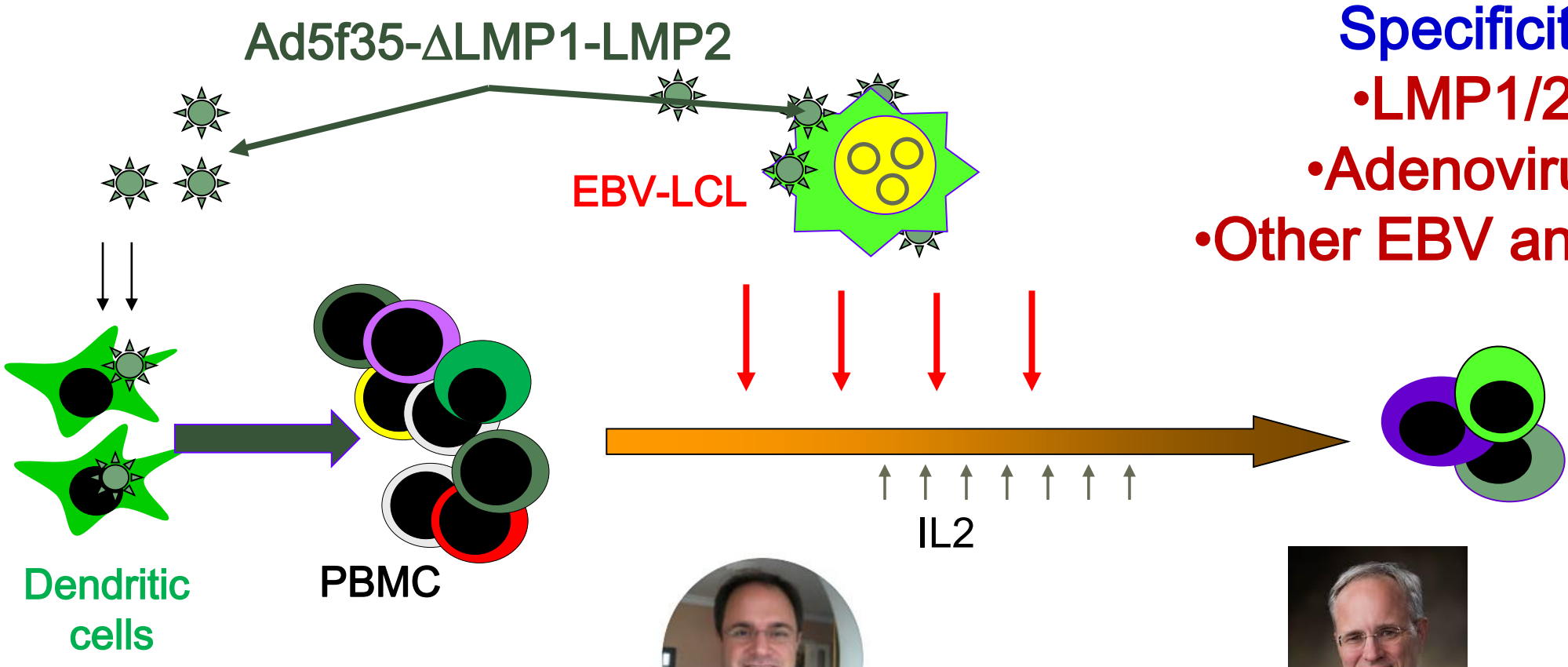
LCL-activated EBVSTs prevent and treat EBV-associated lymphoma after HSCT

- Low doses
  - 2 x 10^7 per m^2
  - Expand exponentially
  - Prevent PTLD
  - CR in 90%
  - No toxicity
  - No GVHD
    - More limited TCR repertoire

- Hodgkin and non-Hodgkin lymphoma outside transplant setting
  - Poor efficacy
  - Lack specificity for type 2 latency antigens



Targeting type 2 latency antigens



Specificity

- LMP1/2
- Adenovirus
- Other EBV antigens

Uluhan Sili, MD, PhD

Steve Gottschalk, MD

Ad-LMP1/2/LCL-activated T-cells For EBV Type 2 malignancy – HL and NHL

- Two Arms
  - Relapsed
  - Adjuvant post Auto HSCT/chemo
- Dose escalation each arm
  - Level 1: $2x10^7$/m2, $2x10^7$/m2
  - Level 2: $2x10^7$/m2, $1x10^8$/m2
  - Level 3: $1x10^8$/m2, $2x10^8$/m2
- No lymphodepletion

Cath Bollard

Bollard et al. J Clin Oncol. 2014 32(8):798-808

Adjuvant Arm

- N=26
- 14 patients post BMT
- 12 post chemo alone
- No toxicities
- 25 remain in remission
  - 1 relapsed 8 wks post CTL
  - CR median of 2.5 years

-All subsequent deaths related to long term effects of chemo/radiotherapy

Patients high risk for relapse at CTL infusion

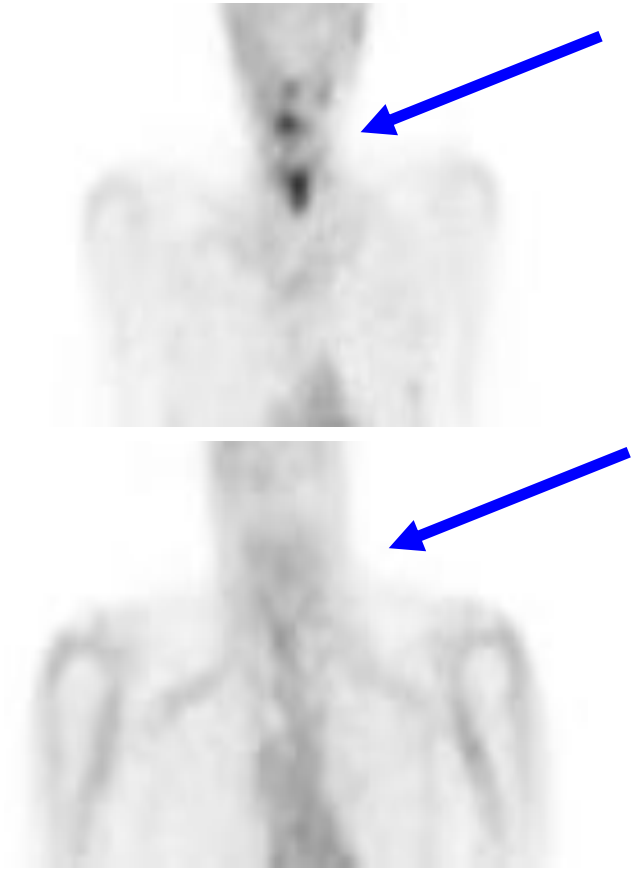
Relapsed Arm

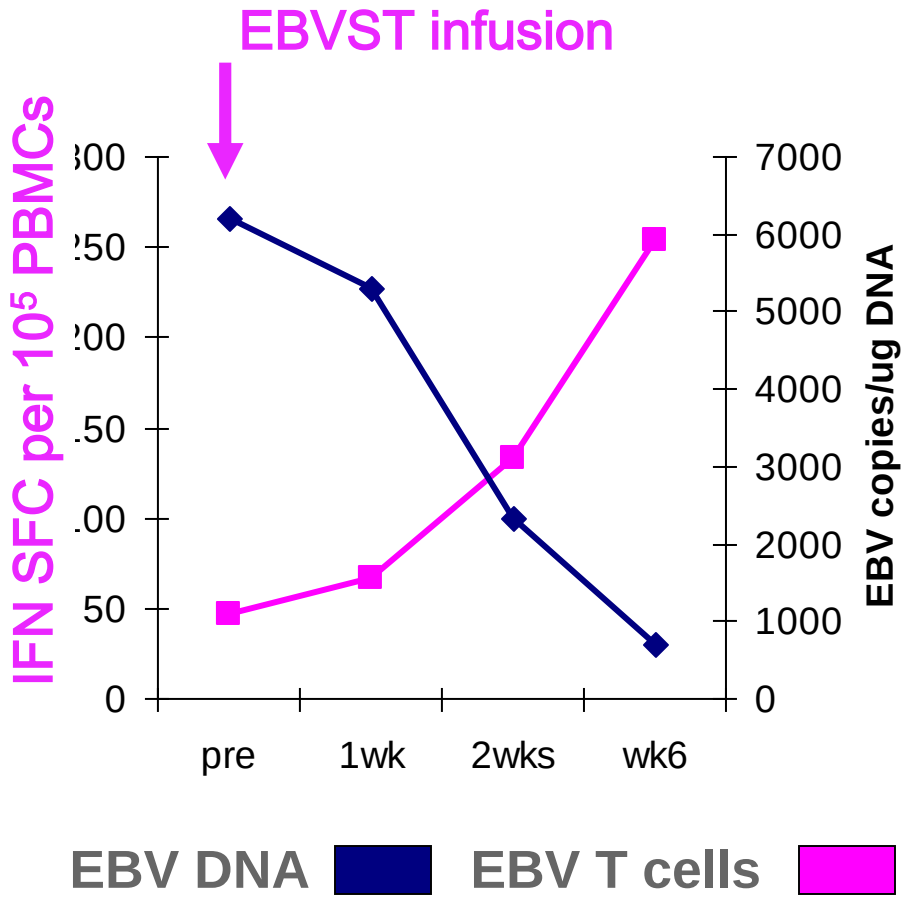
- 23 patients with active disease at time of CTL infusion
  - 10 refractory disease
  - 12 with 2-5 relapses
  - 1 untreated transformation from CLL

Complete Radiological Response
EBV+ve NK-T NHL

Pre CTL

Post CTL





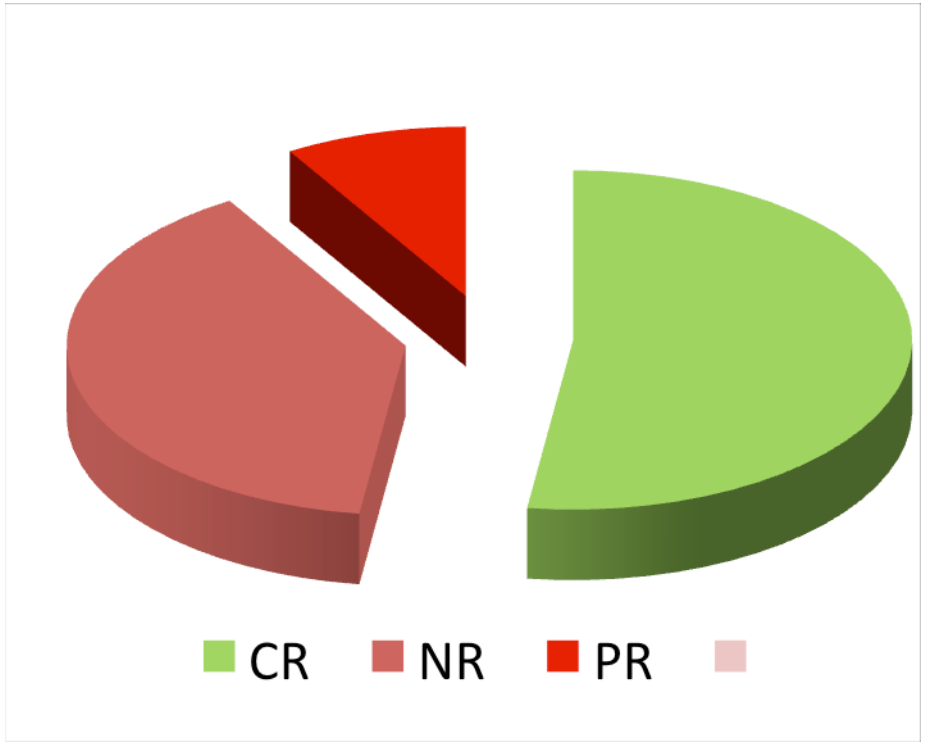
Treatment Arm

Relapsed Disease Arm (n=23)

- No toxicities
- 12 CR (52%)
- 2 very good partial responses
  (up to 36 months)
- 9 no response or progressive disease (39%)

Median clinical response: 1.5y
(range: >6 to >40 months)

Patients with disease at CTL infusion



n =23

Interim summary

- LCL-activated EBVSTs safe and effective for PTLD
- Ad-LMP1/2-LCL-activated EBVSTs effective for HL/NHL

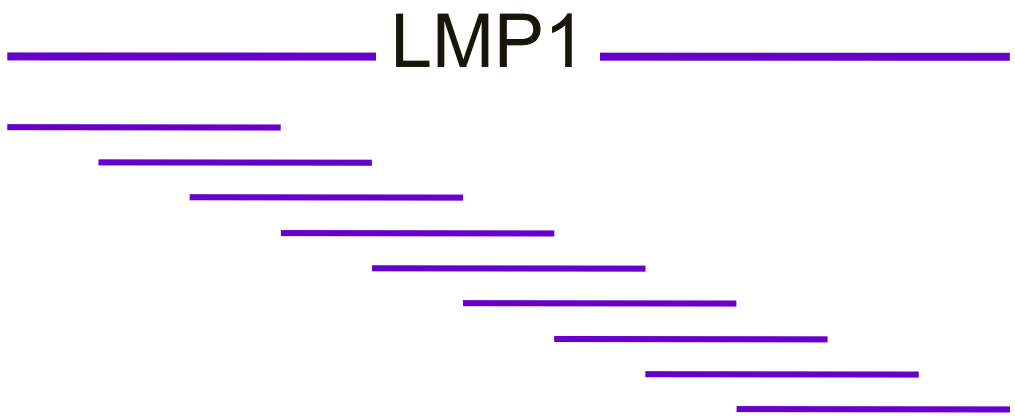
Problems with Manufacturing EBVSTs

- Long (≥12 weeks)
  - 6 wks for LCL, 4 wks for CTL, 2 wks for QC
  - Patients cannot wait
- Complex
  - Live EBV
  - Ad vectors
- Expense of manufacturing time
  - Not a viable healthcare option

HLA-independent method of VST generation

- **Pepmixes**
  - Span entire protein
  - Include all class I epitopes
  - and many class II
  - No knowledge of HLA required
  - Can be combined for GMP testing

15 mers, overlapping by 11 ααs



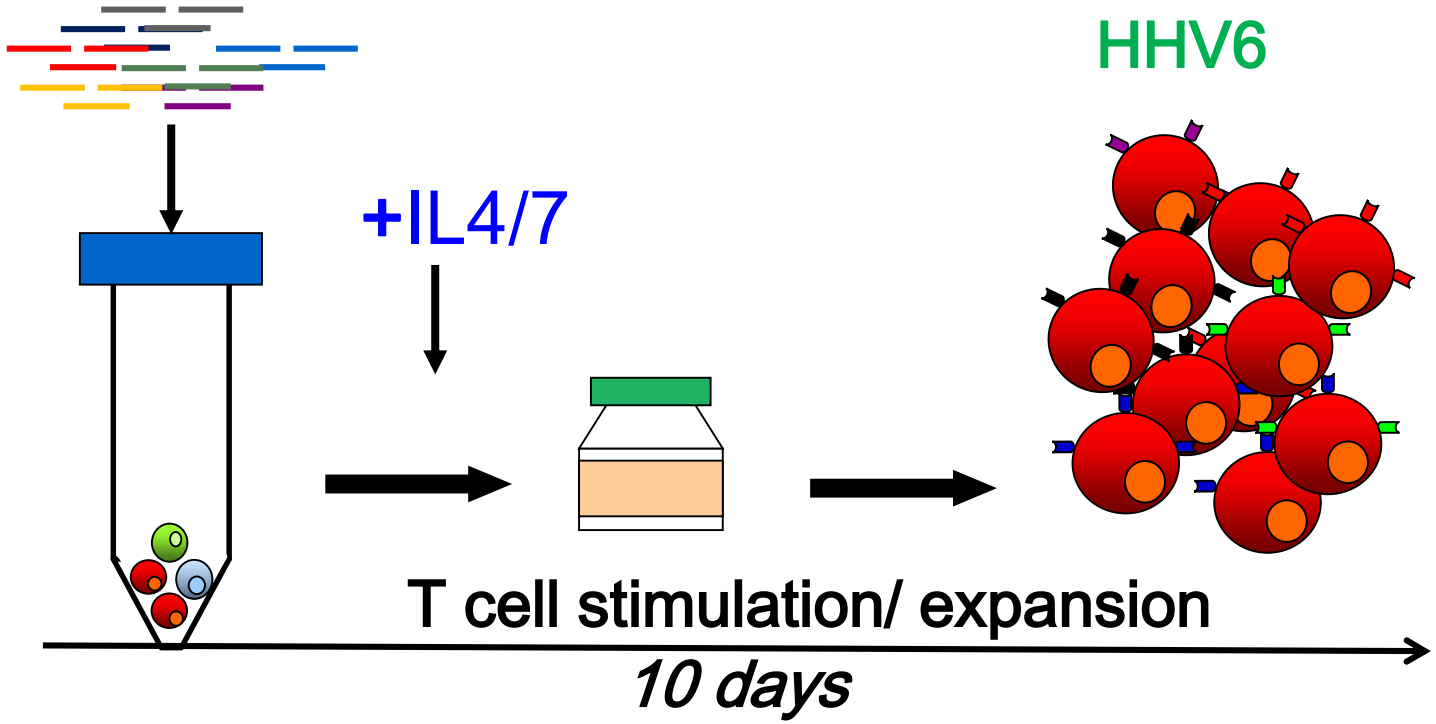
Florian Kern and Hans-Dieter Volk, Charité Institute, Berlin, 2004

Pepmix-activated VSTs targeting 5 viruses

AdV – Hexon, Penton
EBV – EBNA1, LMP2, ZEBRA
CMV – IE1, pp65
BKV – LT, VP1
HHV6 – U11, U14, U90

MVSTs
AdV
EBV
CMV
BK
HHV6

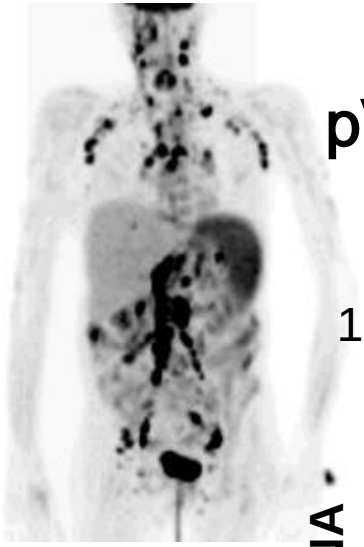
Ann Leen

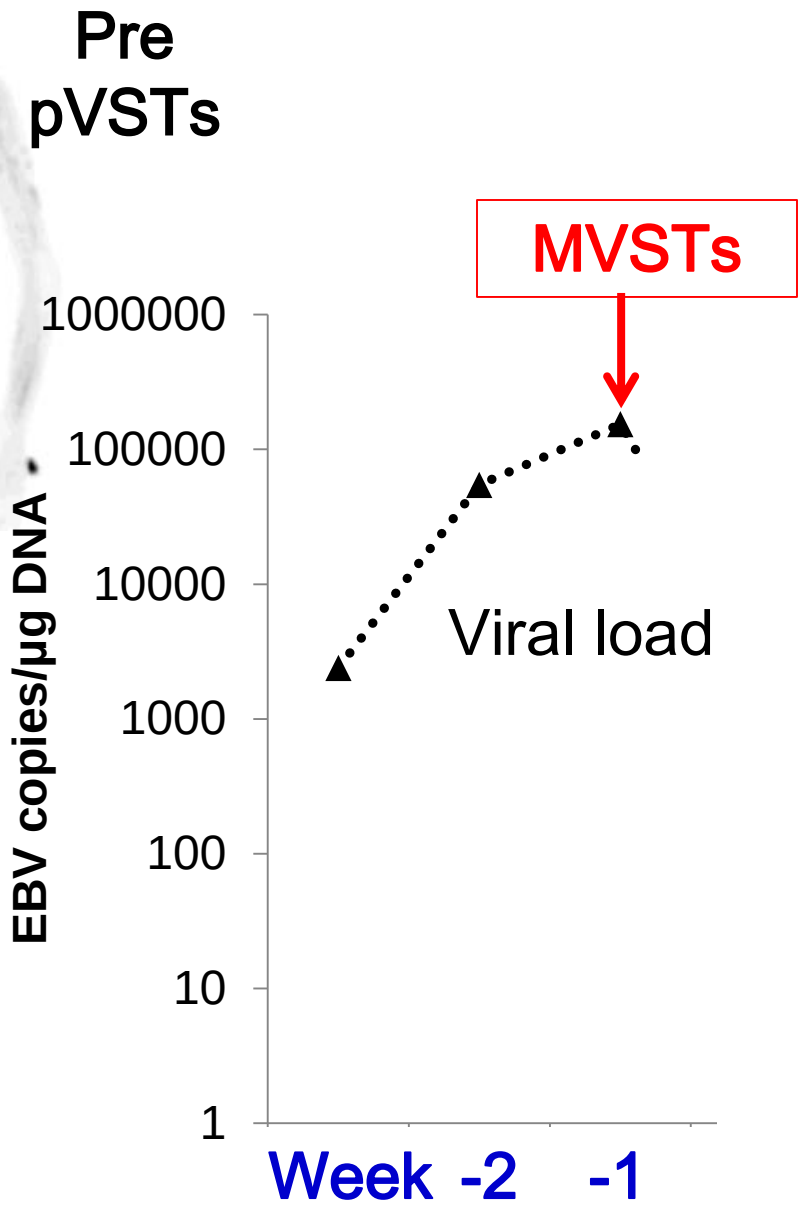


8 HSCT pts treated for 18 infections using donor MVSTs

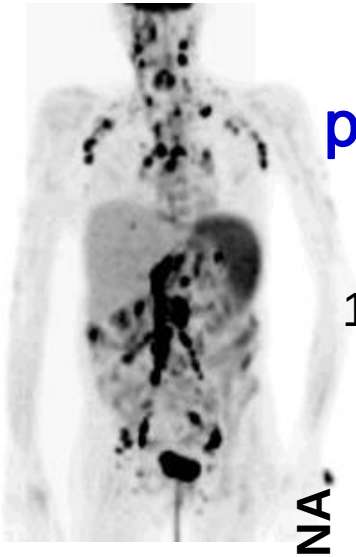
Patient with	AdV	CMV	EBV	BKV	HHV6
1 virus	x				
				x	
2 viruses		x		x	
			x	x	
			x	x	
3 viruses		x	x	x	
			x	x	x
4 viruses		x	x	x	x

Clinical response - Pt with EBV-PTLD

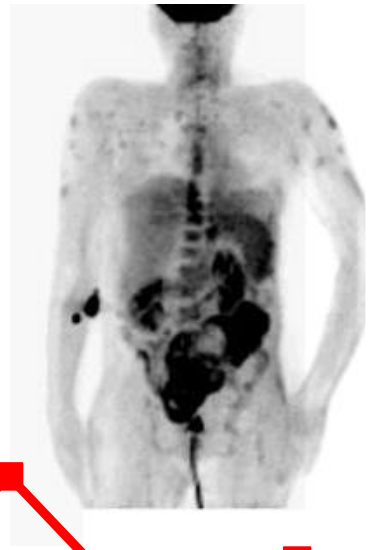




Clinical response - Pt with EBV-PTLD



Pre pVSTs



Post pVSTs

MVSTs

Viral load

EBV T cells

EBV copies/µg DNA

1000000
100000
10000
1000
100
10
1

SFC/5x10^5

600
500
400
300
200
100
0

Week -2 -1 Inf +1 2 3 4 6 8 12

One non responder

Patient with	Adv	CMV	EBV	BKV	HHV6
1 virus	✓				
				✓	
2 viruses		PR		✓	
			✓	✓	
			✓	✓	
3 viruses		✓	✓	✓	
			✓	x	✓
4 viruses		✓	✓	PR	✓

Multivirus-specific T cells after HSCT

- Effective
- Safe
  - 188 donor VST infusions
  - GVHD grade 1 9.1%
  - CRS 1.1%
- Inexpensive, robust manufacturing

– Will pepmix activated EBVSTs work outside the HSCT setting?

Phase I Clinical Trial Design (GRALE)

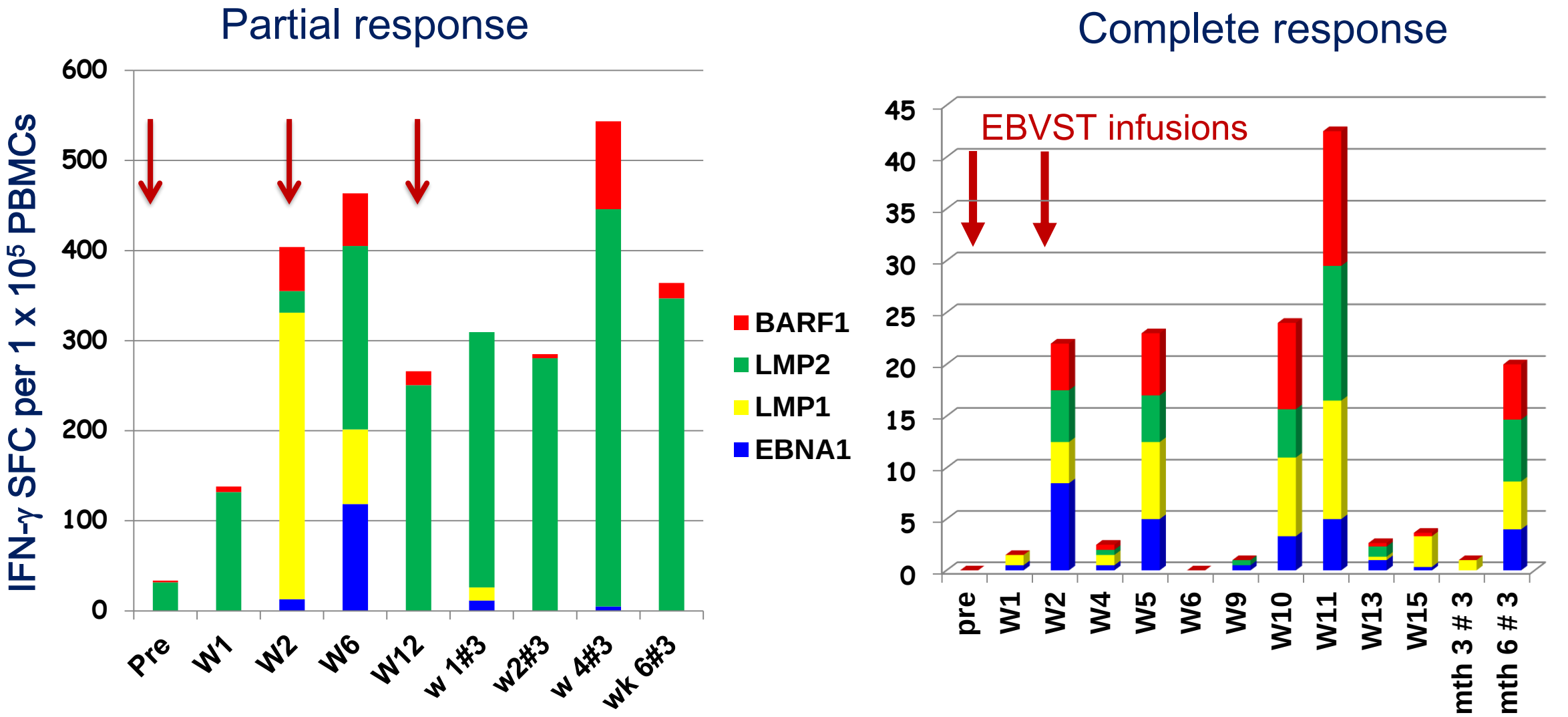
Helen Heslop

- Eligibility
  - EBV+ HL, NHL, T/NK, SCAEBV
- Treated for relapse or as adjuvant therapy for high-risk disease
- No lymphodepletion
- Dose Escalation trial (2 doses, 2 weeks apart)
  1. $2x10^7$ cells/m^2 + $2x10^7$ cells/m^2
  2. $2x10^7$ cells/m^2 + $1x10^8$ cells/m^2
  3. $1x10^8$ cells/m^2 + $2x10^8$ cells/m^2

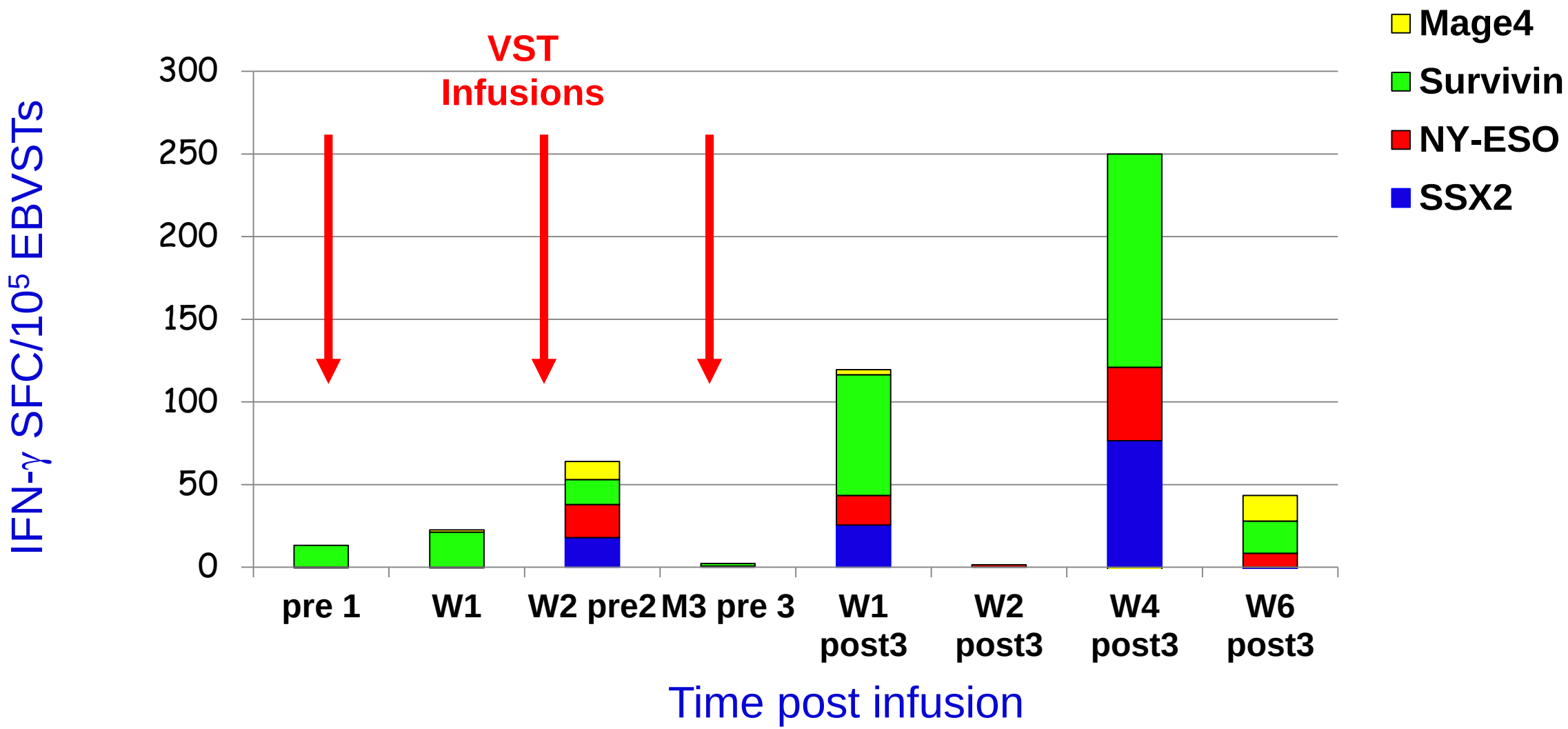
Manufacturing patient EBVSTs with pepmixes

- EBNA1, LMP2, LMP1 and BARF1
- Required dendritic cells and two stimulations
- 6 patients received IL4/7-grown EBVSTs
  - Poor antigen specificity
  - Little expansion after infusion
- 19 received IL7/IL15-EBVSTs
  - Higher antigen specificity

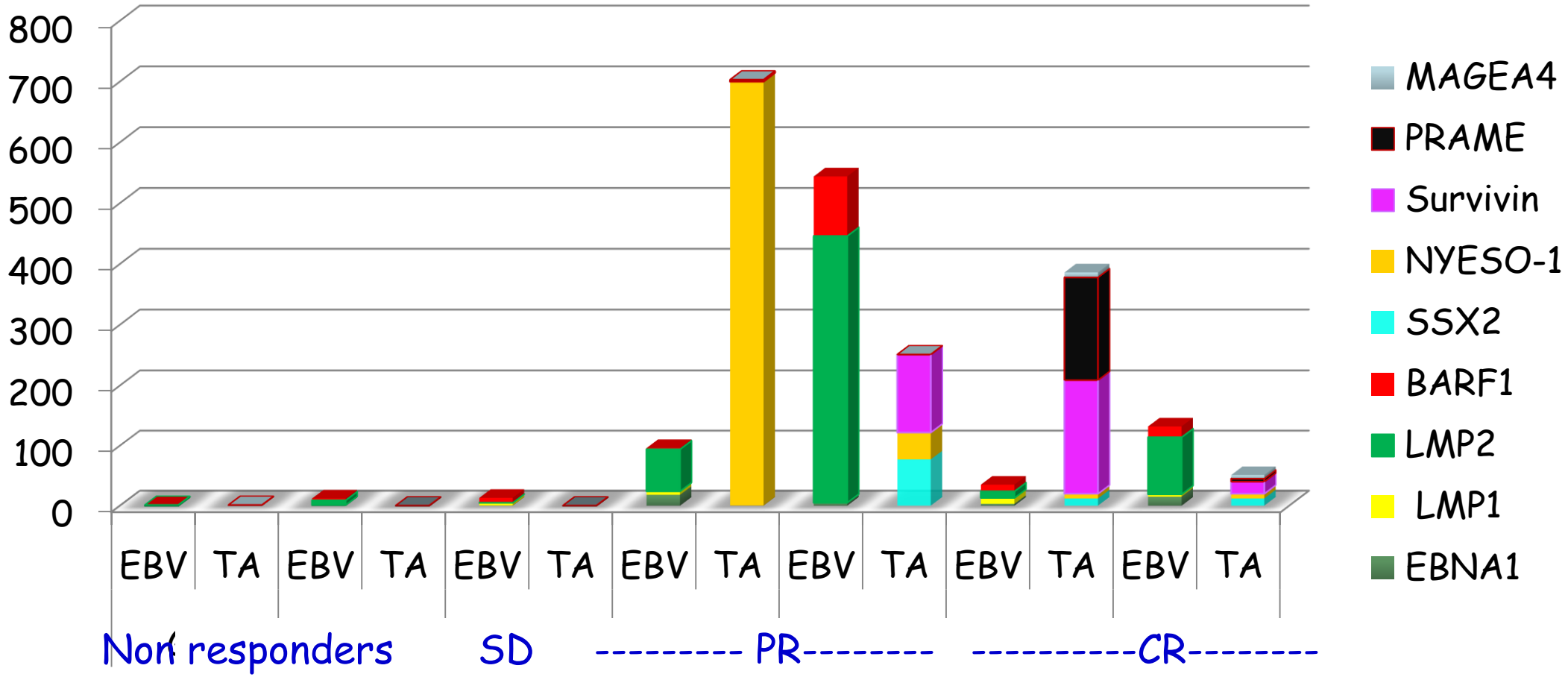
Increased EBVST Frequency post infusion



Epitope Spreading in Responders



Increased frequency of EBVSTs and epitope spreading in responders



Clinical Responses

- 19/22 Adjuvant Patients Remain in Remission
- 14 patients with active disease
  - 4 received IL4/7-expanded EBVSTs
    - 1 SD and 3 PD
  - 10 received IL15/7-expanded EBVSTs
    - 3 CR, 2 PR, 1 mixed, 1 SD and 3 PD

Pepmix versus Ad-LMP-LCL-activation of EBVSTs

- Greater manufacturing success rate
- Higher specificity for type 2 latency antigens
- >50% CR for Ad/LCL-activated
- 30% for pepmix (IL7/15)-activated

- Strategies to improve
  - Specificity
    - Include lytic cycle antigens
  - Lymphodepletion
  - Constitutive IL7 receptor

Allogeneic EBVST banks

- Immediate use, healthy donors
- Effective in solid organ transplant recipients
  - Tanzina Haque and Dorothy Crawford, London, 2001
- Partially HLA-matched
- 33 patients, failed standard therapies
- No GVHD or graft rejection
- 64% response rate (CR and PR) at 5 weeks

Clinical Protocol Multi-VSTs after HSCT

- Single center, phase II study
- Refractory EBV, CMV, AdV, BKV or HHV-6
- Matching VST to recipient
  - Matched at least 1 HLA allele
  - Activity against infecting virus through shared allele

– Overall response rate 92% (100% for EBV)

Ifigeneia Tzannou and Bilal Omer

Banked allogeneic EBVSTs outside the transplant setting?

- Patients who develop EBV+ lymphoma
  - 70% RR
  - 30% CR
  - 40% PR/SD
- Epitope spreading

Rayne Rouce

Summary

- Donor EBVSTs are safe and effective after HSCT
- Banked EBVSTs as effective post HSCT
- Challenges remain outside the HSCT setting
  - Improved specificity and activity
  - Banked allogeneic EBVSTs also effective
    - Induce epitope spreading
    - Need to improve persistence
      - Provide rejection resistance

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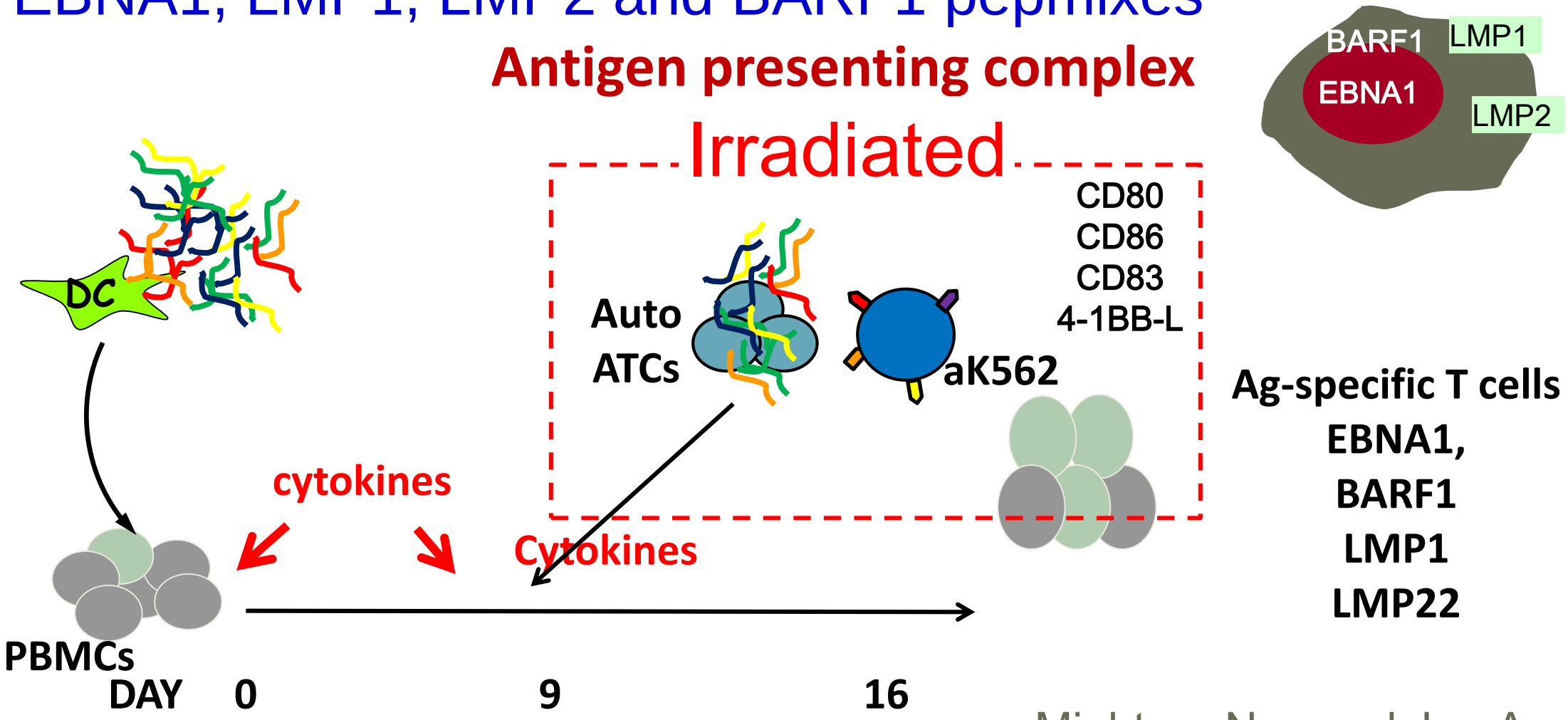
Pepmix activated T-cells for HL/NHL

- EBNA1, LMP1, LMP2 and BARF1 pepmixes



Minhtran Ngo and Jun Ando