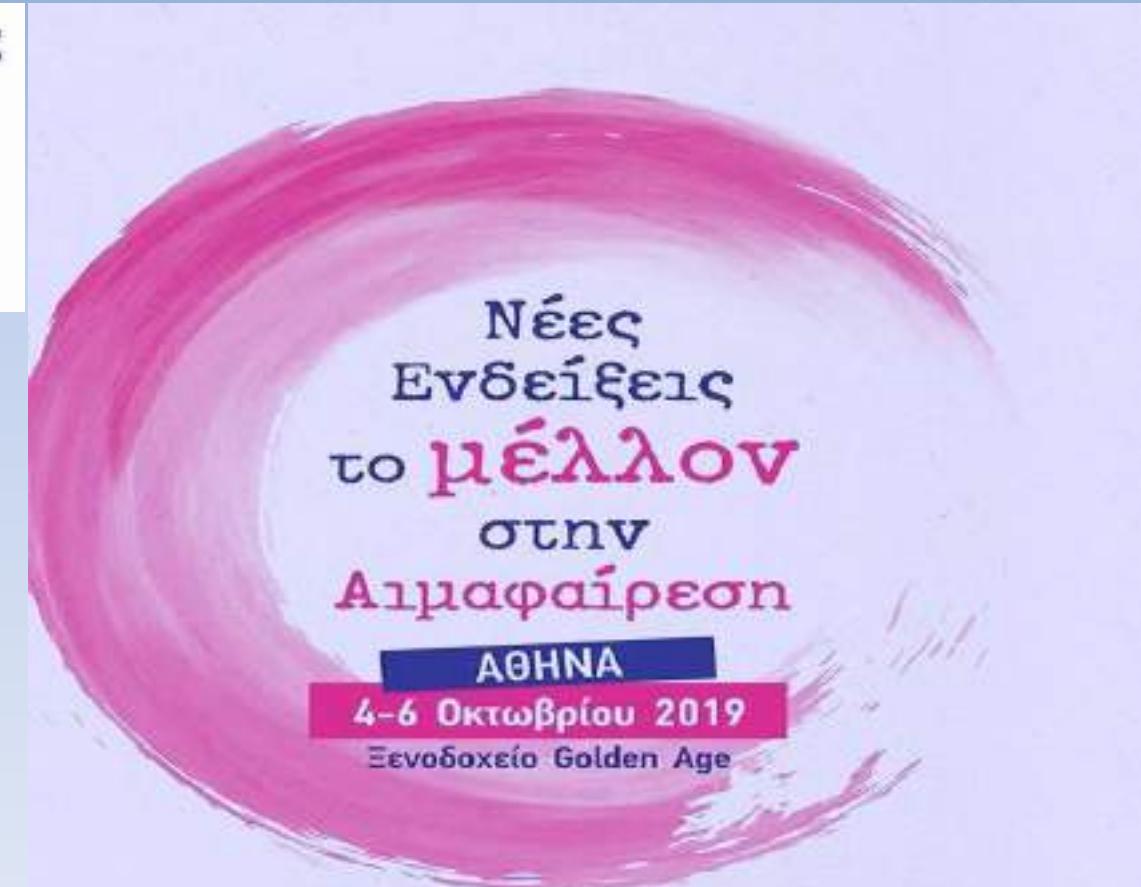


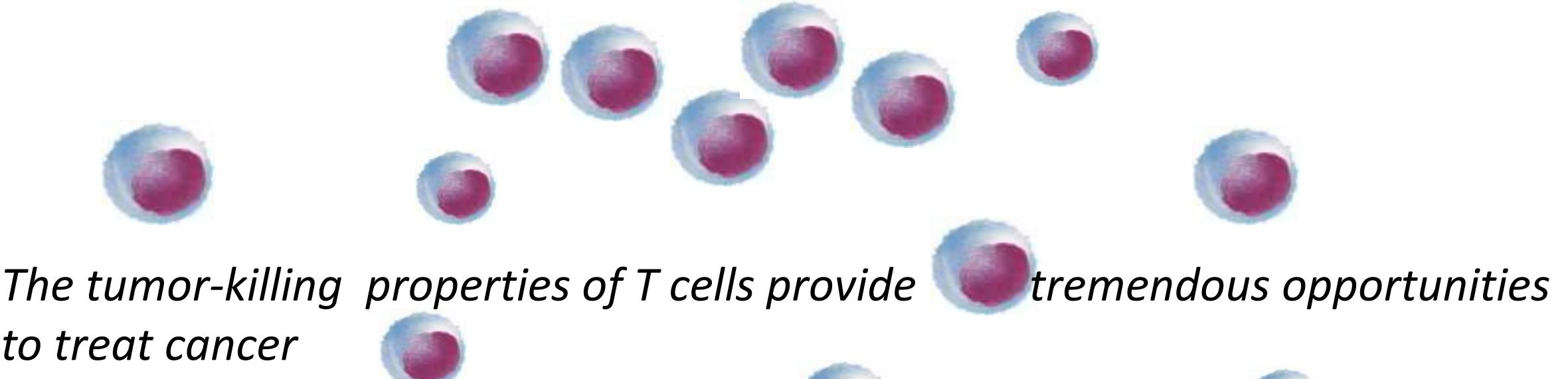
Κυτταρική ανοσοθεραπεία για αντιμετώπιση των ευκαιριακών λοιμώξεων και λευχαιμικών υποτροπών μετά από αλλογενή μεταμόσχευση αιμοποιητικών κυττάρων



A new era in disease treatment : “living drugs” and personalized therapies

*The developmental pathway of cell therapies as approved drug differs substantially from the conventional pharmaceutical model; **each product is customized** to each individual treated, rather than being prepared in bulk and a standardized form*

T-cells as a platform to generate “living drugs”



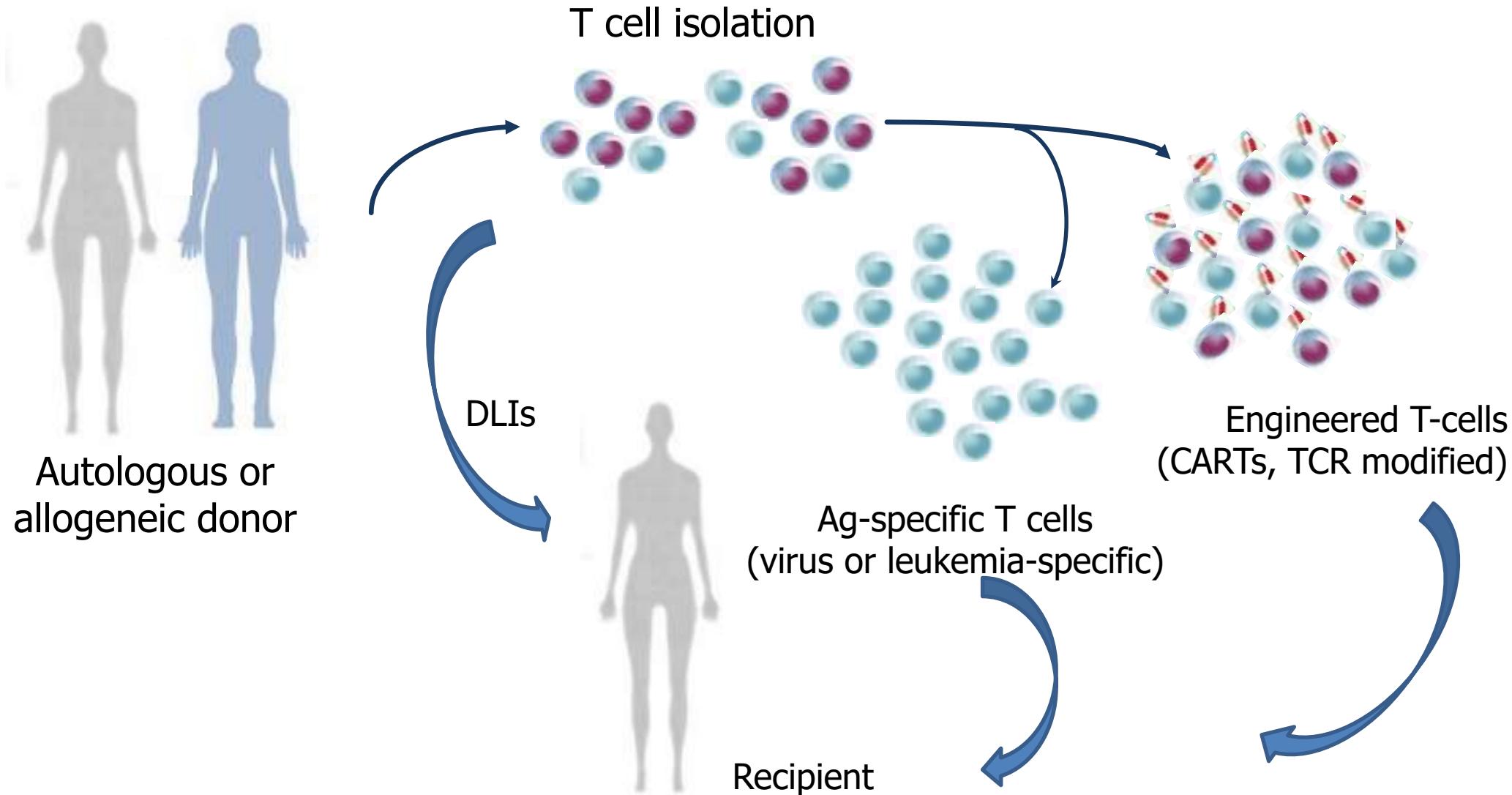
The tumor-killing properties of T cells provide tremendous opportunities to treat cancer

Hematopoietic Stem Cell Transplantation

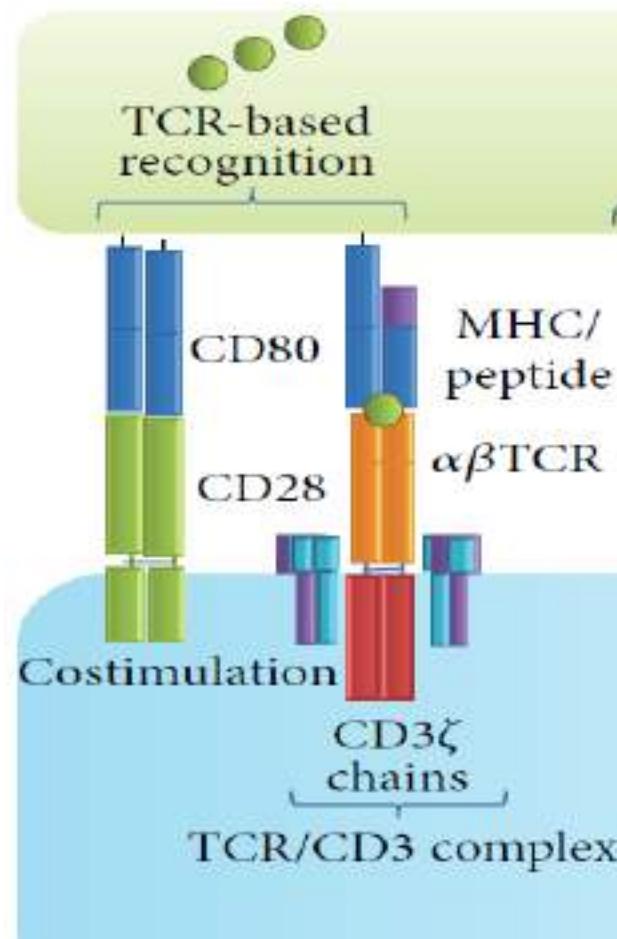
- T-cell containing HSC grafts: lower probability of leukemic relapse over T-cell depleted HSC grafts
- Donor lymphocyte infusions (DLIs) : induce durable remissions



Adoptive T cell therapy : a “living” drug therapy



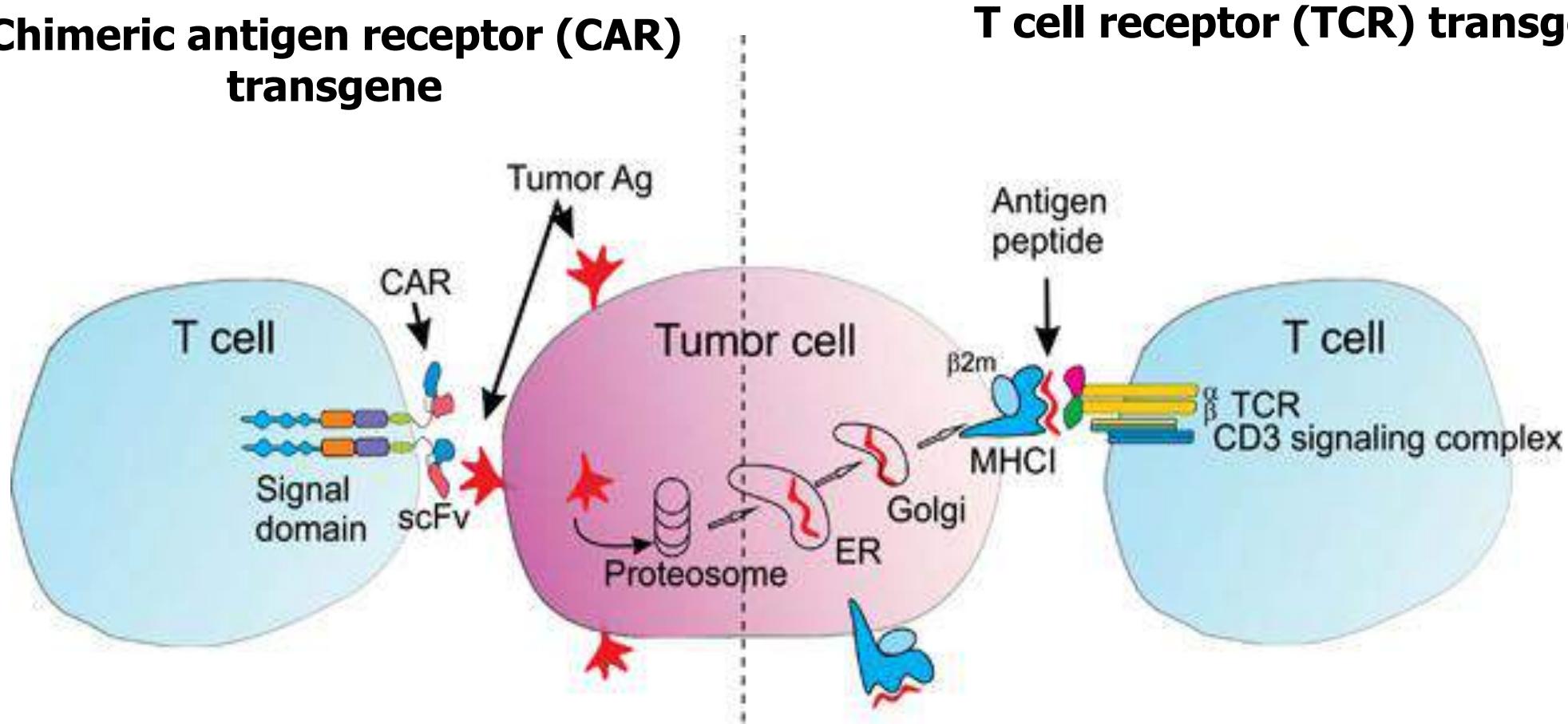
T-cell receptor (TCR)



TCR : *dictates antigen specificity*

Genetic engineering of T cells : redirecting specificity

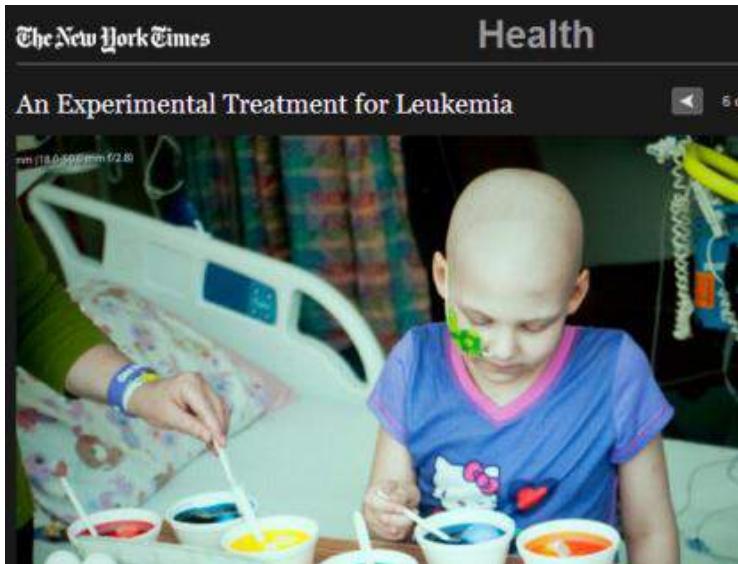
Chimeric antigen receptor (CAR) transgene



T cell receptor (TCR) transgene

CAR : Chimera, part antibody and part T-cell receptor

Genetically modified, autologous T cells with redirected specificity to tumor antigens may combine advantages of:
Antibody therapy (*specificity*) • Cellular therapy (*amplified response*) • Vaccine therapy (*memory activity*)



CAR T cells: Transforming the Clinical Outcomes of Patients with B-cell Hematologic Malignancies

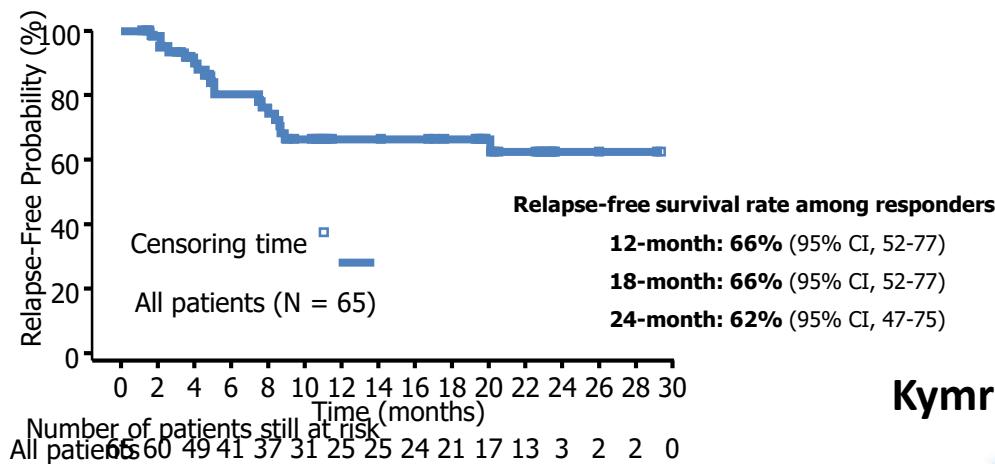


Yescarta (Gilead)

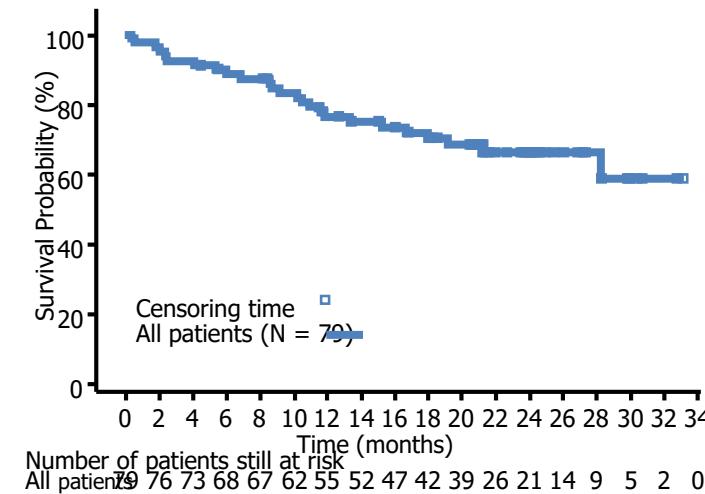
ORIGINAL ARTICLE

Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma

S.S. Neelapu, F.L. Locke, N.L. Bartlett, L.J. Lekakis, D.B. Miklos, C.A. Jacobson, I. Braunschweig, O.O. Oluwale, T. Siddiqi, Y. Lin, J.M. Timmerman, P.J. Stiff, J.W. Friedberg, I.W. Flinn, A. Goy, B.T. Hill, M.R. Smith, A. Deol, U. Farooq, P. McSweeney, J. Munoz, I. Avivi, J.E. Castro, J.R. Westin, J.C. Chavez, A. Ghobadi, K.V. Komanduri, R. Levy, E.D. Jacobsen, T.E. Witzig, P. Reagan, A. Bot, J. Rossi, I. Navale, Y. Jiang, J. Aycock, M. Elias, D. Chang, J. Wiezorek, and W.Y. Go

**Kymriah (Novartis)****Overall survival rates among all infused patients**

- **12-month:** 76% (95% CI, 65-85)
- **18-month:** 70% (95% CI, 58-79)
- **24-month:** 66% (95% CI, 54-76)



ORIGINAL ARTICLE

Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia

S.L. Maude, T.W. Laetsch, J. Buechner, S. Rives, M. Boyer, H. Bittercourt, P. Bader, M.R. Verneris, H.E. Stefanski, G.D. Myers, M. Qayed, B. De Moorloose, H. Hiramatsu, K. Schlis, K.L. Davis, P.L. Martin, E.R. Niemecik, G.A. Yanik, C. Peters, A. Benichou, N. Boissel, F. Mechennaud, A. Bahfuzzi, J. Krueger, C.H. June, B.L. Levine, P. Wood, T. Tarari, M. Leung, R.T. Mueller, Y. Zhang, K. Sen, O. Letwohl, M.A. Pulsipher, and S.A. Grupp

(Updated, Grupp S, ASH 2018)

**Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma**

Stephen J. Schuster, M.D., Michael R. Bishop, M.D., Constantine S. Tam, M.D., Edmund K. Waller, M.D., Ph.D., Peter Borchmann, M.D., Joseph P. McGuirk, D.O., Ulrich Jäger, M.D., Samantha Jaglowski, M.D., Charalambos Andreadis, M.D., Jason R. Westin, M.D., Isabelle Fleury, M.D., Veronika Bachanova, M.D., Ph.D., S. Ronan Foley, M.D., P. Joy Ho, M.B., B.S., D.Phil., Stephan Mielke, M.D., John M. Magenau, M.D., Harald Holte, M.D., Ph.D., Serafino Pantano, Ph.D., Lida B. Pacaud, M.D., Rakish Awasthi, Ph.D., Jufen Chu, Ph.D., Öglem Anak, M.D., Gilles Salles, M.D., Ph.D., and Richard T. Maziarz, M.D., for the JULIET Investigators*

Gene-modified T cell therapy

Spectacular responses in treating cancer, but also.....

Drawbacks		
Clinical Safety	Serious Adverse Events (SAEs)	<i>Cytokine release syndrome (CRS), neurotoxicity, on-target but off-tumor toxicities (B aplasia-CAR CD19; autoimmunity due to epitope cross-reactivity)</i>
	Fatalities caused by therapy	√
	Mutagenesis risk	√
Clinical Impact	Specificity	<i>Single targeted Ag: high relapse rate due to Ag-negative escape</i>
	Duration of clinical benefit	?
Applicability	Finding suitable target Ag	<i>Challenging</i>
	Regulatory approval	<i>Difficult</i>
	Estimated treatment cost	<i>Genetic modification</i> <i>Requirement for hospitalization & use of Abs</i>

Outside T cell engineering – Ag-specific T cells

Targeting through their native TCR specific



Viral Ags



Tumor-associated Ags
(TAAs)

Antigen-specific T-cells against pathogens

Pharmacological treatment of viral infections

Limitations

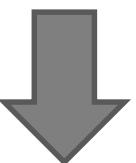
- suboptimal efficacy
- toxicity
- growth of viral resistance
- for many viral infections there are limited or no effective prophylactic or therapeutic pharmacotherapies



Encephalitis



enormous human and financial cost



Adoptive immunotherapy with virus-specific T-cells

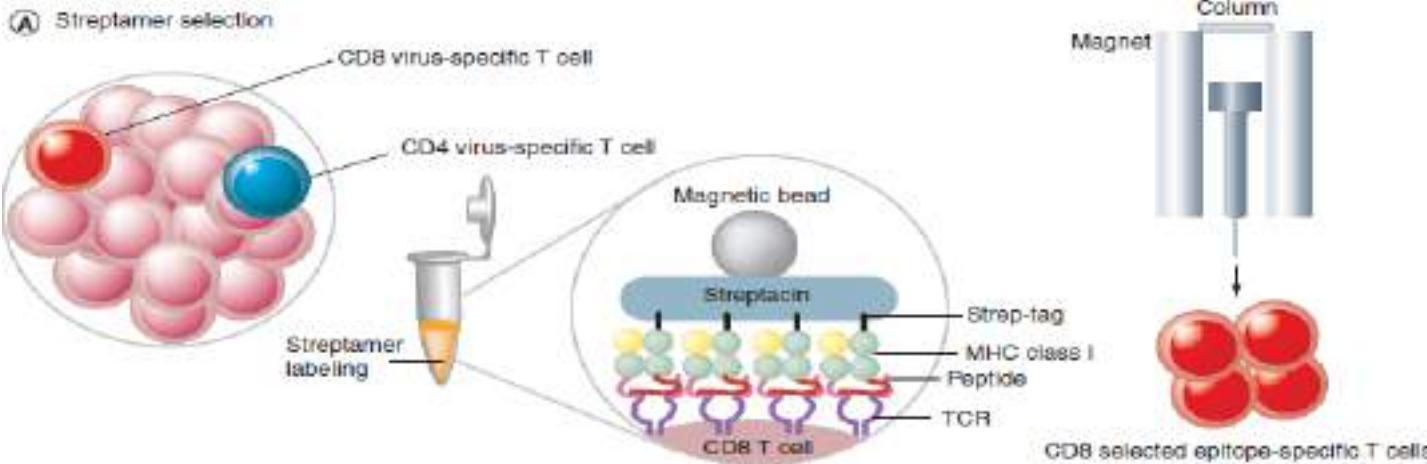
Strategies to generate virus-specific T-cells (VSTs)

Direct enrichment of VSTs

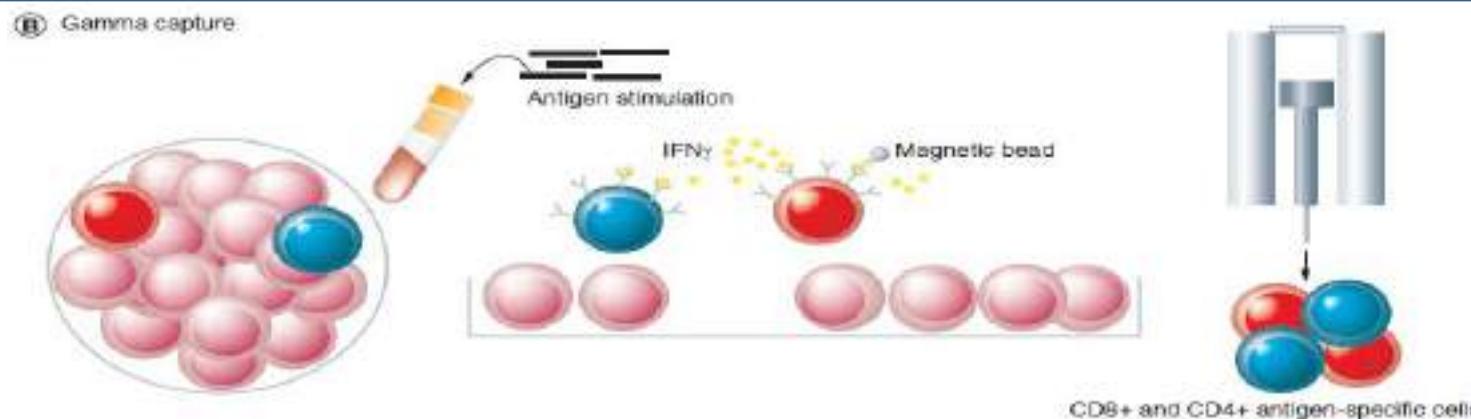
- multimer selection
- IFN- γ -capture

Ex vivo expansion of VSTs

Direct enrichment of virus-specific T cells (VSTs)



Multimer/Streptamer selection



IFN- γ capture

Direct enrichment of VSTs

Pros

- rapid manufacturing (~4-24h)

Cons

Streptamer

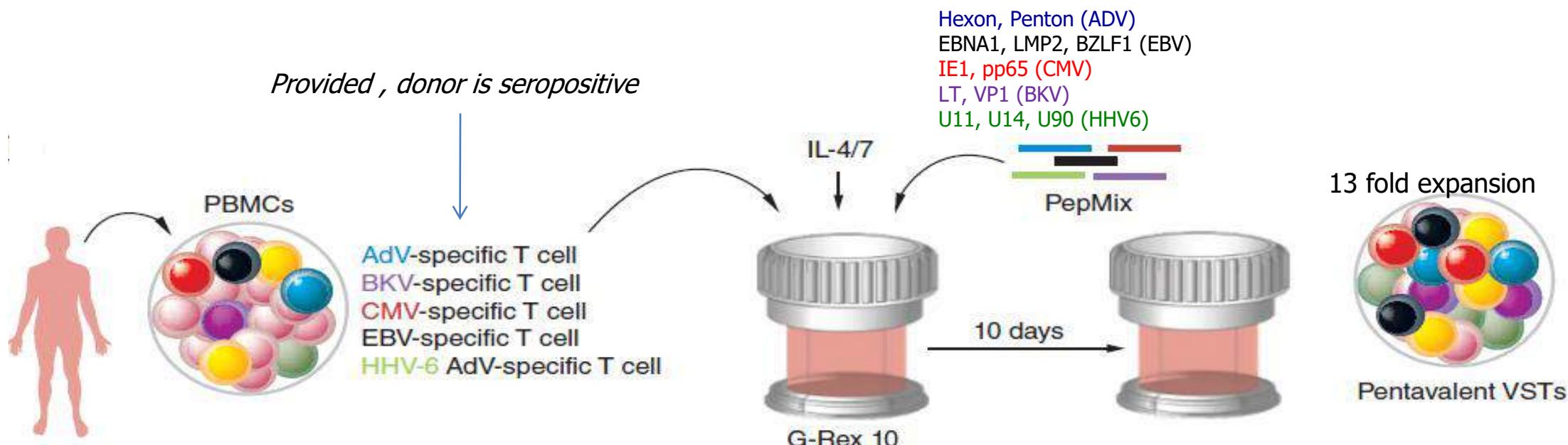
- selection of CD8+ T cells
- restricted to certain HLA types & well-characterized viruses

Streptamer and gamma-capture:

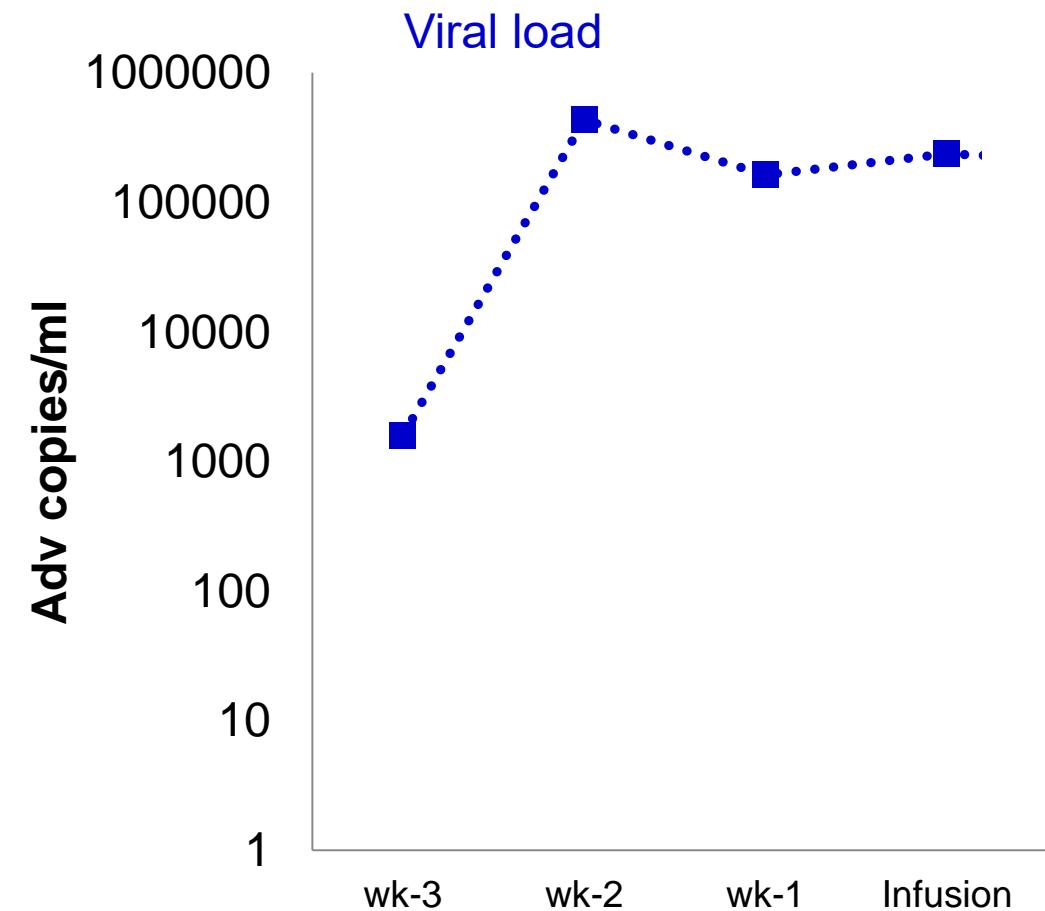
- large volume of blood
- high frequency of circulating VSTs (EBV, CMV)

Ex vivo expanded VSTs

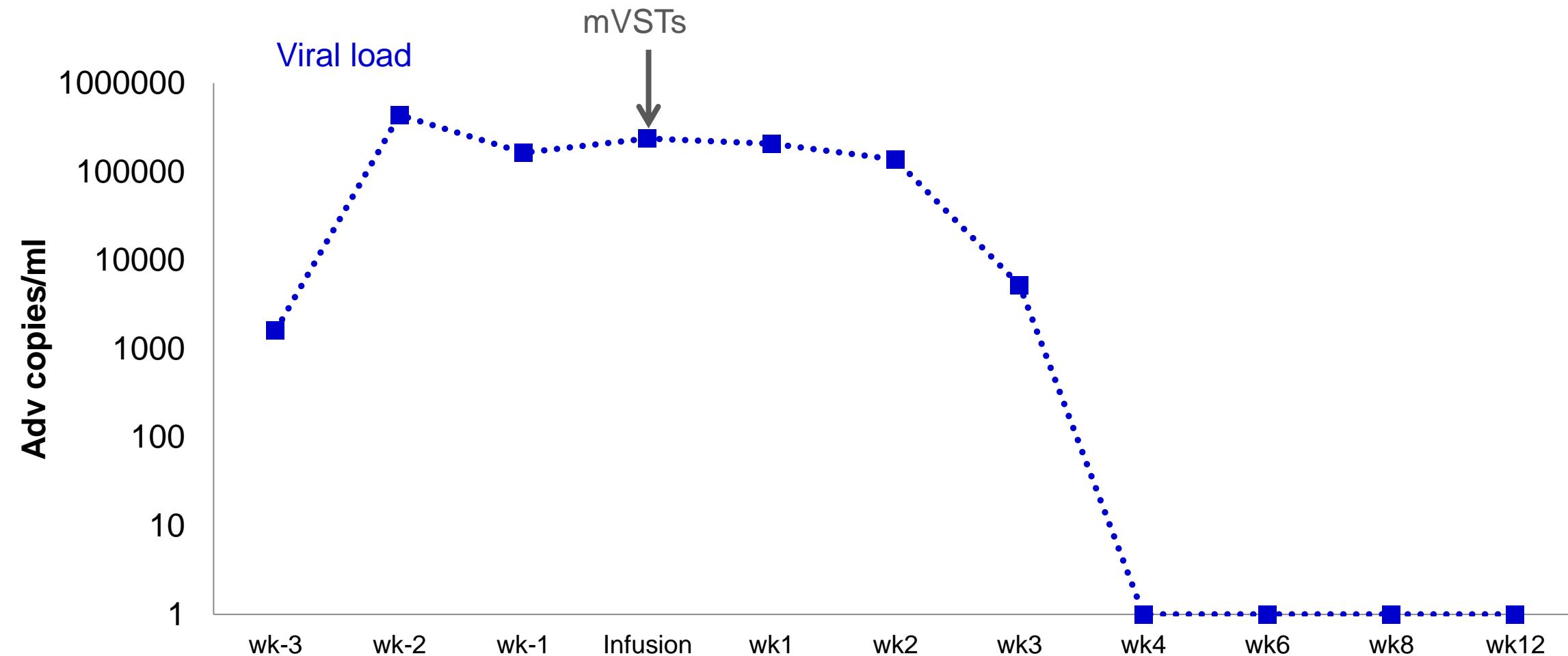
Targeting up to 5 viruses by a single product using a rapid and simplified procedure



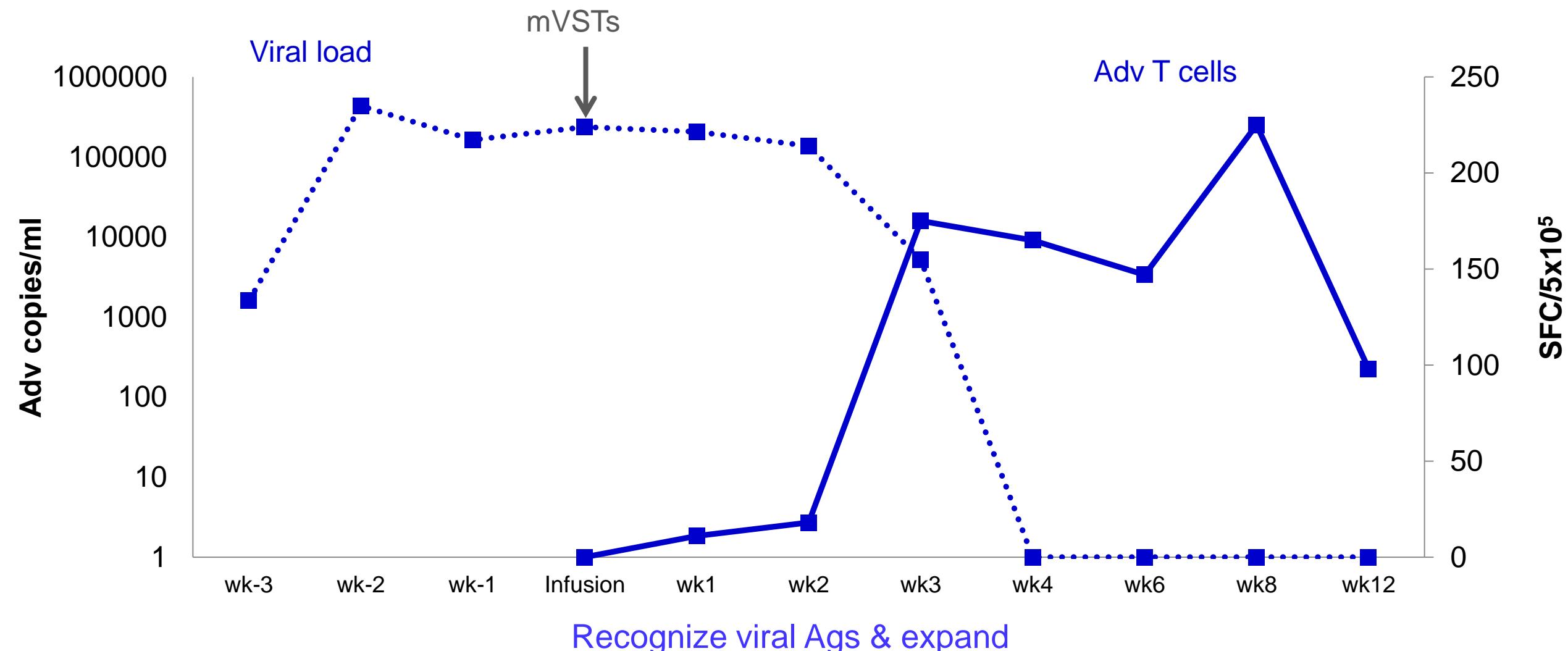
Clinical response – pt with adenoviral infection



Clinical response – pt with adenoviral infection



Clinical response – pt with adenoviral infection



Summary Outcomes: 92% response rate

Patient with	Adv	CMV	EBV	BKV	HHV6
1 virus (3/12)	✓				
				✓	
				✓	
2 viruses (4/12)		PR		✓	
			✓	✓	
			✓	✓	
3 viruses (2/12)	✓				X
		✓	✓	✓	
			✓	X	✓
4 viruses (3/12)		✓	✓	PR	✓
	✓	✓		X	✓
	✓	✓		✓	✓

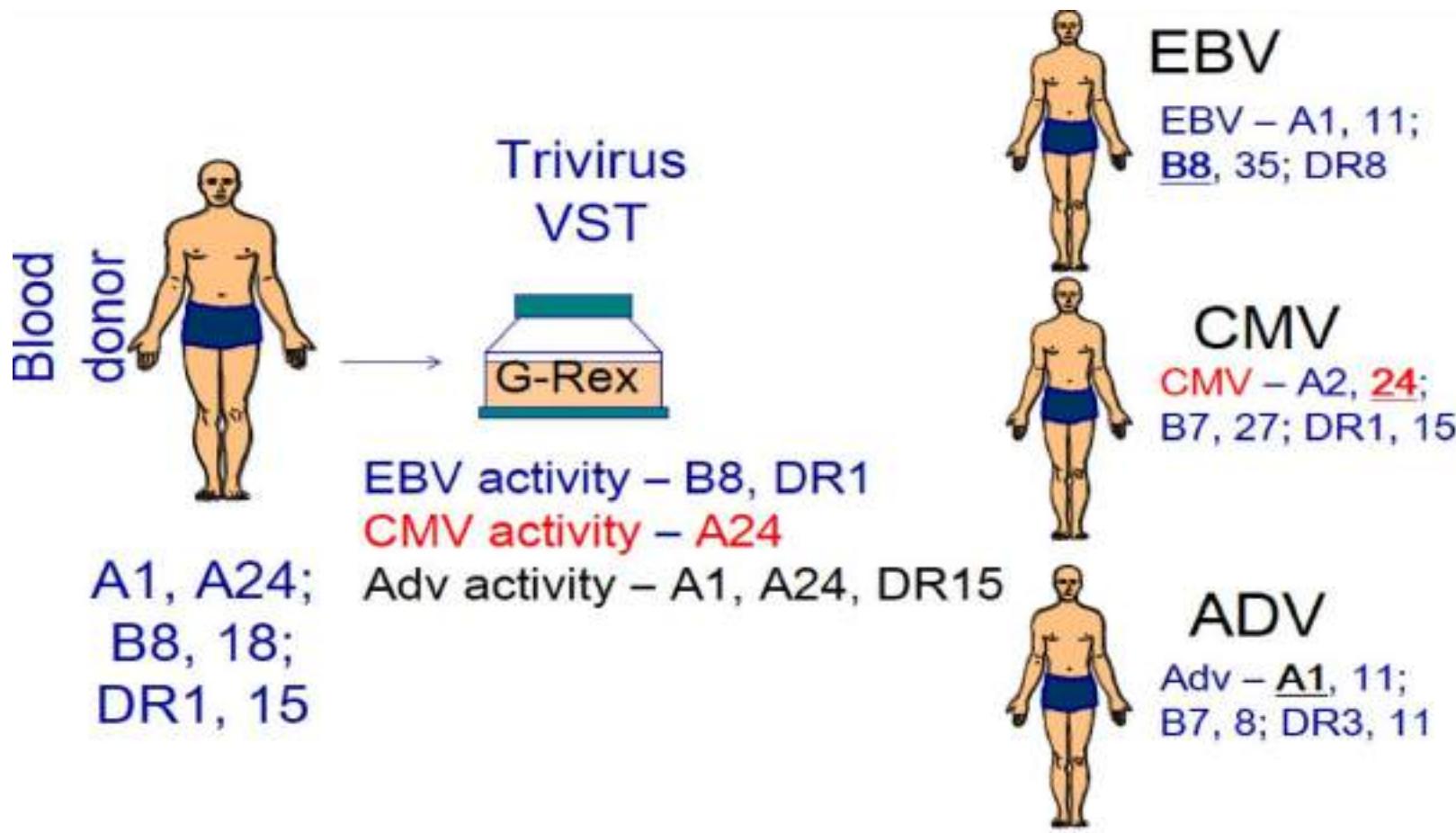
The only cause of failure was if the donor was seronegative for the infecting virus

Multi-VSTs -limitations

- i) custom manufacturing precludes the immediate availability of VSTs
- ii) requirement for seropositive donors
- iii) patients on steroids $\geq 0.5\text{-}1\text{mg/kg}$ are excluded from receiving VSTs

Third-party virus-specific T-cells

- Banks of HLA-matched VSTs from seropositive donors
- The most closely matched line with activity against the infecting virus through a shared HLA-antigen can be infused as an immediately available, “**off the shelf**”, product



Off-the-Shelf Virus-Specific T Cells to Treat BK Virus, Human Herpesvirus 6, Cytomegalovirus, Epstein-Barr Virus, and Adenovirus Infections After Allogeneic Hematopoietic Stem-Cell Transplantation

Ifigeneia Tzannou, Anastasia Papadopoulou, Swati Naik, Kathryn Leung, Caridad A. Martinez, Carlos A. Ramos, George Carrum, Ghadir Sasa, Premal Lulla, Ayumi Watanabe, Manik Kuvalekar, Adrian P. Gee, Meng-Fen Wu, Hao Liu, Bambi J. Grilley, Robert A. Krance, Stephen Gottschalk, Malcolm K. Brenner, Cliona M. Rooney, Helen E. Heslop, Ann M. Leen, and Bilal Omer

2017 by American Society of Clinical Oncology

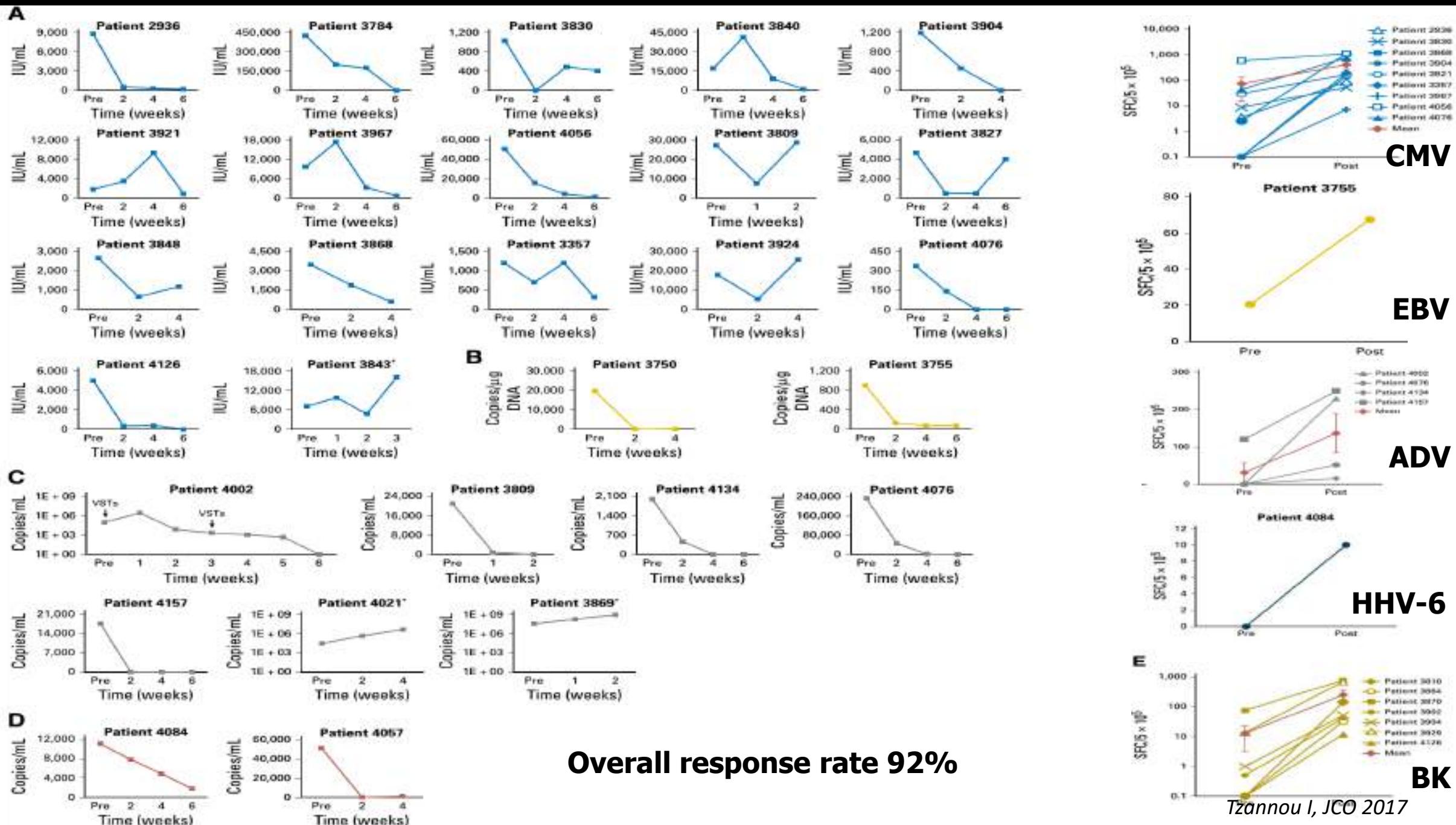
- Bank of VSTs recognizing 5 common viral pathogens : EBV, CMV, ADV, BK, HHV-6
- 38 patients with 48 drug-refractory infections

Overall response rate 92%

CMV	92%
EBV	100%
ADV	71%
BK	100%
HHV/6	67%

VST tracking by epitope profiling revealed persistence of functional VSTs of third-party origin for up to 12 weeks

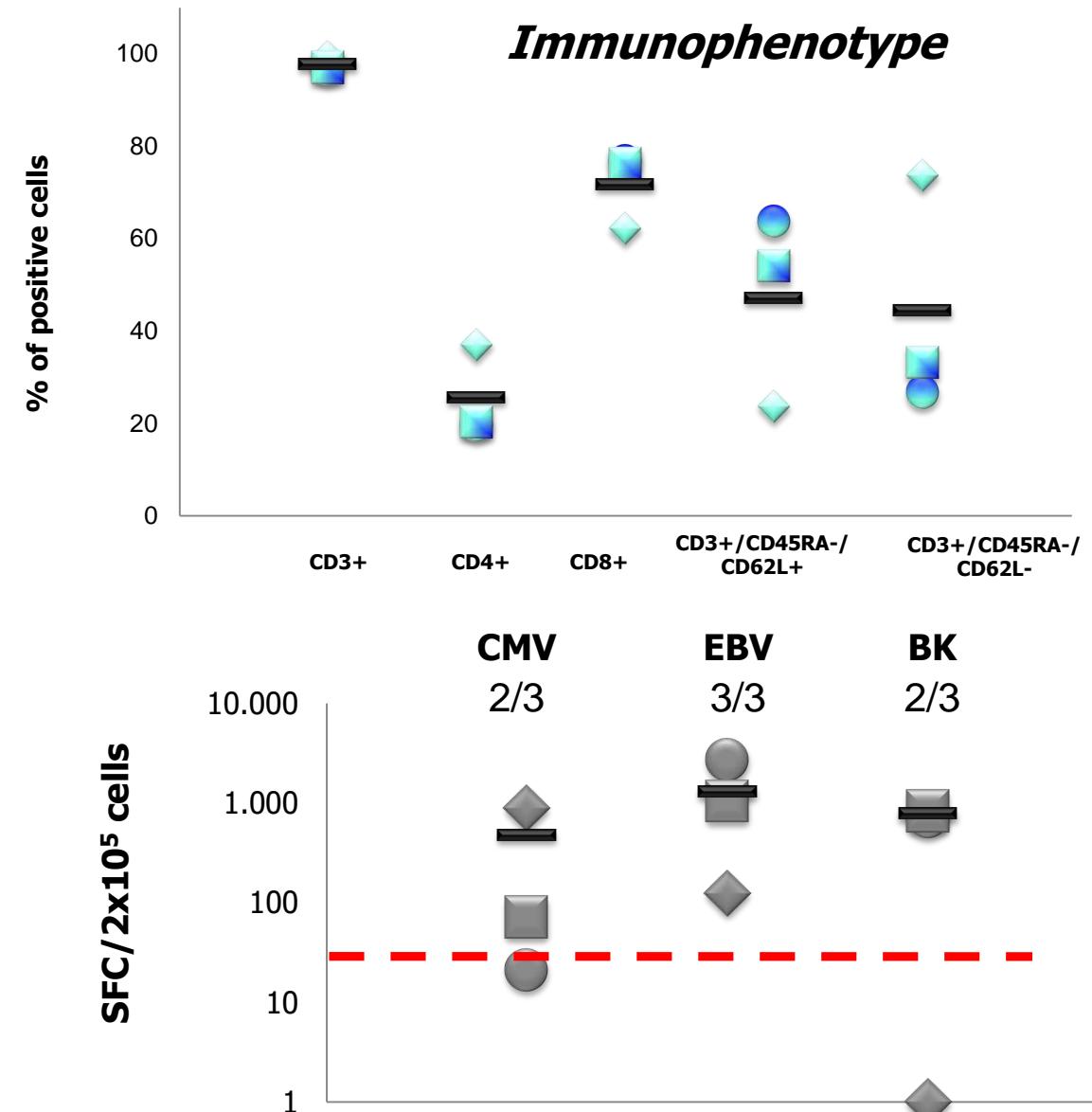
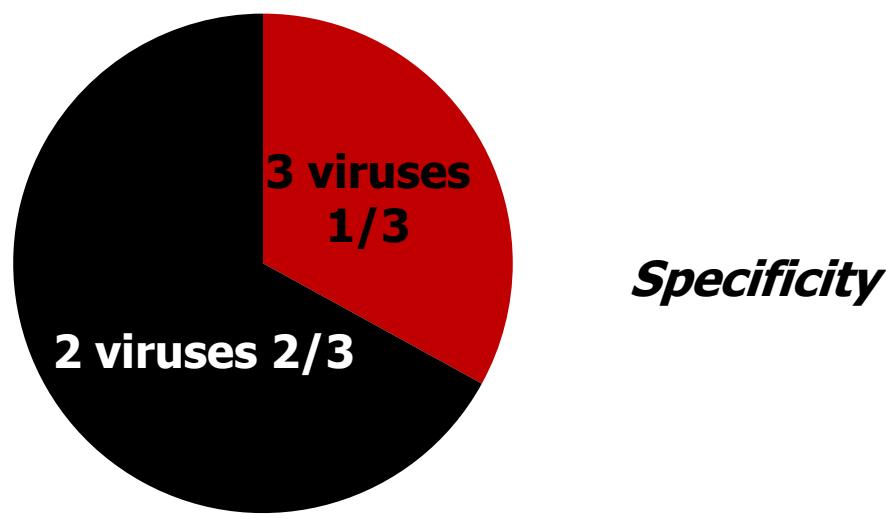
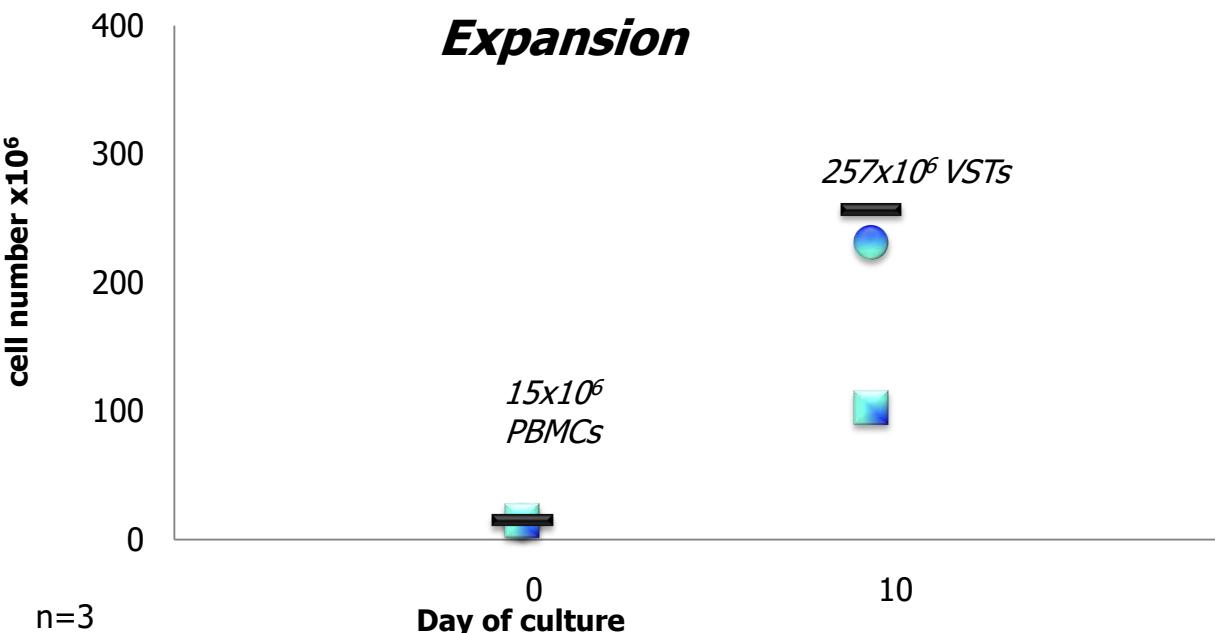
Off-the-shelf T cells for CMV, EBV ADV HHV-6, BK



Adoptive immunotherapy with Virus-Specific T cells (VSTs) in Greece: where do we stand?

Tri-VSTs:*Rapidly produced multivirus-specific T cells for the management of viral infections from CMV, EBV and BKV, post allogeneic hematopoietic cell transplantation*

Validation of GMP production of tri-VSTs (against CMV, EBV, BK)



Tri-VSTs: Rapidly produced multivirus-specific T cells for the management of viral infections from CMV, EBV and BKV, post allogeneic hematopoietic cell transplantation

Protocol Code: tri-VSTs

European Clinical Trials Database EudraCT #:
2014-004817-98

Authorization from the Office of Production
Control and Product Circulation of EOF for the
production of cellular products for human use
(EOF, #52759/12-09-2018)

Clinical Trial Approval :
25/06/2019, EOF:IS-067-19



EOM

CFs

ΕΟΦ/
IRB/ ΕΕΔ

EudraCT#

Πρωτόκολλο
CFs

CAT

Άδεια
Παραγωγής

Άδεια Υπουργείου
Ανάπτυξης

Κάτοψη χώρων

'Έλεγχος
εγκατάστασης/
μηχανημάτων

Ασφαλιστήριο
συμβόλαιο

Αίτηση

Διορισμός
υπευθύνων

Κατάλογος
οργάνων

Παράβολο
3.000

Συνοδευτική επιστολή

Εγχειρίδιο ερευνητή

IMPD

CRO

Παράβολο 1.500

Αίτηση εργαστηρίου

Υπεύθυνη
δήλωση
νοσοκομείου

Αίτηση
επιστημόνων

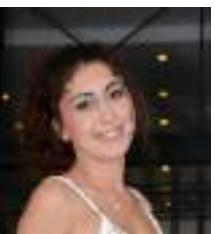
Παράβολο 500

ARTICLE



Clinical-scale production of *Aspergillus*-specific T cells for the treatment of invasive aspergillosis in the immunocompromised host

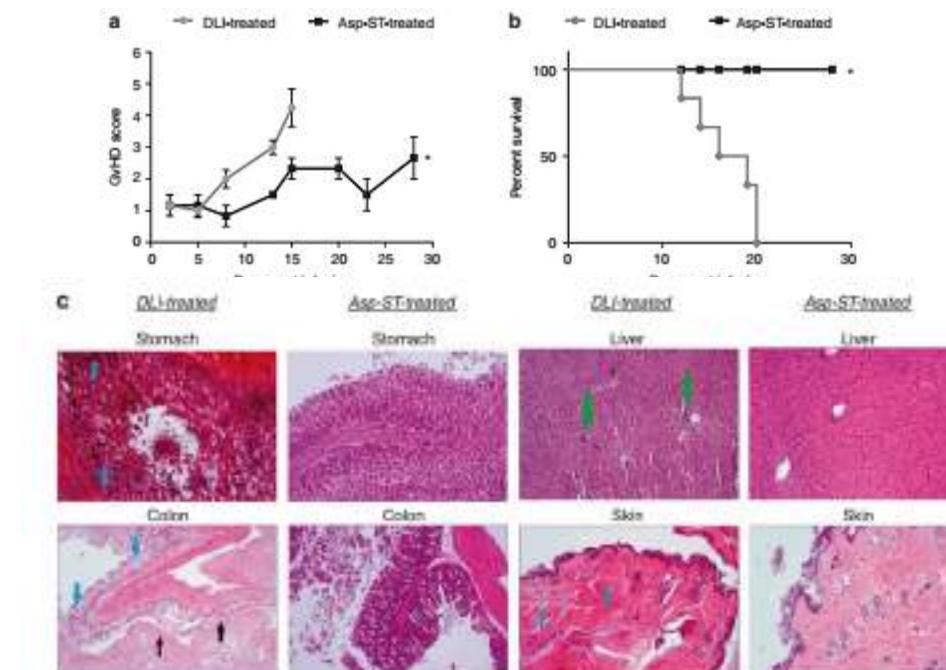
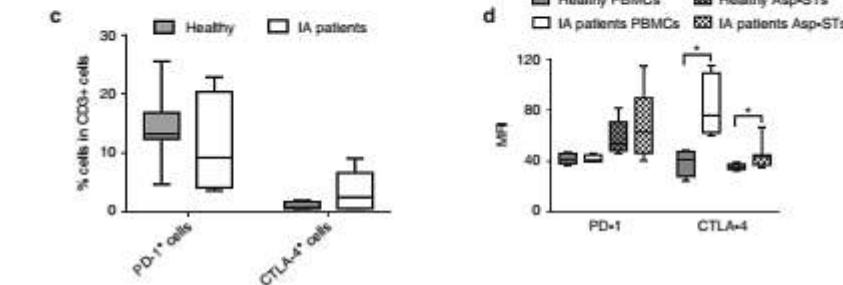
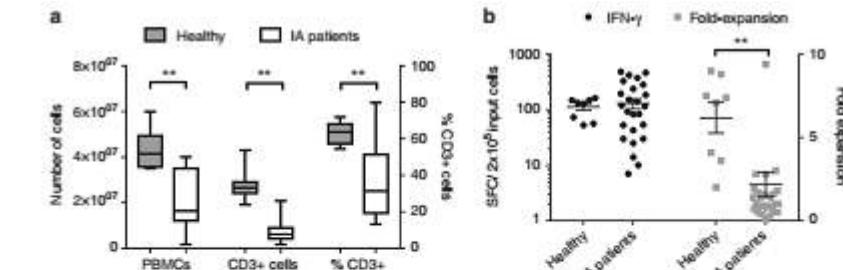
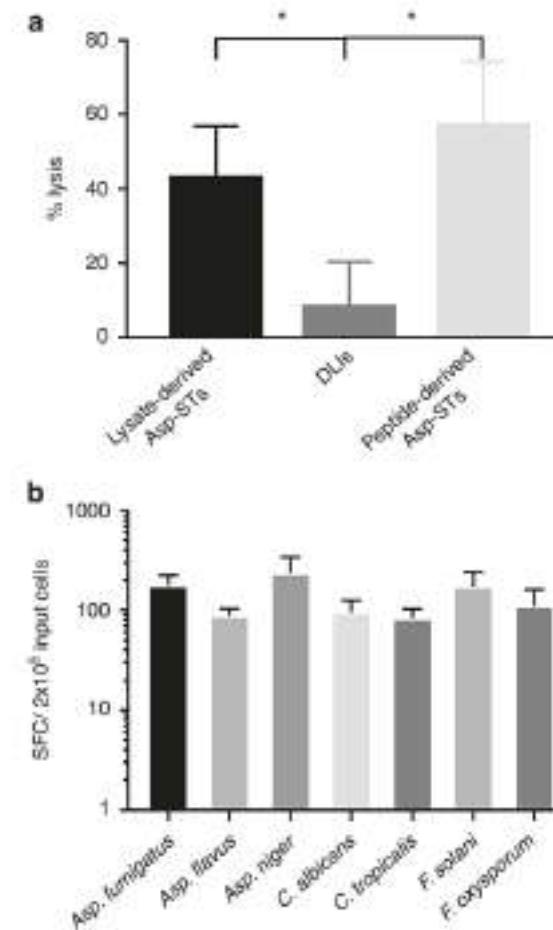
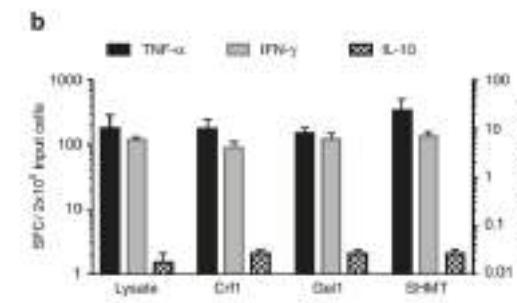
Anastasia Papadopoulou¹ · Maria Alvanou^{1,2} · Kiriakos Koukoulias^{1,2} · Evangelia Athanasiou¹ · Andriana Lazaridou¹ · Nikolaos Savvopoulos^{1,2} · Panayotis Kaloyannidis³ · Anthi-Marina Markantonatou⁴ · Timoleon-Achilleas Vyzantiadis⁴ · Minas Yiagou² · Achilles Anagnostopoulos¹ · Evangelia Yannaki^{1,5}



IKY



ΙΔΡΥΜΑ ΚΡΑΤΙΚΩΝ ΥΠΟΤΡΟΦΙΩΝ
STATE SCHOLARSHIPS FOUNDATION



“Cerberus” T cells: A single Glucocorticoid-resistant T Cell Product To Simultaneously Target Multiple Pathogens In Immunocompromised Patients



A.Papadopoulou
Advanced Research Grant 2018



Specificity against viruses
(CMV, EBV, AdV, BKV)



Specificity against fungi
(Aspergillus fumigatus)

Resistance to
glucocorticoids

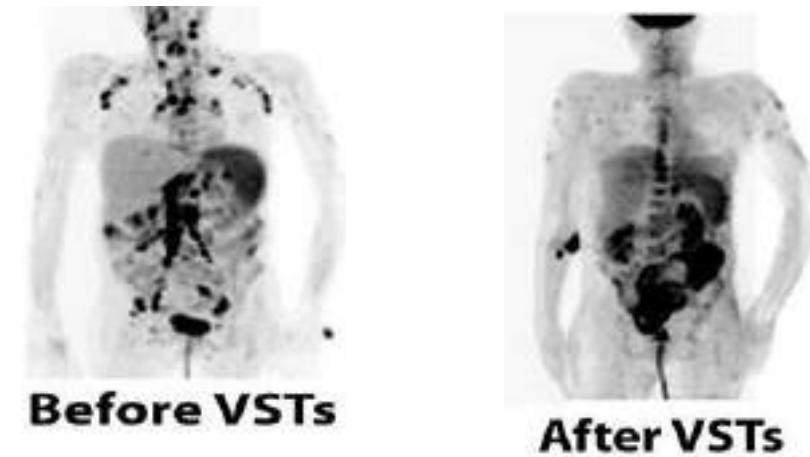
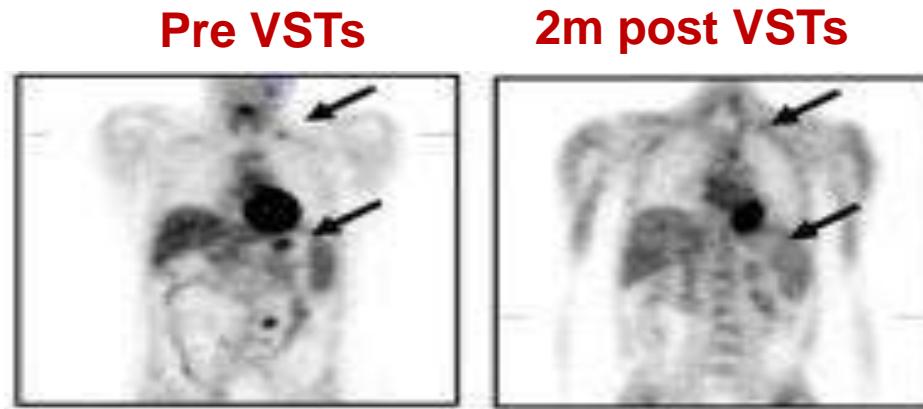
- Generation of a CRISPR/Cas9 system targeting the glucocorticoid receptor (GR) gene
- Generation & functional in vitro and in vivo characterization of Cb-STs
- Product optimization for clinical development

Antigen-specific T-cells against leukemia/cancer

VSTs beyond infections –virus-associated malignancies

Targeting EBV+ tumors:

- ✓ EBV-associated post-transplant lymphoproliferative disease (EBV-PTLD)
- ✓ Highly immunogenic EBV lymphoma (post HSCT & solid organ transplant) *Sun Q, Br J Haematol 2002; Bollard CM, Blood 2007*



Bollard CM, Blood 2007

Papadopoulou A et al, Sci Transl Med. 2014;6(242):242ra83.

VSTs beyond infections : virus-associated malignancies

Targeting less immunogenic EBV+ malignancies outside transplant setting:

- ✓ EBV-Hodgkin's lymphoma in immunocompetent individuals

Bollard CM, JCO 2014

- ✓ NK-T lymphoma

Cho SG, Mol Ther 2015

- ✓ nasopharyngeal carcinoma

Comoli P, JCO 2005; Chia WK, Mol Ther 2014

- ✓ progressive multiple sclerosis

Pender MP, JCI Insights 2018

Targeting human papillomavirus (HPV)+ tumors:

- ✓ cervical cancer

Stevanović S, JCO 2015

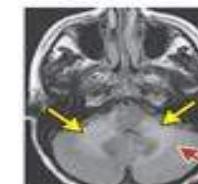


Targeting JC virus with BK-virus-specific T cells:

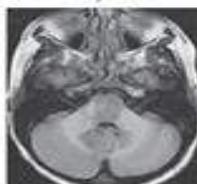
- ✓ Progressive multifocal leukoencephalopathy

Muftuoglu M, N Engl J Med 2018

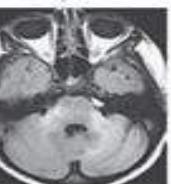
Pre VSTs



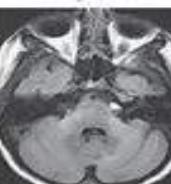
Day 21



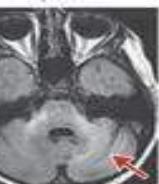
Day 58



Day 135



Day 258



Tumor-associated Ags (TAAs)

Viral antigens are the most immunogenic but, aside from EBV in lymphoma or PTLD, are rarely found in hematologic malignancies

Tumor-associated Ags (TAAs):

- widely expressed by leukemic cells and are in some instances, associated with induction of immune responses corresponding with clinical efficacy
- overexpressed on tumors compared with normal tissues

Ideal TAA: universally & selectively expressed on tumor cells

- **Leukemia-associated Ags (LAAs):**

- ✓ Wilms' tumor protein (WT1)
- ✓ Preferentially expressed antigen in melanoma (PRAME)
- ✓ Proteinase 3 (PR3)
- ✓ Hyaluronanmediated motility receptor (HMMR/Rhamm)
- ✓ Survivin

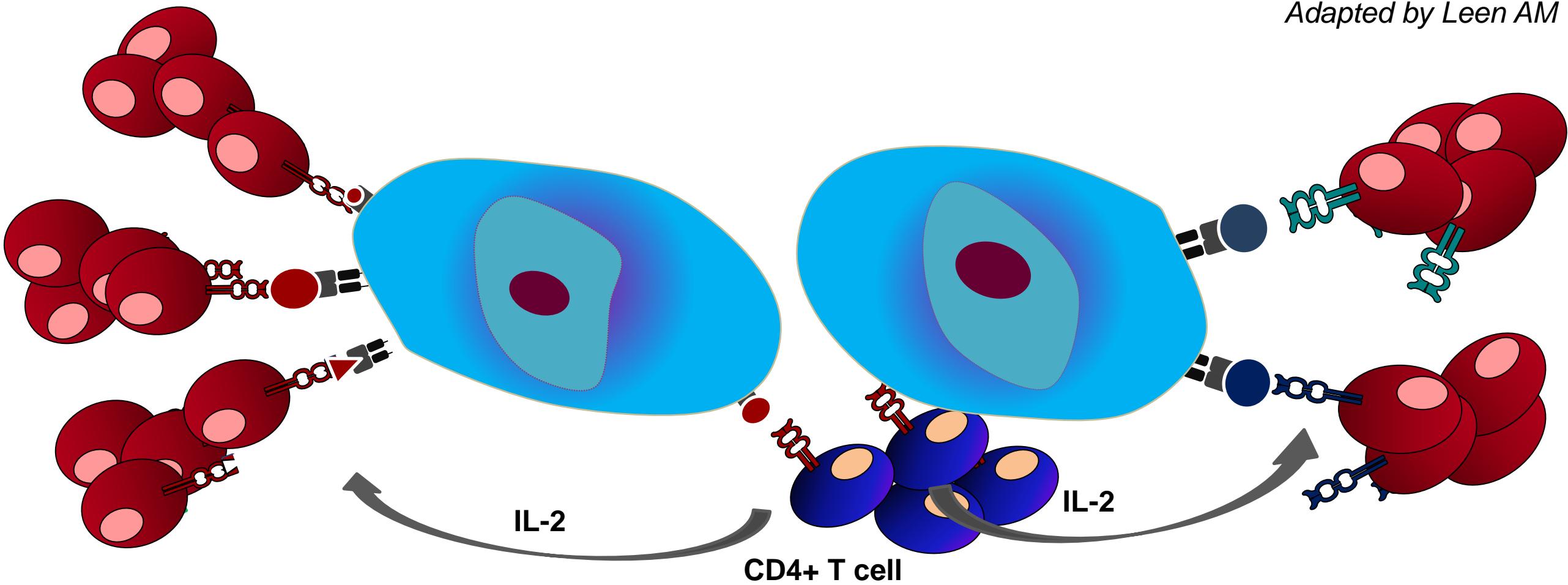
Leukemia-specific T-cells : limitations and challenges

- requirement for high numbers of APCs
- time- and labor-intensive / costly
- lack of practicality and efficacy in generating individualized patient and single HLA class I-restricted antigen-specific T cell products
- frequently anergic or tolerant status of “self-reactive” T cells targeting non- viral LAAs
- tumor escape mechanisms, rather commonly occurring with single-epitope restricted leukemia T-cells
- challenging the generation of ex vivo expanded allogeneic T cells, derived from a naïve population

Isolation of T-cells specific for non viral tumor-associated antigens has been less successful

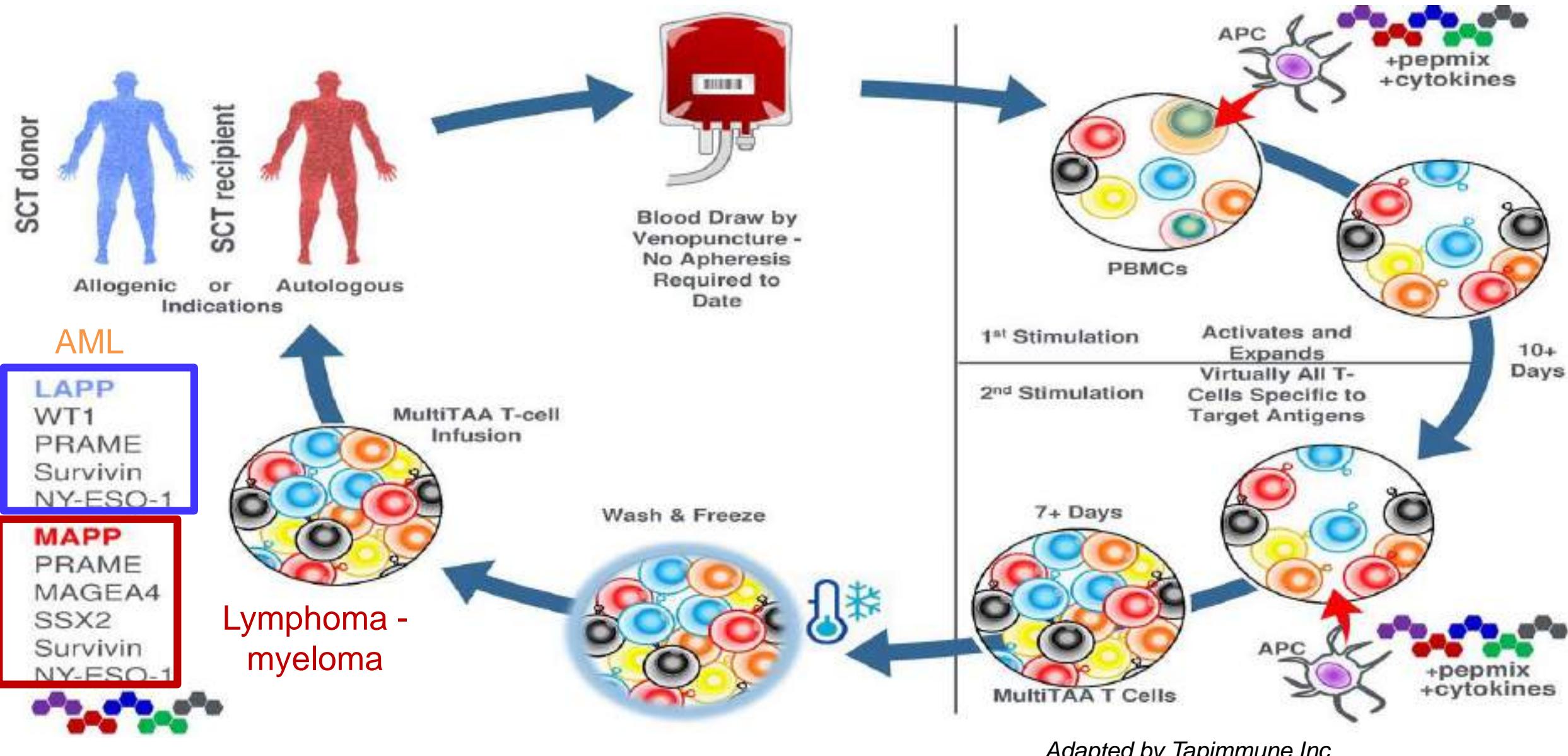
The IDEAL tumor-targeting T cell product

Adapted by Leen AM



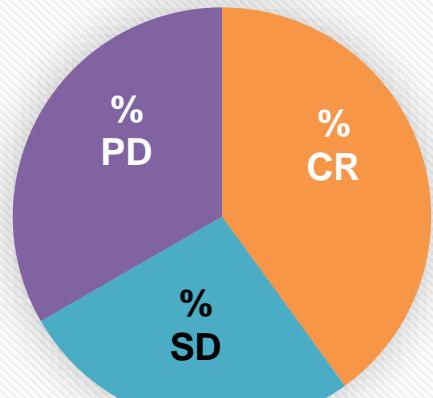
Polyclonal (CD4+ & CD8+) T cells that target
multiple tumor antigens in a single cell therapy product

Generating TAA-specific T cells



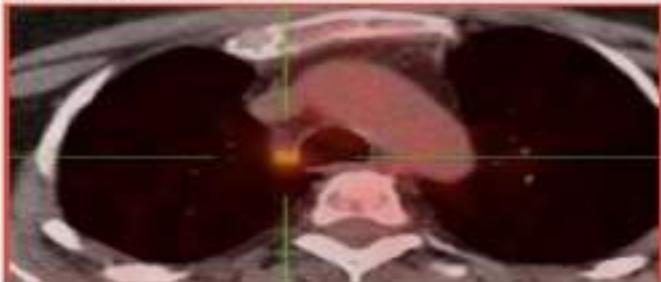
Compelling response rates– Lymphoma

Pts with active disease

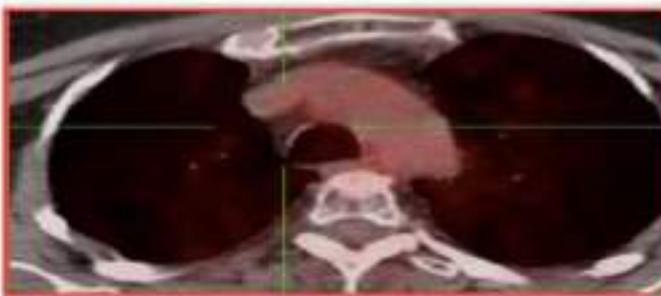


15 pts who failed 4 lines of prior therapy

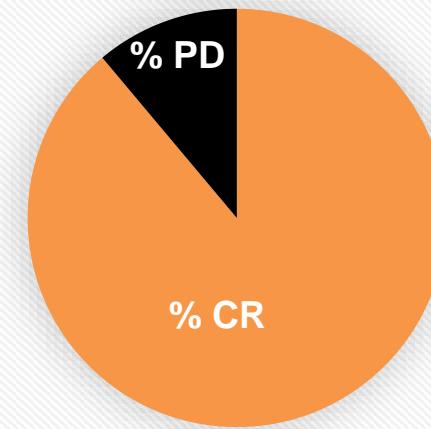
Pre-Infusion



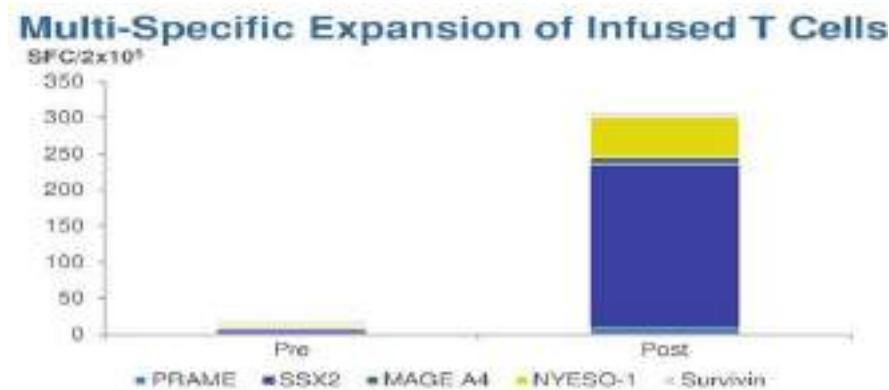
Post-Infusion



Pts in remission post HSCT



16/18 pts in remission

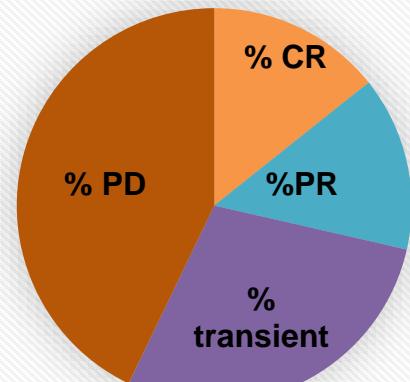


Tapimmune Inc

Carrum G; ASBMT Biology of Blood & Marrow Transpl March 2019
ASH Clinical news, 1st May 2019

Compelling response rates– AML/MDS, multiple myeloma

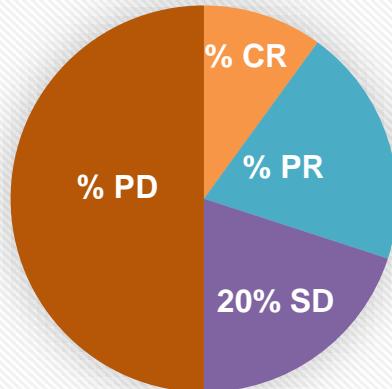
Pts with active disease



7 pts who failed 3 lines of prior therapy
1 CR; 1 PR; 2 transient benefit; 3 PD

AML/MDS

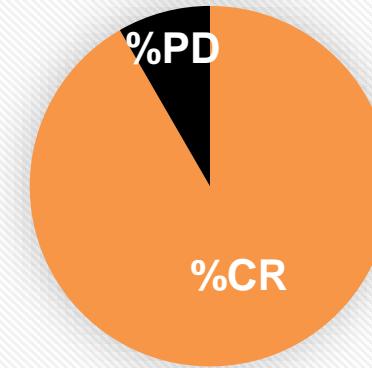
Pts with active disease



Multiple myeloma

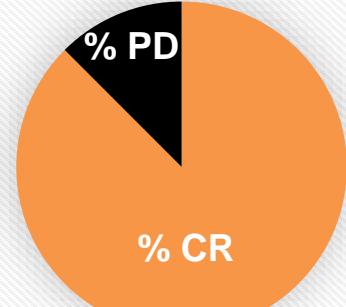
Lulla P; ASBMT Biology of Blood & Marrow Transpl 2019

Pts in remission post HSCT



11/12 pts in remission

Pts in remission post HSCT



No infusion-related cytokine release syndrome (CRS), neurotoxicity or GvHD



DEVELOPING AN “ALL IN ONE” T-CELL THERAPY PRODUCT TO SIMULTANEOUSLY FIGHT LEUKEMIC RELAPSE AND INFECTIONS POST HEMATOPOIETIC STEM CELL TRANSPLANTATION

Ideal T-cell product : target viral antigens & LAAs (LEVIs)

Simultaneously fight leukemia relapse and viral infections

- Non-genetically engineered
- No alloreactivity
- Target multiple epitopes of various Ags to eliminate the risk of tumor escape

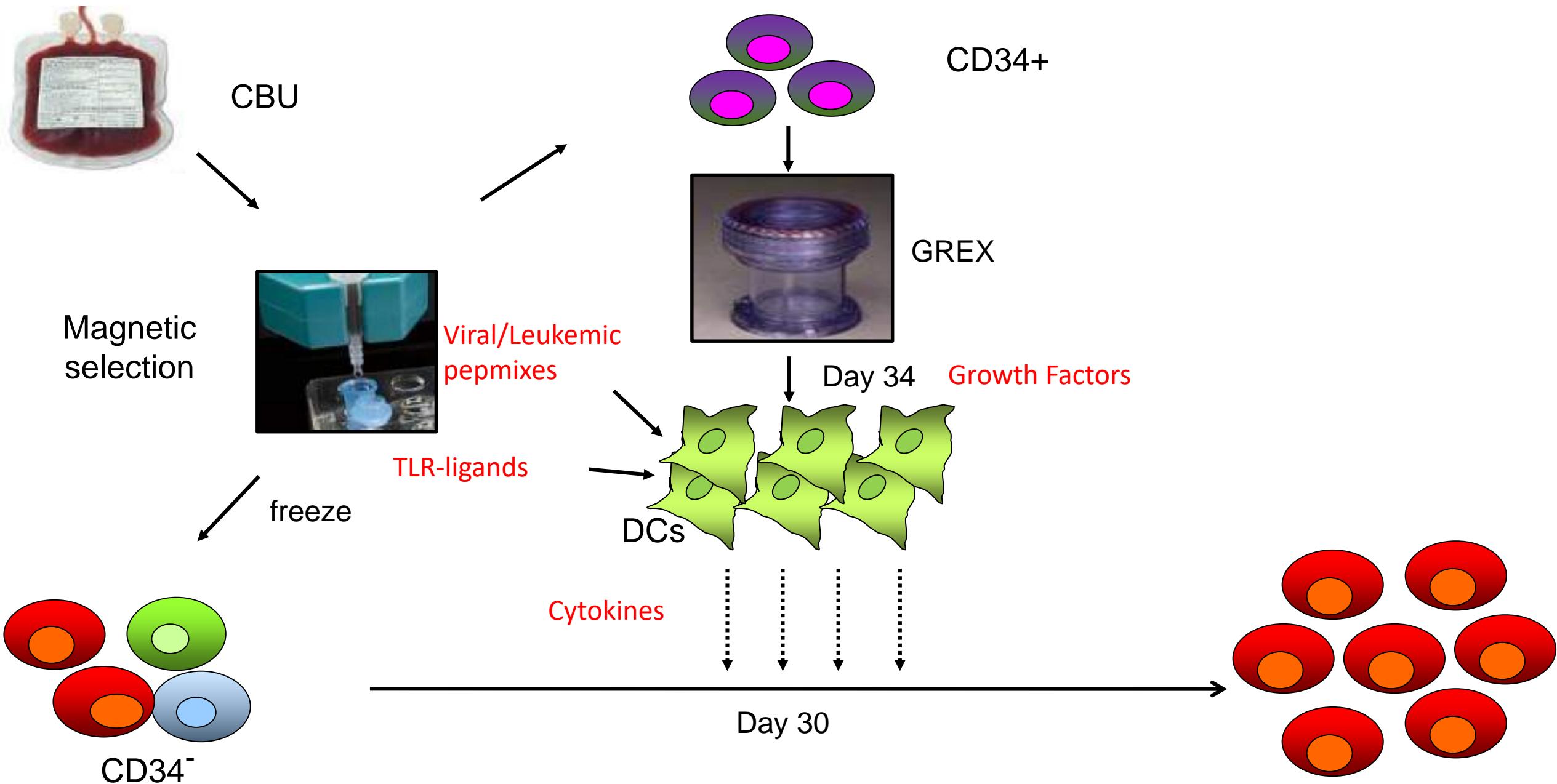
AIM

Generation of an “all-in-one” T cell product from nonusable cord blood units (CBUs) which simultaneously recognize multiple common viruses (*EBV, CMV, BK, AdV*) and LAAAs (*PRAAME, WT1*)

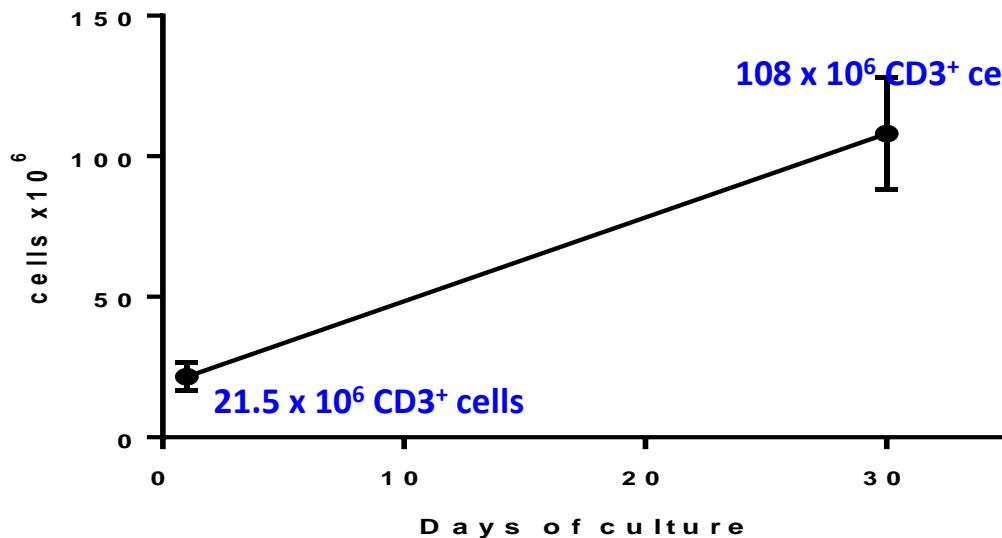
Circular economy in medicine

6/10 CBUs unsuitable for clinical use

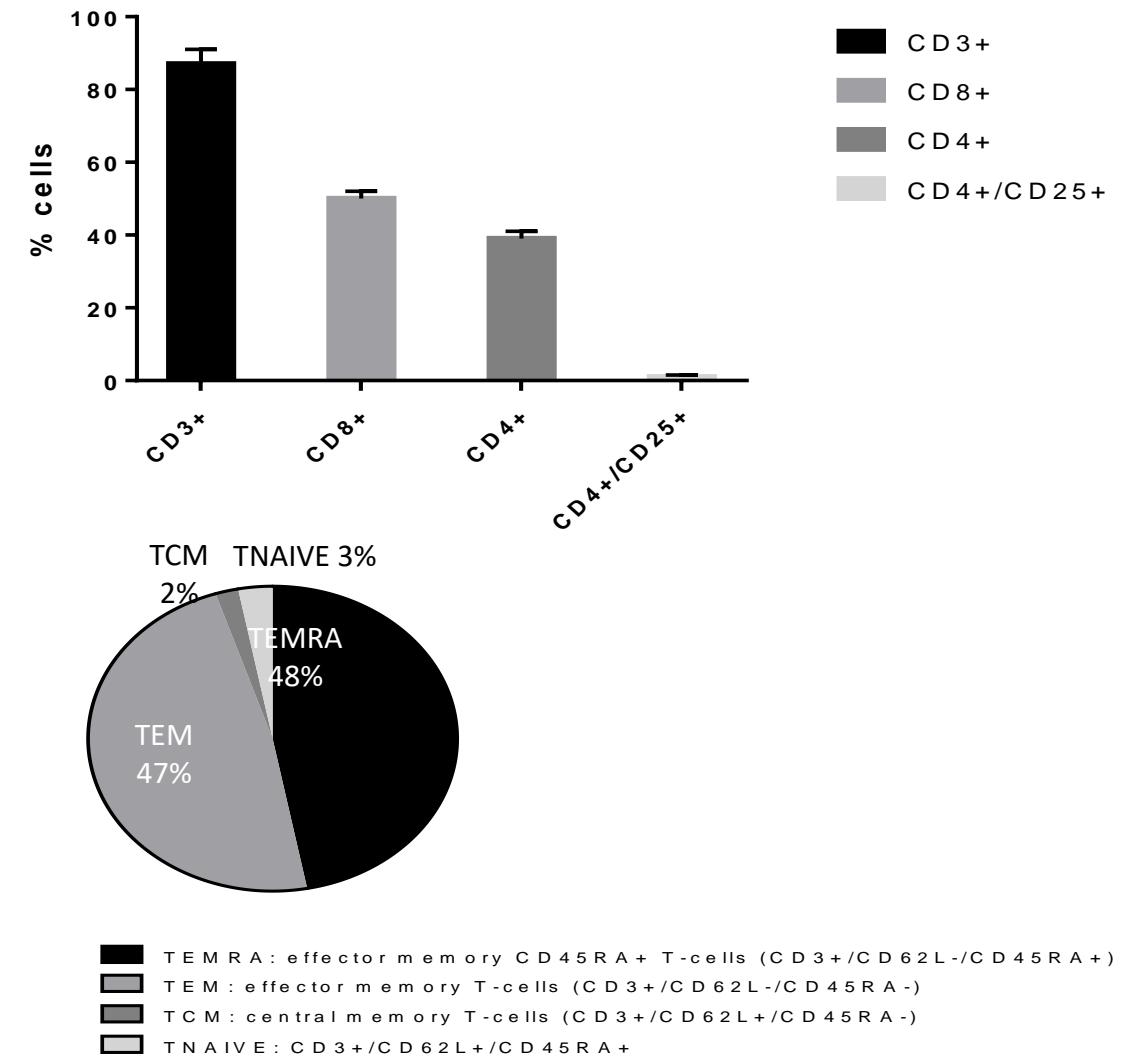
Experimental design



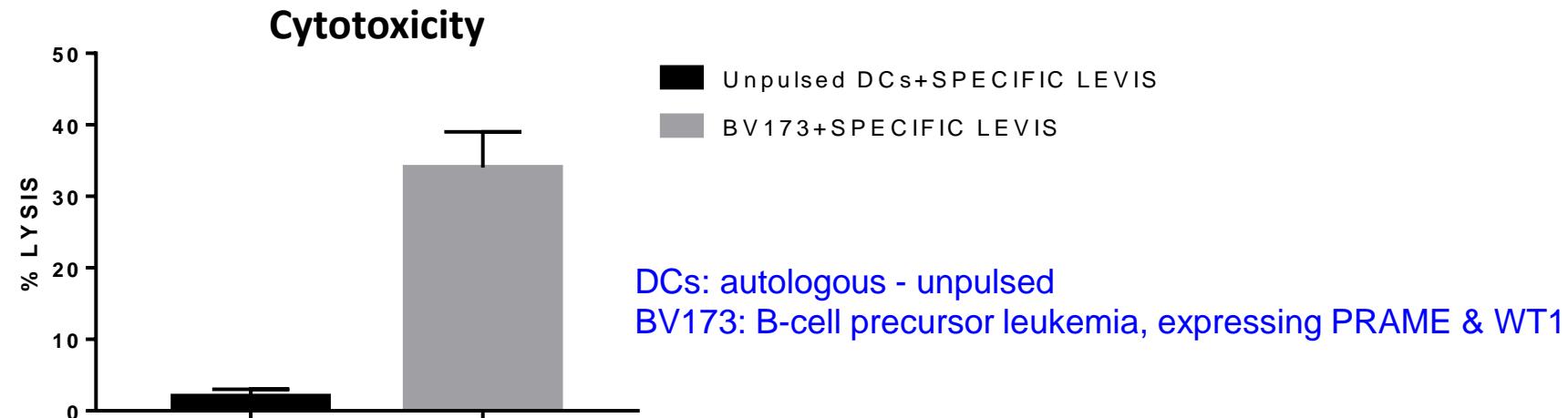
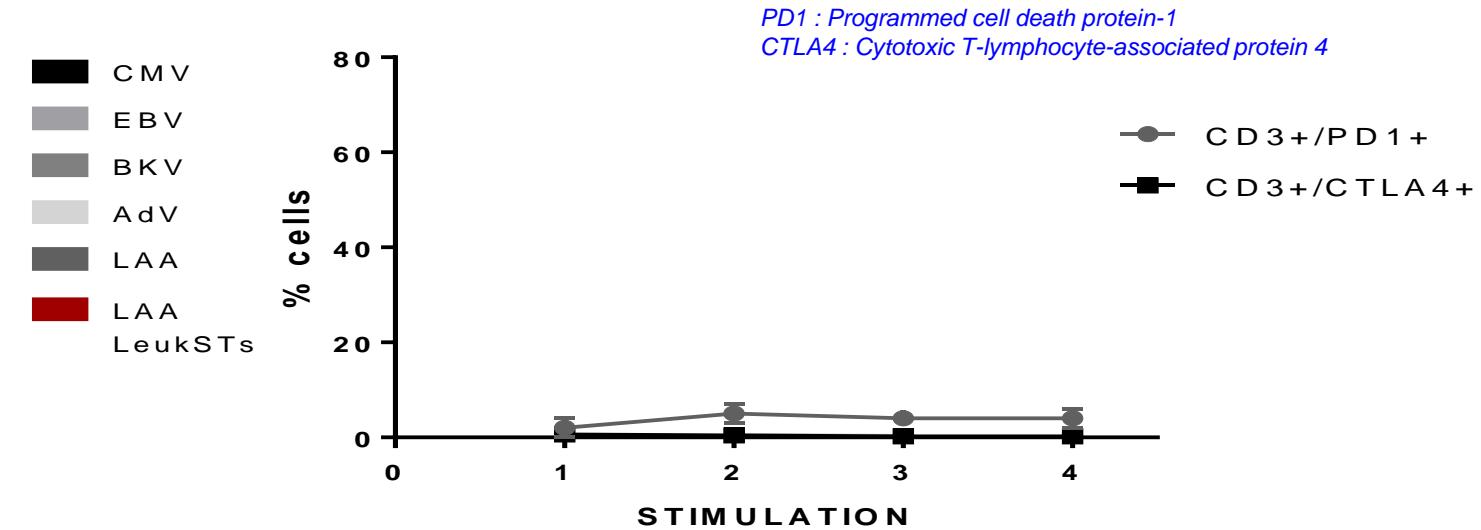
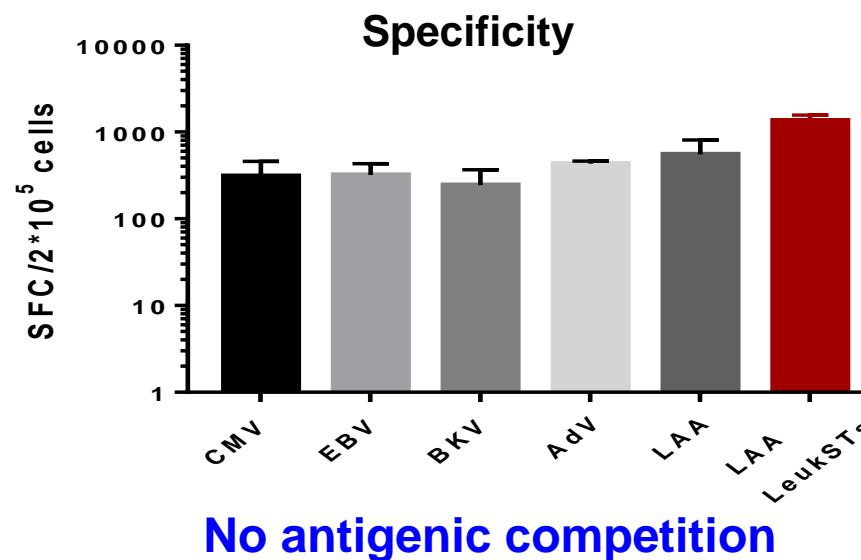
Expansion and Immunophenotypic characterization



N=4



Lack of antigenic competition and exhaustion/anergy



Re-qualifying non-usable CBUs : a paradigm of circular economy

- ❖ The production of clinically relevant numbers of third party LEVIS feasible
- ❖ **Leuk-STs :**
 - ✓ non-genetically engineered
 - ✓ polyclonal
 - ✓ express memory markers
 - ✓ target plethora of epitopes of two LAAs
 - ✓ “off-the-shelf” → readily available

CARs vs Ag-specific T cells

		Genetically-engineered	Non-genetically engineered
Clinical Safety	Serious Adverse Events (SAEs)	√	X
	Fatalities caused by therapy	√	X
	Mutagenesis risk	√	X - natural T cells
Clinical Impact	Specificity	Single	Multiple
	Epitope spreading	Observed once	√
	Duration of clinical benefit	Limited ?	Increased ?
Applicability	Regulatory approval	Difficult	Easy
	Estimated treatment cost	\$ 350.000 -475.000	Lower - no genetic modification

Gene and Cell Therapy Center, Hematology – HCT Unit, G.Papanikolaou Hospital, Thessaloniki



Ευχαριστώ!