Targeting High-Risk Leukemias with Redirected WT1-Specific T-Cells

Aude Chapuis, MD

Fred Hutchinson Cancer Research Center

Seattle

Background: High-Risk Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)

- Definition of <u>high-risk AML</u>:

  - Unfavorable Cytogenetics/FISH/molecular abnormalities:

    - Del 5q; del 7q; abnormalities involving 3q, 9q, 11q, 20q, 21q, 17p; t(6;11); t(15;17); complex karyotype; FLT3-ITD mutation.

  - Refractory / beyond 1st remission.

  - AML caused by previous chemotherapy (therapy-related).

  - AML arising for a previous hematologic disorder.

- Definition of <u>high-risk MDS</u>:

  - MDS with IPSS score >1.5 and/or

  - Unfavorable cytogenetics.

- **For these patients, the probability of post-HCT <u>relapse</u> (>95% fatal) and/or <u>death</u> is >50% within 2 years** (Gyurcokza B., JCO 2010; Deeg J., Blood 2002; Gratwohl A., BMT 1996; Radich J., Semin. Hematol. 2010).

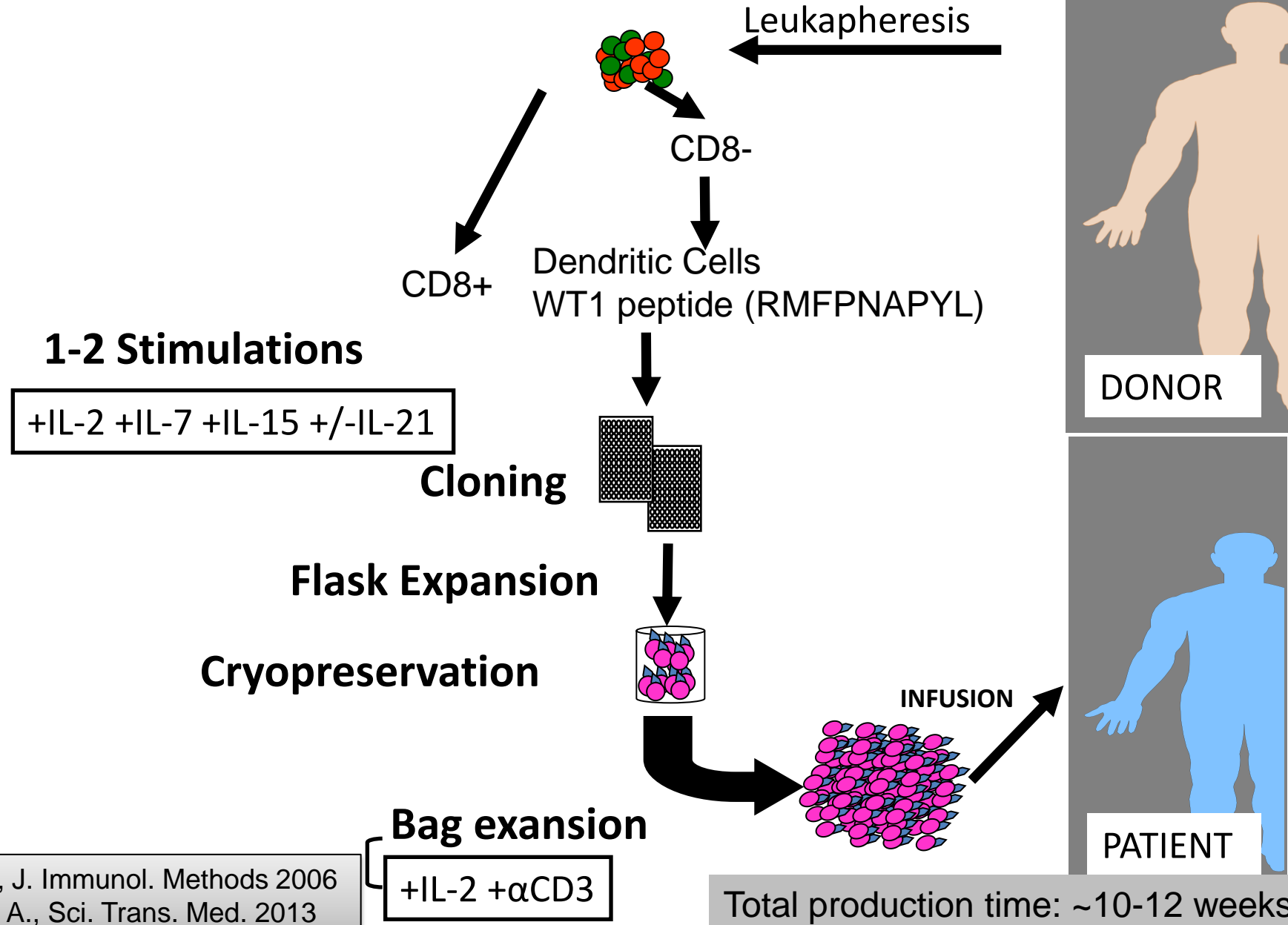# Leukemias that relapse post-HCT have a very poor prognosis

- Current post-HCT treatment options include withdrawal of immunosuppression, re-induction chemotherapy, donor lymphocyte infusion from the original HCT donor, second non-myeloablative transplant.

- Patients with early post-HCT relapse have a worse prognosis with shortened survival compared to patients who relapse later.

- **Trials designed to prevent relapse in patients with high-risk leukemias constitute a high priority, but no predictably effective therapy exists**.

Mielcarek M. et al,. 2007
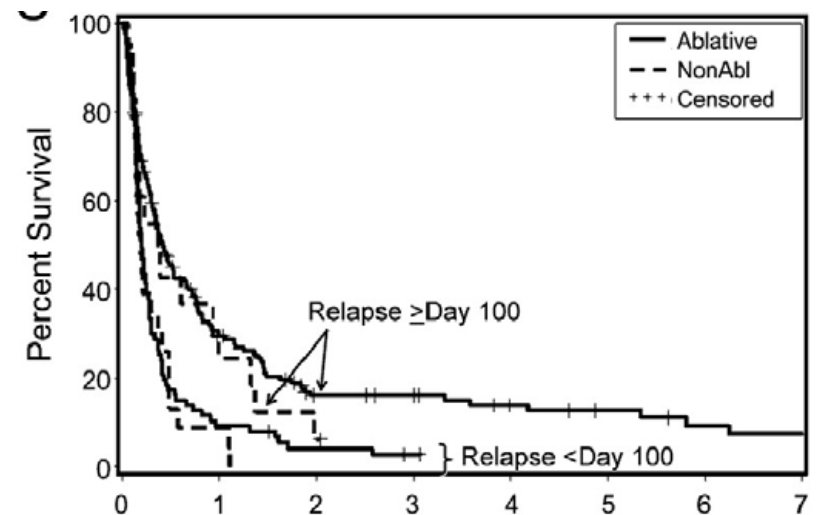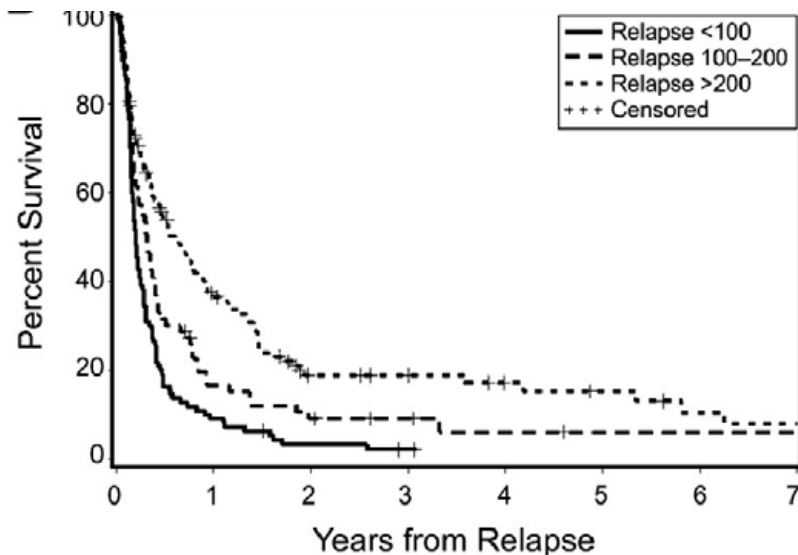
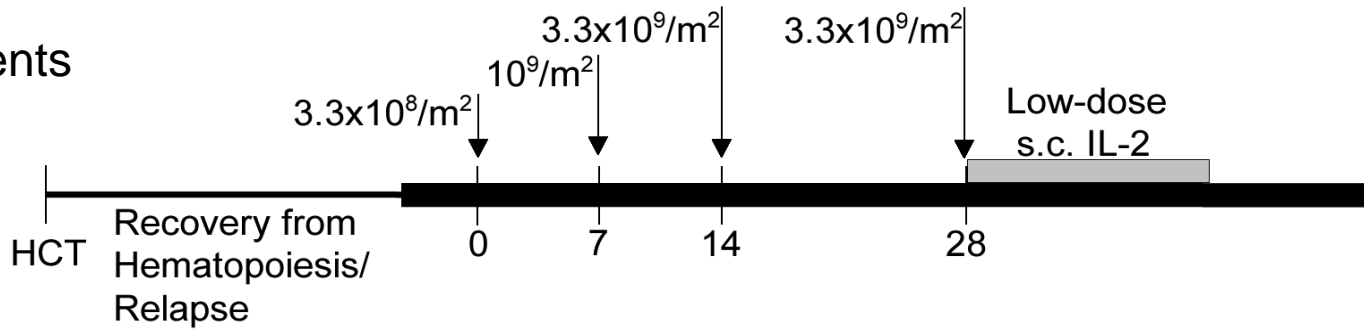# Rationale for targeting WT1 in patients with high-risk leukemias

- WT1, a zinc finger protein that regulates gene expression
  - originally characterized as a gene associated with Wilm's tumor (Pritchard-Jones K., *Nature* 1990).

- Over-expressed (10->1000 fold) in AML, ALL, CML, MDS blasts
  -higher levels correlated with worse prognosis (Bergman L., *Blood* 1997).

- Low-level expression in adult kidney podocytes, testis sertoli cells, mesothelial lung cells (mesodermal origin) and CD34^{+} progenitor cells (Inoue K., *Blood* 1997).

- CD8^{+} T-cells can distinguish difference in protein expression between physiologic WT1 expression levels and leukemic cells (Gao L et al., 2000).

- WT1 vaccine studies have resulted in anti-tumor responses, including some long-term complete remissions in solid tumors and leukemias (Oka Y., *Immunotherapy* 2010; Van Tendeloo *PNAS* 2010; Ochsenreither S., *J Immunother* 2010).

- No toxicities to normal tissues expressing physiologic levels of WT1 have ever been reported.

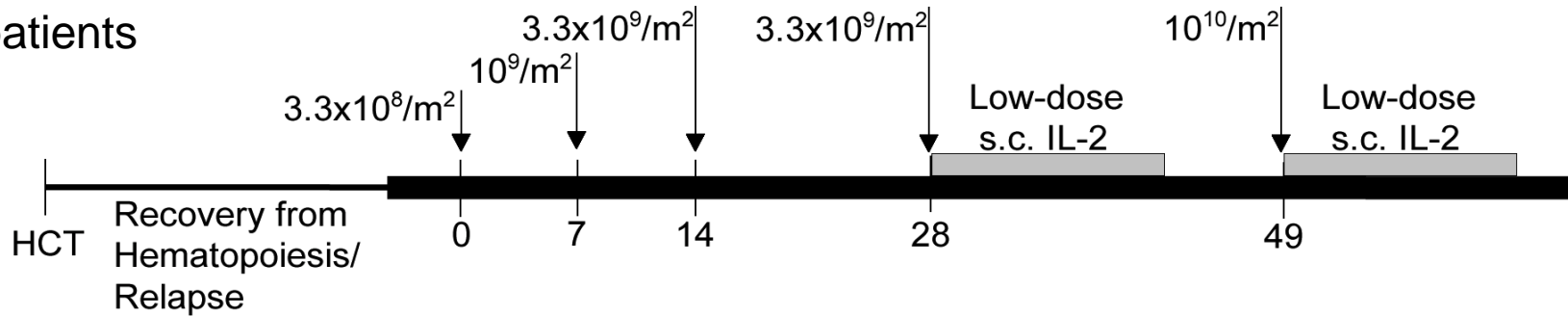# Generation of WT1-specific CD8⁺ T cell clones from donor PBMC: Methods

Leukapheresis

CD8-

CD8+

Dendritic Cells
WT1 peptide (RMFPNAPYL)

1-2 Stimulations

+IL-2 +IL-7 +IL-15 +/-IL-21

Cloning

Flask Expansion

Cryopreservation

INFUSION

Bag exansion

+IL-2 +αCD3

DONOR

PATIENT

Ho W.Y., J. Immunol. Methods 2006
Chapuis A., Sci. Trans. Med. 2013

Total production time: ~10-12 weeks

Infusion of donor-derived WT1-specific CD8$^+$ T cell clones: Treatment plan

First 2 patients

$3.3 \times 10^8/m^2$ $10^9/m^2$ $3.3 \times 10^9/m^2$ $3.3 \times 10^9/m^2$

Low-dose s.c. IL-2

HCT — Recovery from Hematopoiesis/ Relapse

0   7   14   28

Second 2 patients

$3.3 \times 10^8/m^2$ $10^9/m^2$ $3.3 \times 10^9/m^2$ $3.3 \times 10^9/m^2$ $10^{10}/m^2$

Low-dose s.c. IL-2   Low-dose s.c. IL-2

HCT — Recovery from Hematopoiesis/ Relapse

0   7   14   28   49

Last 7 patients

$3.3 \times 10^9/m^2$ $10^{10}/m^2$ $10^{10}/m^2$

Low-dose s.c. IL-2

HCT — Recovery from Hematopoiesis/ Relapse

0   14   28
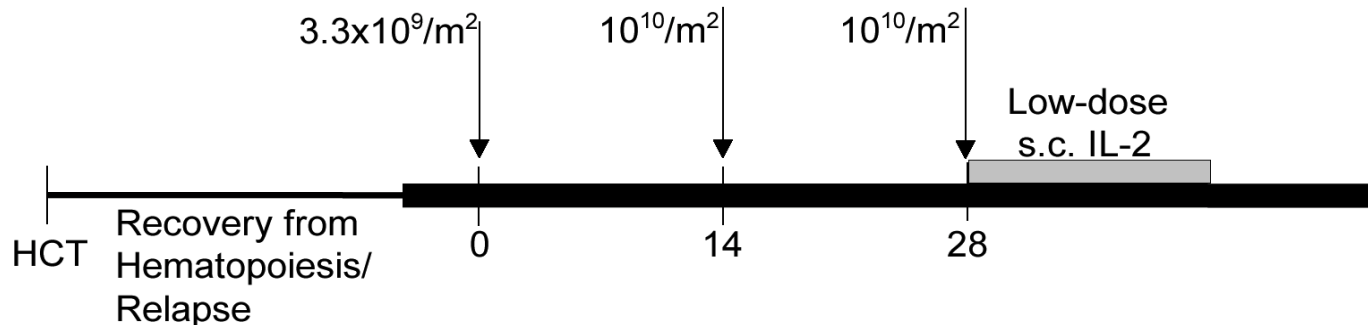
Infusion of donor-derived WT1-specific CD8+ T cell clones: Results

- 11 patients with high-risk or relapsed leukemias received escalating doses of WT1-specific T cells for a maximum dose of 10^{10} cells/m^2.

- No injuries were observed to tissues expressing physiologic levels of WT1.

- Evidence of direct albeit transient anti-leukemic activity.

Patient 15

Legend: %Blasts; %WT1+ T-cells

Chapuis AG., Ragnarsson G., Sci. Trans. Med. 2013
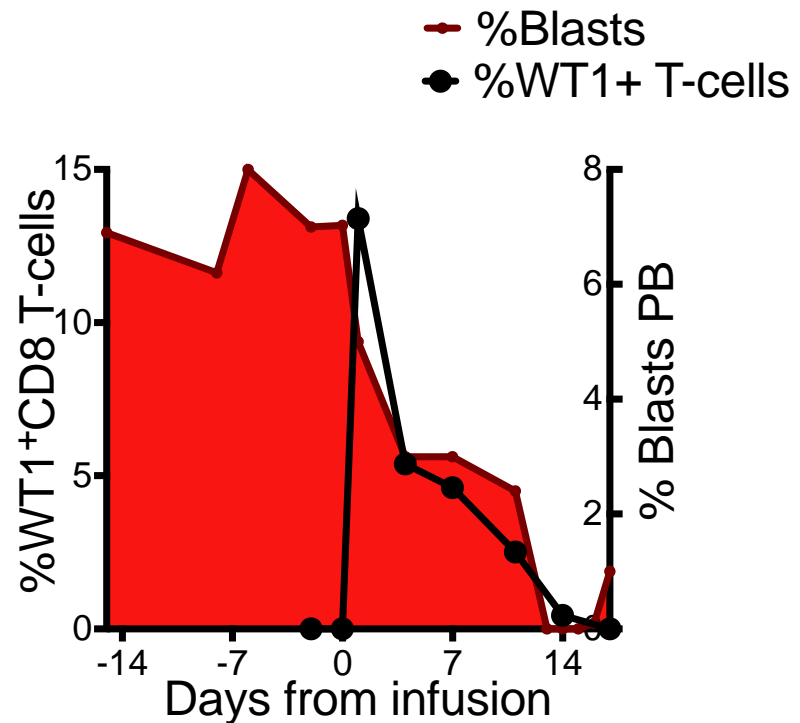
Variability in the avidity of infused clones
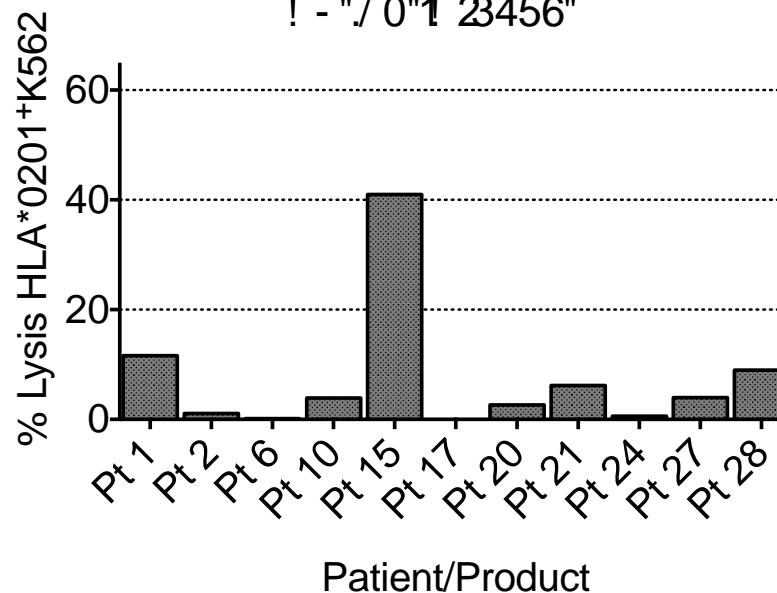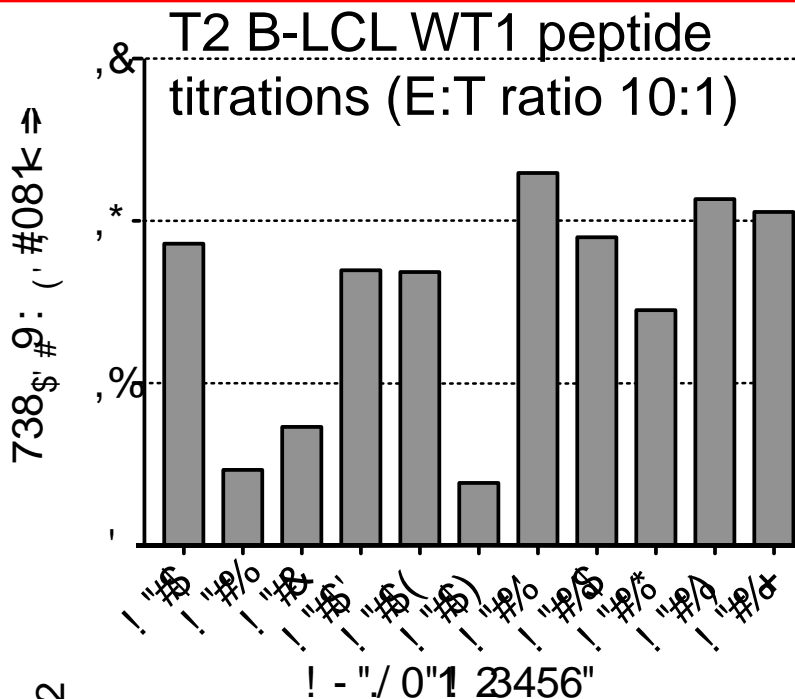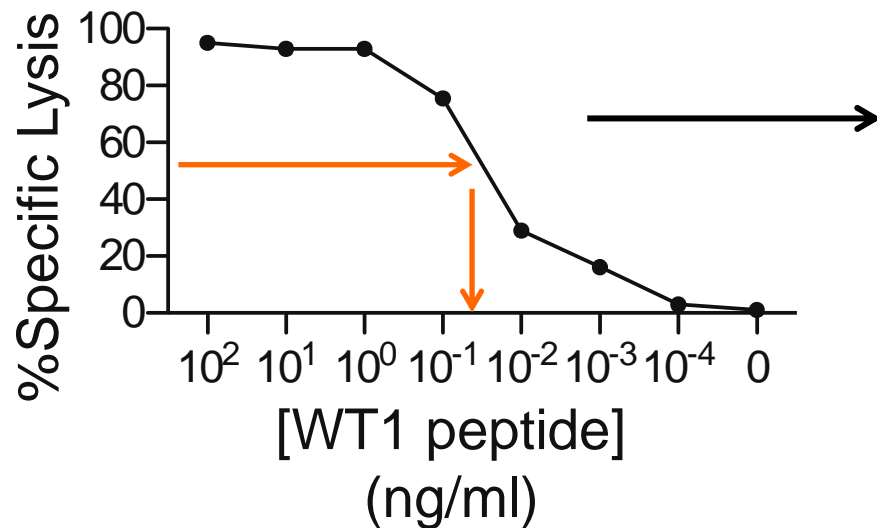
Rationale for targeting WT1 with a <u>naturally occurring</u> TCR of higher affinity

- Although the most avid clone (based on WT1 peptide titrations) from each patient/donor pair was selected for infusion, the avidities obtained were variable.

- T cells expressing higher affinity TCRs exhibited improved recognition of WT1^{+} target cells.

As TCR affinity is a major determinant of T-cell avidity, expressing a higher affinity TCR is associated with better recognition of leukemic cells

Selection of a naturally occurring high-affinity HLA A*0201-restricted WT1-specific TCR

Relative affinity of the 'parent C4 clone' compared to clones administered to patients:

The K_D of C4 is 2.98 nM

Legend:
- ■ P1
- ▲ P2
- ▼ P6
- ◆ P10
- ● P15
- □ P18
- △ P20
- ▽ P21
- ◇ P22
- × C4

T. Schmitt   WT1 tetramer titration (Normalized to the maximal tetramer binding - Bmax)
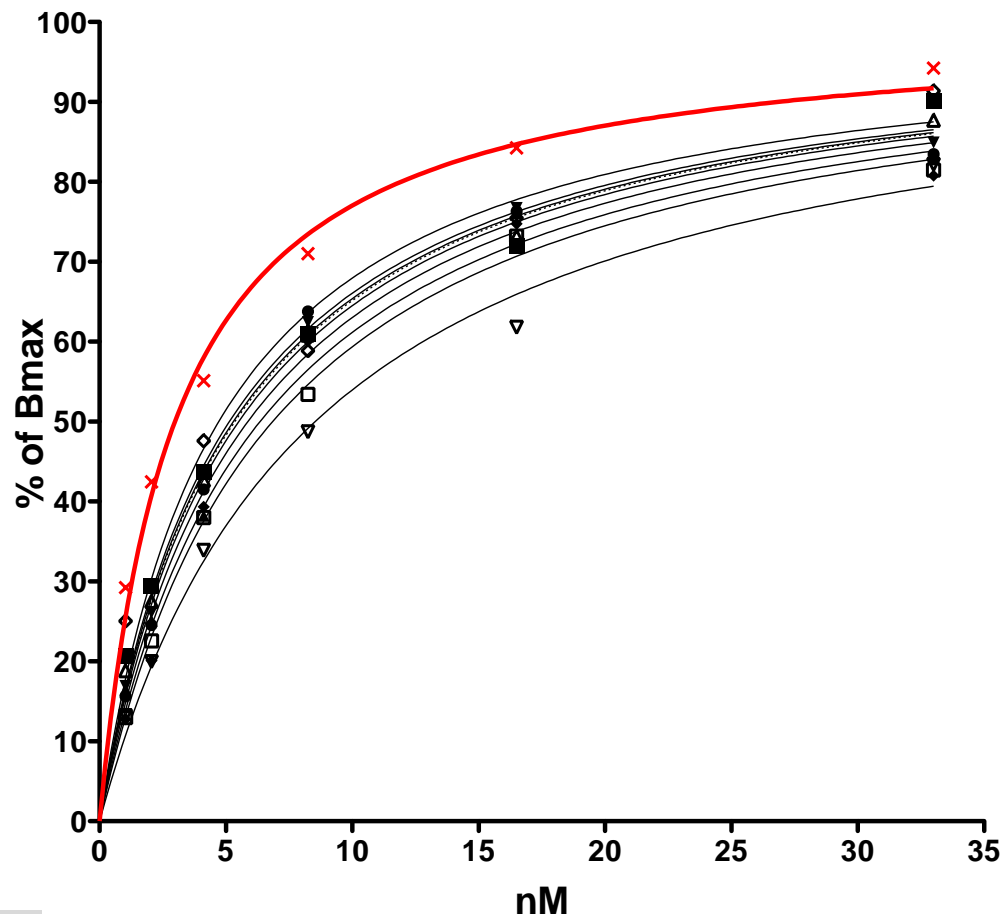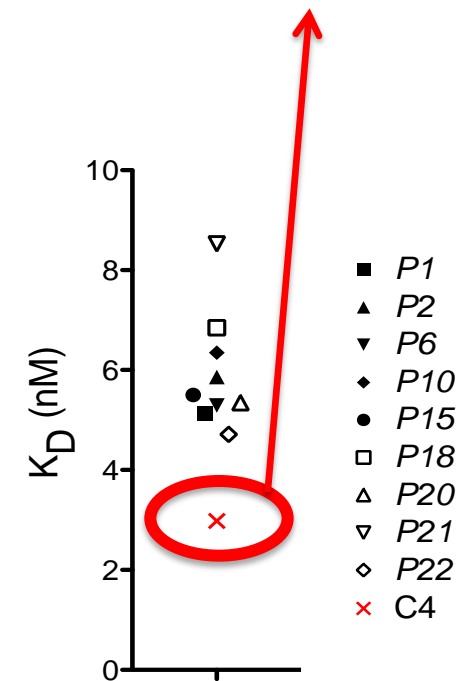
Insertion of the TCR$_{C4}$ in a lentiviral SIN vector with a strong internal promoter

5'SIN LTR - Ψ – cPPT - MSCV U3 - TCRβ - P2A - TCRα - WPRE - 3'SIN LTR

MSCV U3 promoter 7914...8298
cPPT 7773...7896
RRE 7048...7289
1...1816 C4beta–P2A–C4alpha
RU5 6205...6386
RSV 5973...6204

pRRLSIN-C4B-P2A-A

1818...2416 WPRE
2498...2731 SIN18 deletion of U3 R and U5
2932...3138 SV40\pA/ORI
3299...3757 F1\ORI
AmpR 3889...4746
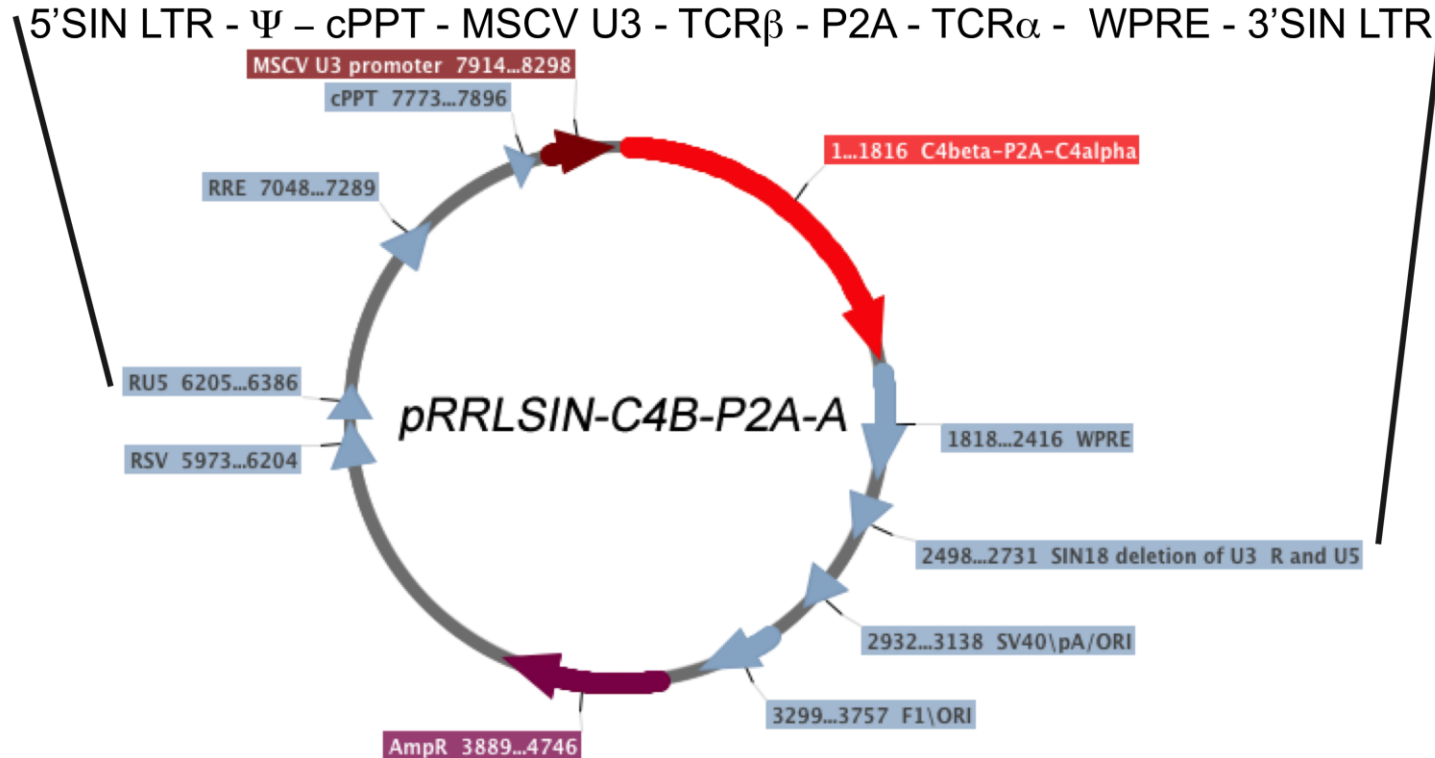
SIN LTR = Self-inactivating long terminal repeat
Ψ = Packaging signal
cPPT = Central polypurine tract
MSCV = MSCV U3 promoter
TCRβ = Beta chain of the WT1-specific TCR
P2A= 2A element from the porcine teschovirus
TCRα = Alpha chain of the WT1-specific TCR
WPRE = Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element

T. M. Schmitt

Relative avidities of the parent C4 clone and TCR$_{C4}$ CTL

T2 B-LCL WT1 peptide titrations (E:T ratio 10:1)

Lysis of HLA A*0201 K562 (E:T ratio 5:1)

Lysis of fresh HLA A*0201+ Leukemia cells
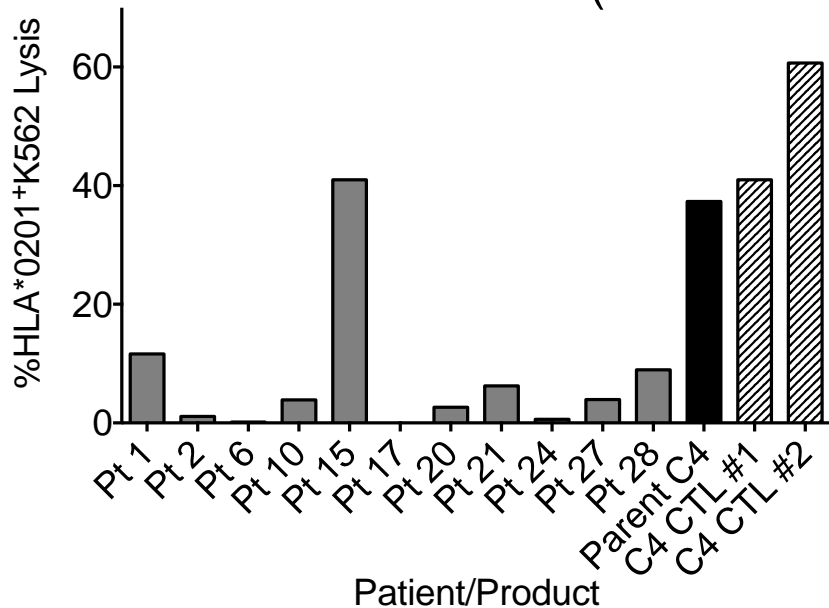
Generation of TCR$_{C4}$-transduced products for infusion

1. **Stimulation with HLA A*0201-restricted EBV/CMV peptide**
2. **Transduce with TCR$_{C4}$ Lentiviral supernatant**

   +IL-2 +IL-7 +IL-15
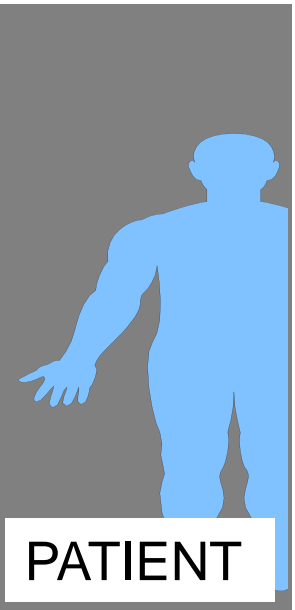
3. **Sort on virus- and WT1-multimer$^+$ cells**
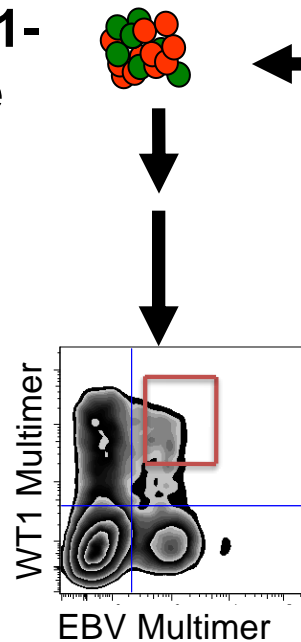
4. **Flask expansion**

   +IL-2+αCD3

5. **Bioreactor expansion**

   +IL-2+αCD3
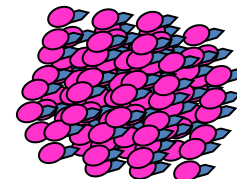
Total production time: ~5 weeks

G-mobilized Leukapheresis

DONOR

WT1 Multimer

EBV Multimer

INFUSION

PATIENT

Leukemia trial: Patient characteristics -update

Pt#	M/F	Age	EBV/CMV	Disease	Leukemia WT1 expression*	Disease Characteristics at 1st CTL infusion
1	M	56	EBV	AML, chloroma 5y after 1st myeloablative HCT	ND	PET+ Chloroma
2	F	50	CMV	AML, 2nd HCT for relapse	YES	MRD by flow (0.03%)
3	M	48	EBV	High-risk AML	ND	NED
4	M	25	EBV	High-risk AML	YES	NED
5	M	48	EBV	High-risk AML	YES	70% peripheral blasts
6	F	20	CMV	High-risk AML2nd HCT for relapse	ND	NED
7	F	33	EBV	High-risk AML	YES	MRD by flow (0.08%)
8	F	62	EBV	High-risk AML	ND	MRD by flow (0.26%)
9	F	67	CMV	High-risk AML	YES	MRD by FISH (0.3%)
10	M	74	EBV	High-risk AML	ND	NED
11	M	59	EBV	High-risk AML	ND	NED
12	F	55	EBV	High-risk AML	ND	NED
13	F	59	EBV	High-risk AML	ND	NED
14	M	17	EBV	High-risk AML, relapse after 1st HCT	ND	MRD by flow (0.02%)

*>250-fold expression compared to normal donor marrow

TCR$_{C4}$-transduced EBV/CMV-specific CD8$^+$ T-cells – plan of treatment

1st Phase: Dose escalation (5 patients completed 4 infusions):
Arm 1: Preventative treatment after HCT.
Arm 2: Treatment of detectable disase.

Day of Treatment

| 0 | 14 | 28 | 42 | 56 |

Monitoring → Evaluation

SC IL-2

HCT | Recovery of Hematopoiesis | 1 x 10^9 | 3.3 x 10^9 | 1 x 10^{10} | 1 x 10^{10} | WT1-specific T Cell Dose (per m^2)

2nd Phase (7 patients):

Day of Treatment

| 0 | 14 | 28 |

Monitoring → Evaluation

SC IL-2

HCT | Recovery of Hematopoiesis | 1 x 10^{10} | 1 x 10^{10} | WT1-specific T Cell Dose (per m^2)

Protocol FHCRC #2498 P.I. Dr Merav Bar
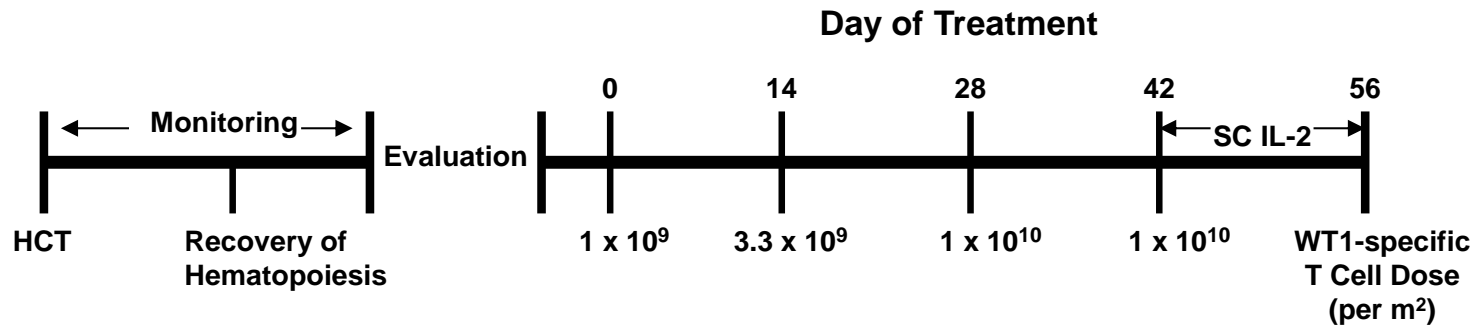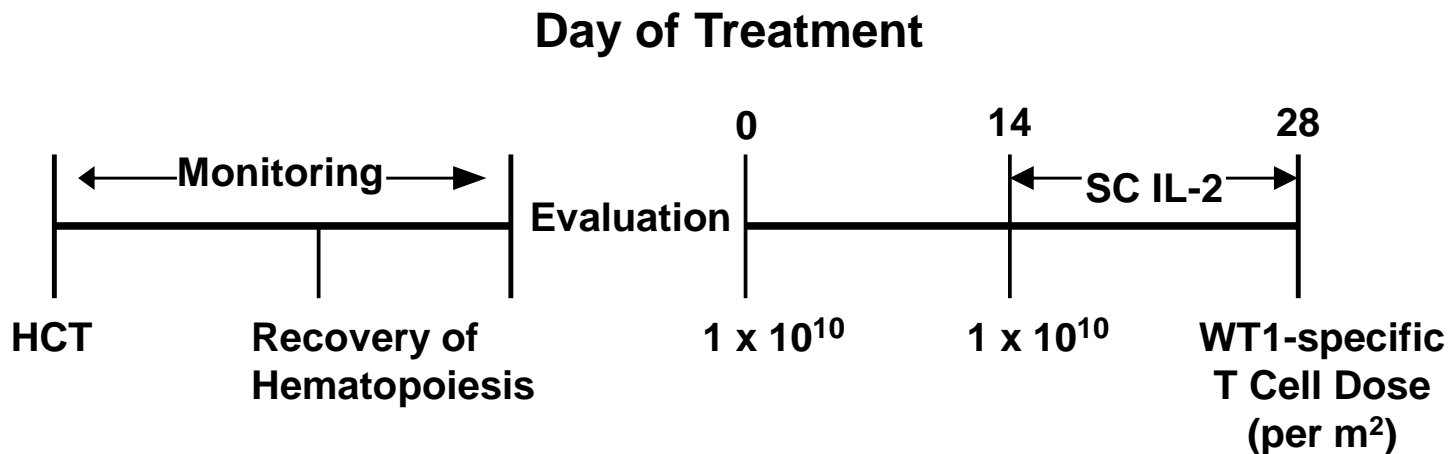
TCR$_{C4}$-transduced EBV/CMV-CTL do not injure normal tissues expressing physiologic levels of WT1

NCI CTCAE v4.0	Grade 3	Grade 4
Fever (within <24 hours of infusion)	2	0
Chills (within <24 hours of infusion)	1	0
Hypotension (within <24 hours of infusion)	1	0
Lymphopenia (<10 days after infusion)	8	1
Thrombocytopenia	3	0
Transient maculo-papular rash	1	0
GVHD	0	0

Preliminary Clinical Outcomes

- 7 pts received TCR$_{C4}$-transduced T cells with **NED post-HCT** either on the prevention arm or after salvage chemotherapy for relapse:
  - All are alive with NED average **240 days** (range 93-525 days) after infusions, **328 days** (range 161-605) days after HCT.

- 3 pts received TCR$_{C4}$-transduced T cells with **MRD post-HCT**:
  - 1 **cleared MRD** and is alive without additional therapy **557** days after infusions.
  - 2 pts continue to have detectable but decreased MRD 183 and 86 days after infusions.

- 4 pts **progressed** during the time they were receiving the TCR$_{C4}$-transduced T cells:
  - 1 had a resistant extramedullary chloroma.
  - 1 had 70% peripheral blasts (increasing rapidly).
  - 2 had received salvage therapy for early relapse post-HCT (1 pt received CMV-specific cells, no persistence).

Assessment of persistence after infusions:
CMV-Specific CTL

3 of 3 patients:

% WT1+ CD8+ T cells:
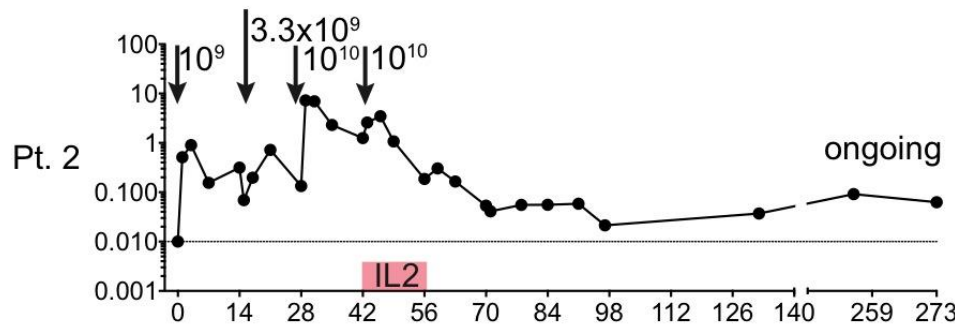
WT1+ CD8+ T cells / µl
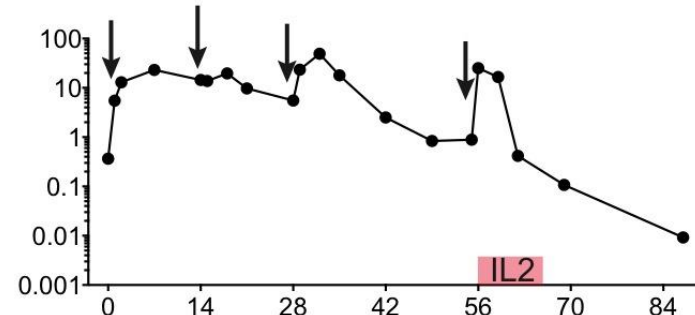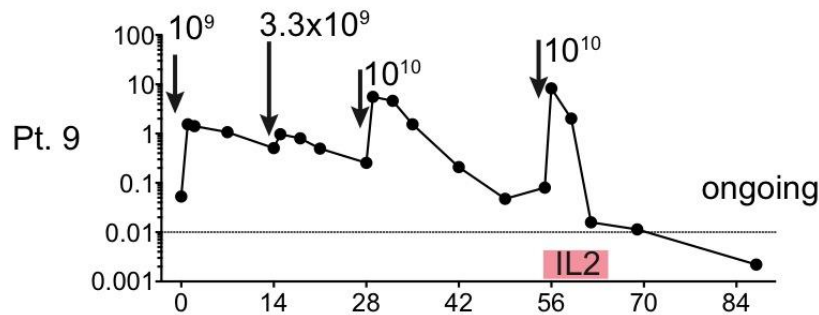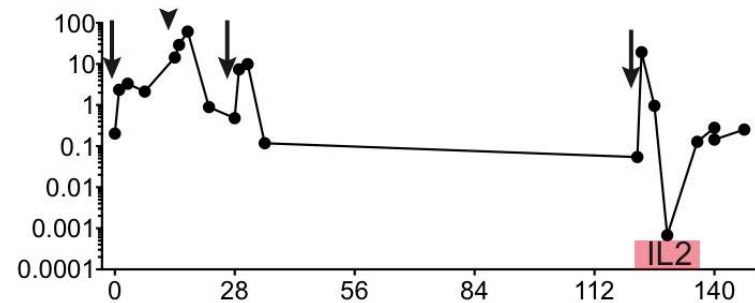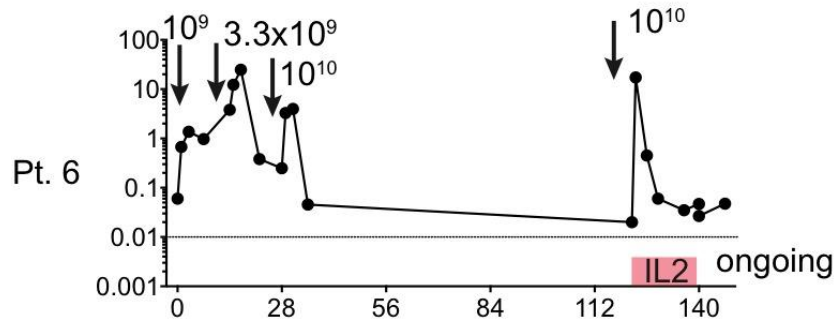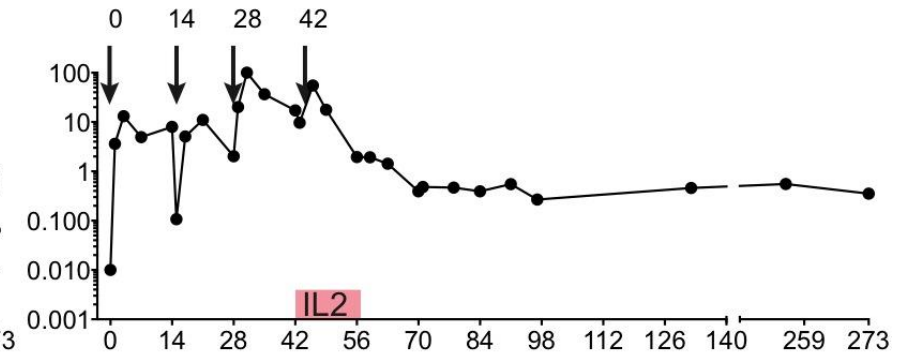
Days after 1st CTL infusion

Days after 1st CTL infusion

Assessment of persistence after infusions:
EBV-Specific CTL

6 of 7 patients who could be followed > 42 days after infusions:
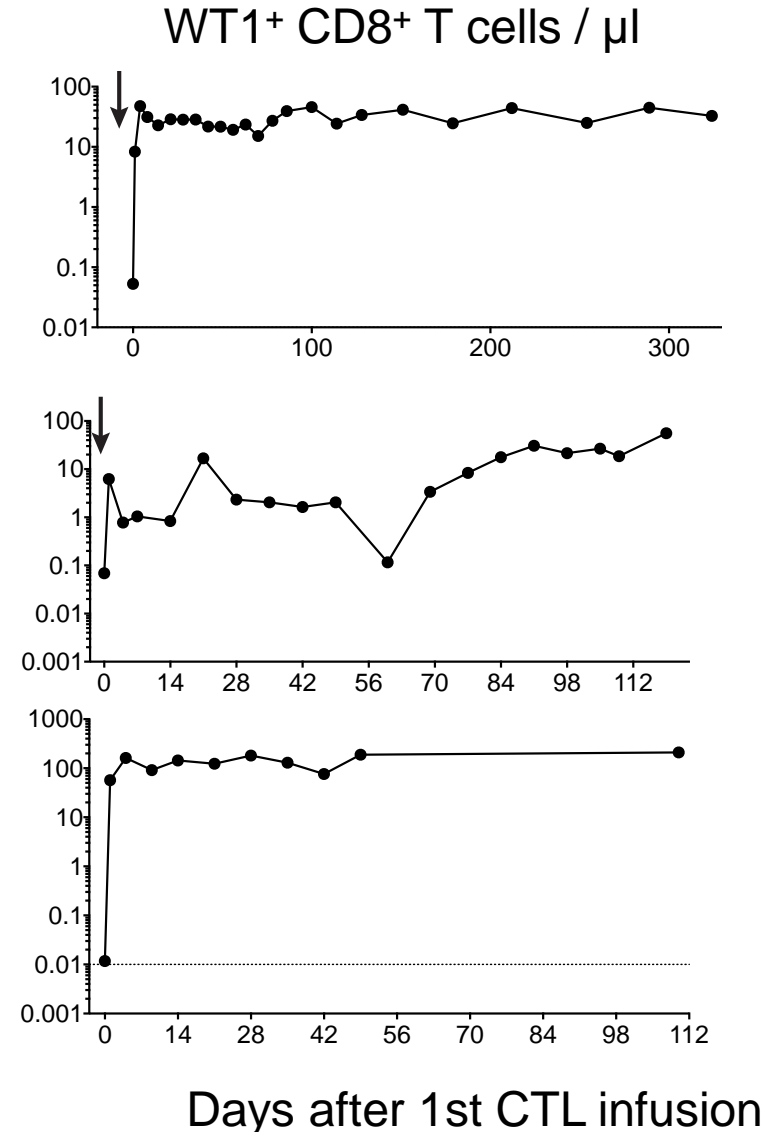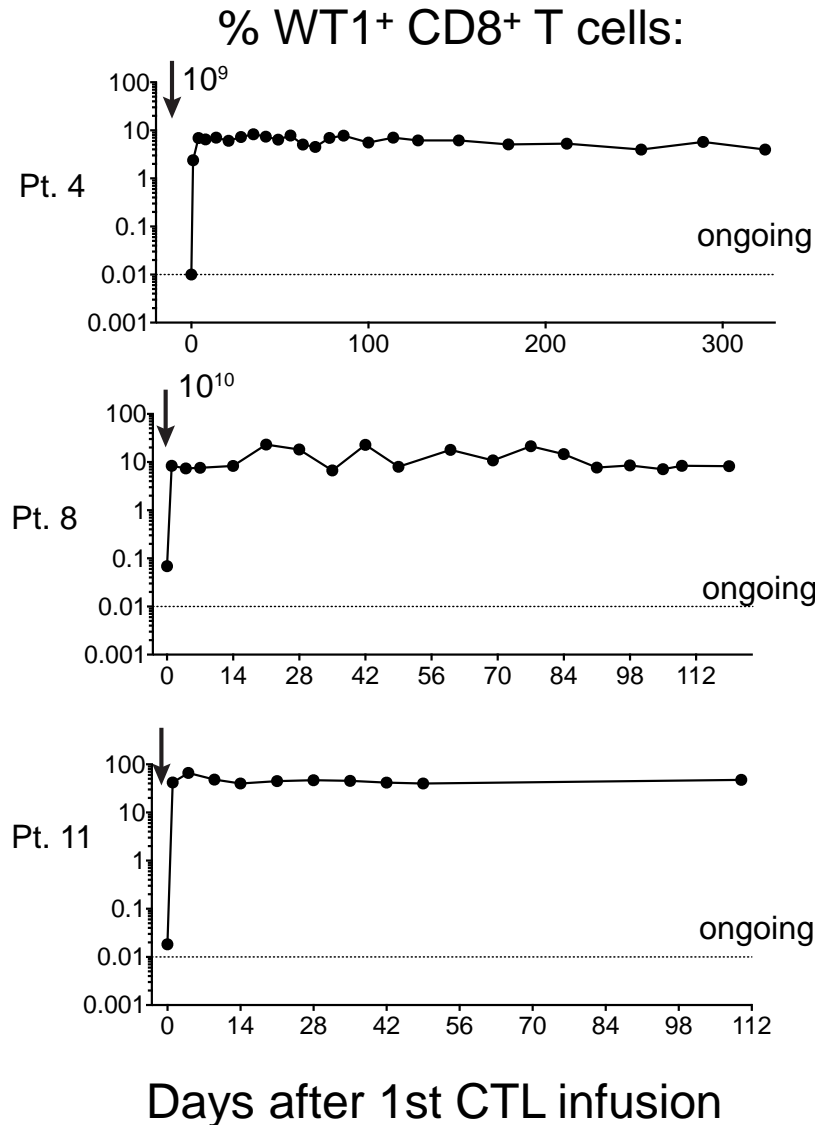
% WT1$^+$ CD8$^+$ T cells:

WT1$^+$ CD8$^+$ T cells / µl

Days after 1st CTL infusion

Days after 1st CTL infusion

Why are EBV-specific CTL persisting longer with higher frequencies *in vivo* than CMV-specific CTL?

- No difference in the expression of <u>surface markers associated with memory</u> (CD28, CD27, CD127, CD62L, CCR7) or <u>activation/exhaustion</u> (PD1, TIM3, 2B4, KLRG1, CD160) on infusion products.

- CMV infection is characterized by <u>continued low-level</u> replication (Sylwester AW, JEM 2005).

- EBV infection establishes latency, infrequent reactivations.

- EBV-specific cells are present in a 'resting state' in normal donors. Secrete IL-2, less perforin upon cognate Ag recognition (Makedonas G, PLOS pathogens 2010).
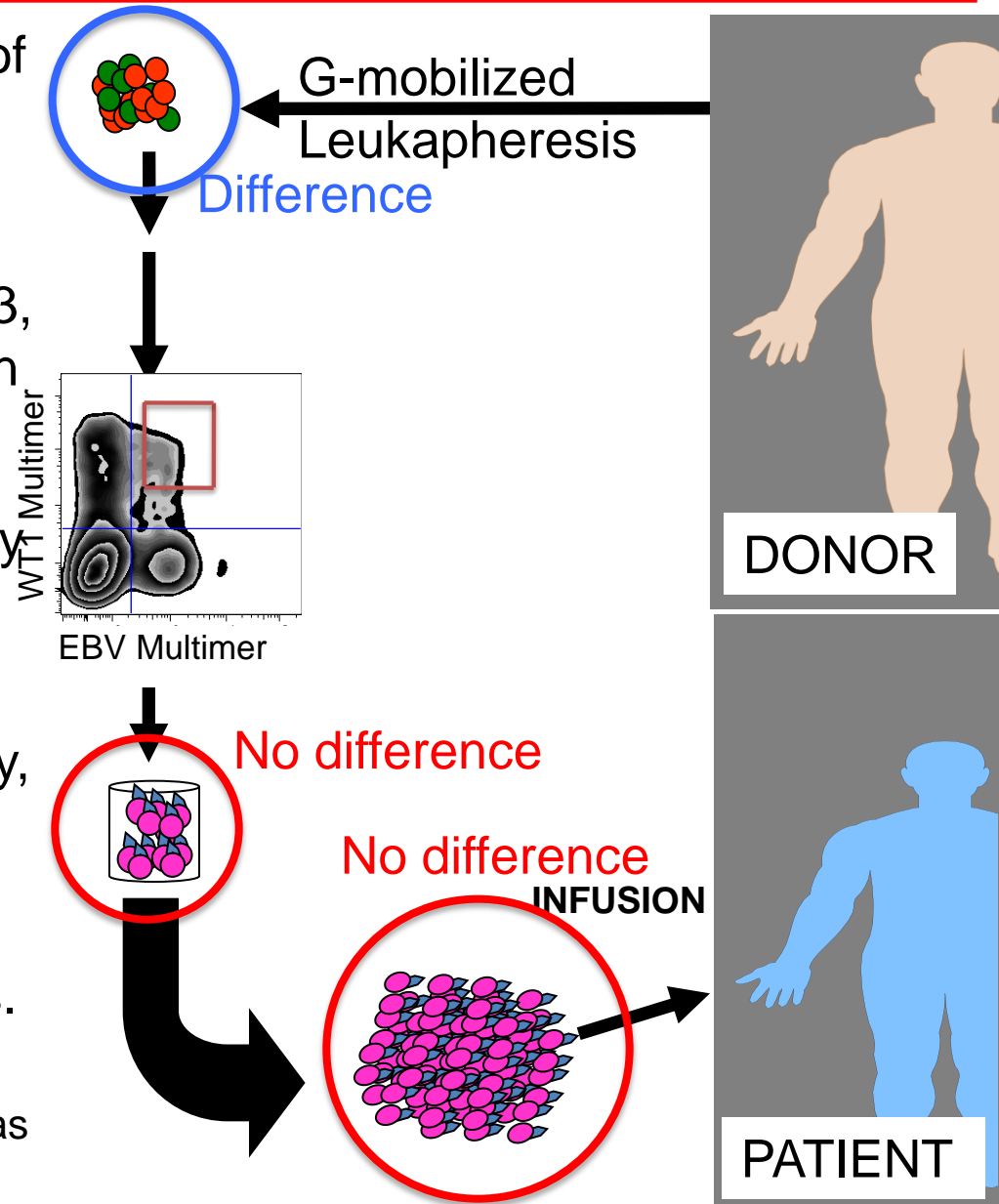
G-mobilized Leukapheresis

Difference

WT Multimer

EBV Multimer

No difference

No difference

INFUSION

DONOR

PATIENT

Why are EBV-specific CTL persisting longer with higher frequencies *in vivo* than CMV-specific CTL?

Donor Pt. 3: **CMV-specific substrate**

Gated on Tet.$^+$ CD8$^+$ T-cells
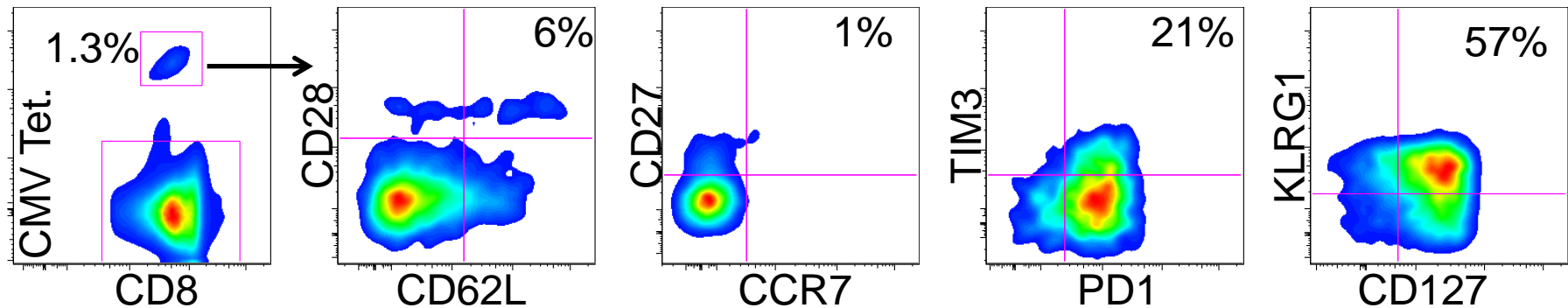
Donor Pt. 4: **EBV-specific substrate**

Gated on Tet.$^+$ CD8$^+$ T-cells
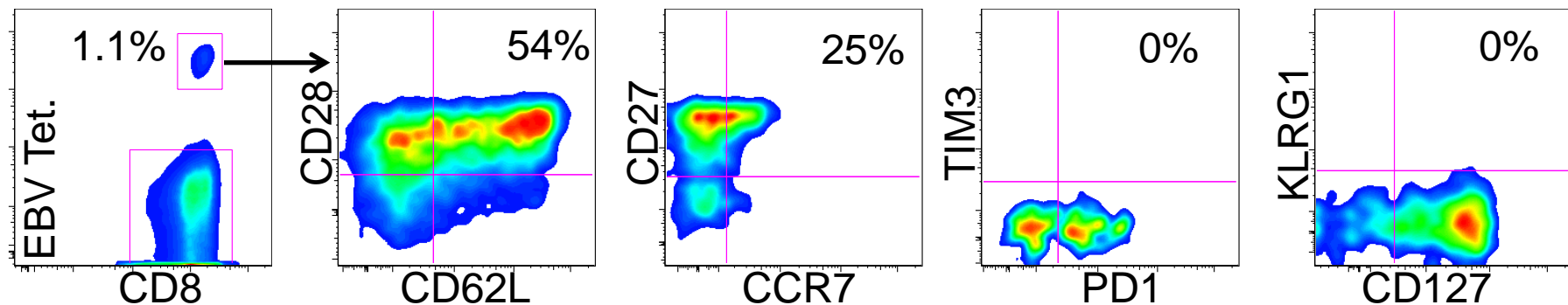
Differential expression of markers associated with memory on donor EBV and CMV-specific T-cells
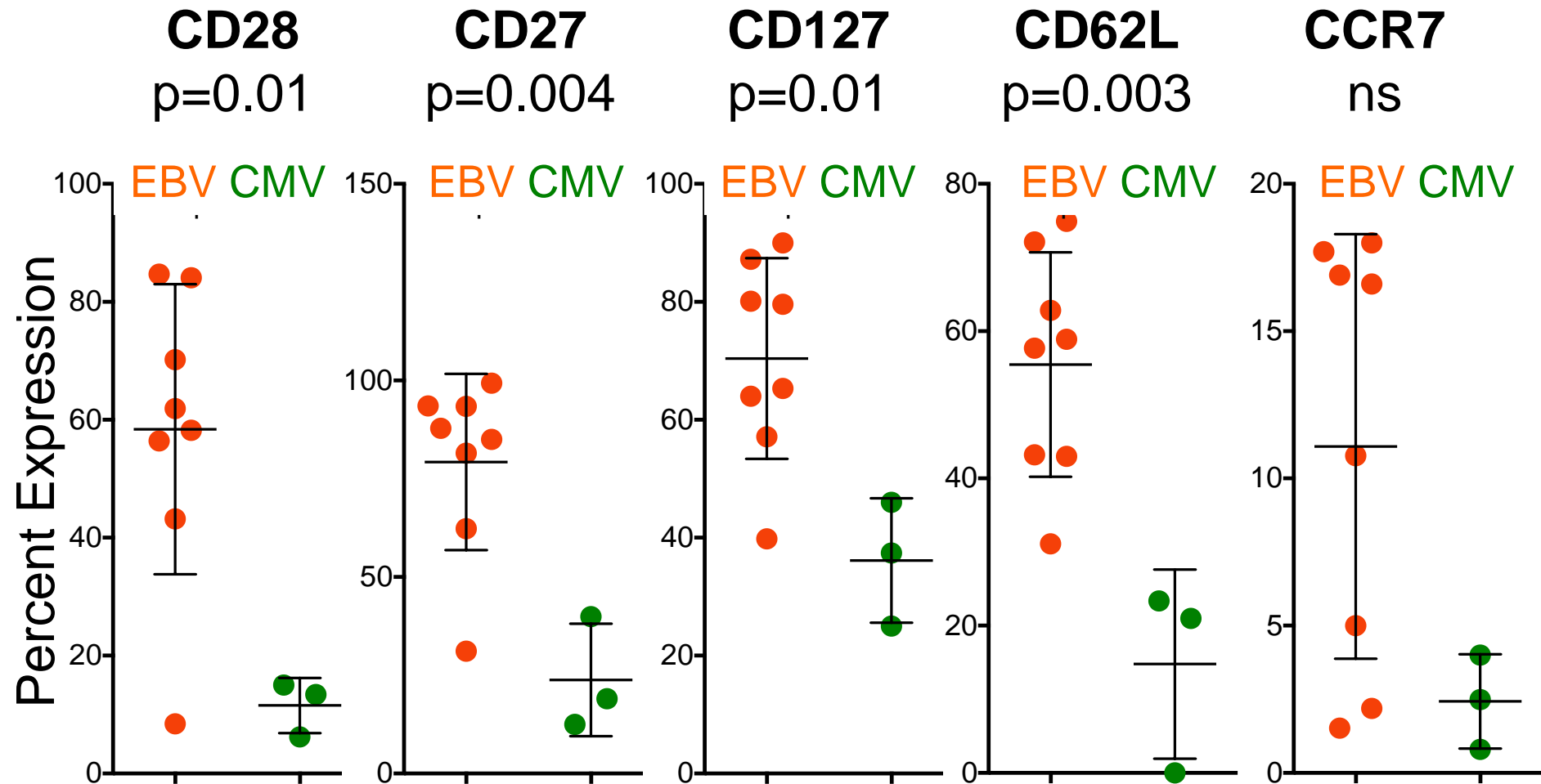
Differential expression of activation/exhaustion markers on donor EBV and CMV-specific T-cells
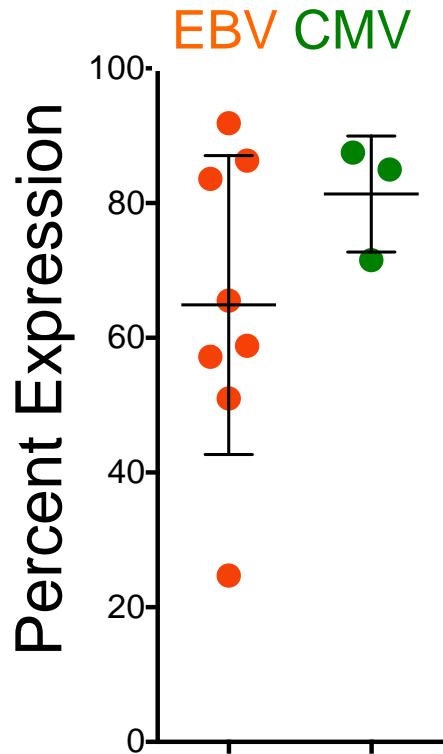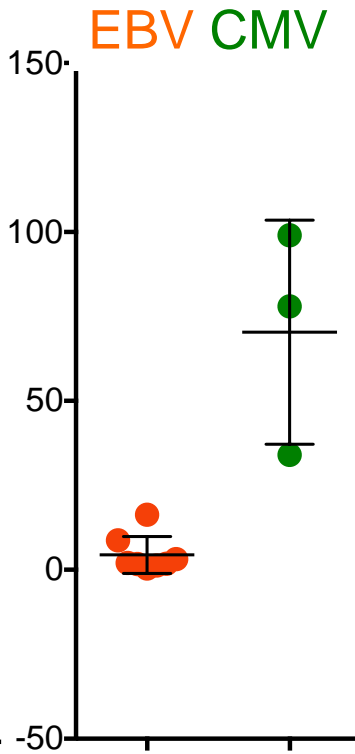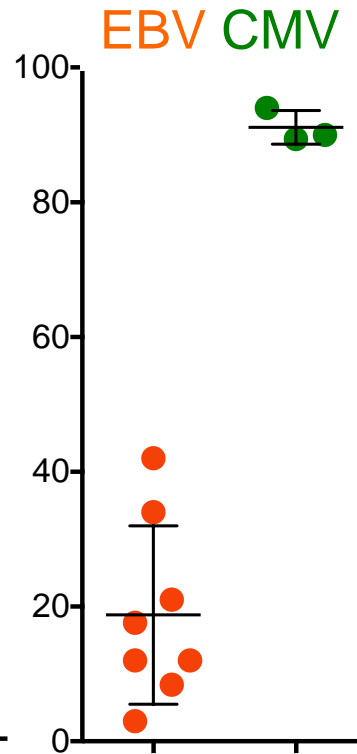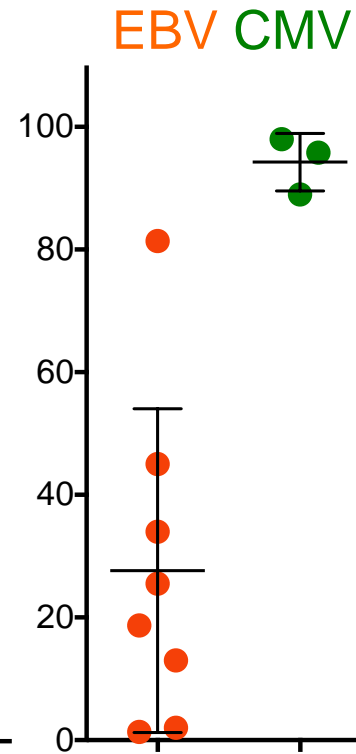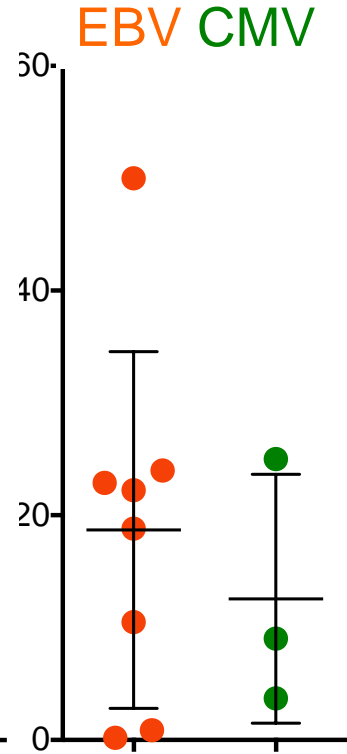
Phenotype of persisting WT1-specific T-cells ~12 weeks after transfer

Pt 8: Received EBV-specifc cells transduced with WT1

Day 84 (12 weeks) after the last infusion

Gated on Tet.⁺ CD8⁺ T-cells

9.4%   0%   0%   8%   57%

WT1 Tet. / CD8

CD28 / CD62L

CD27 / CCR7

TIM3 / PD1

KLRG1 / CD127
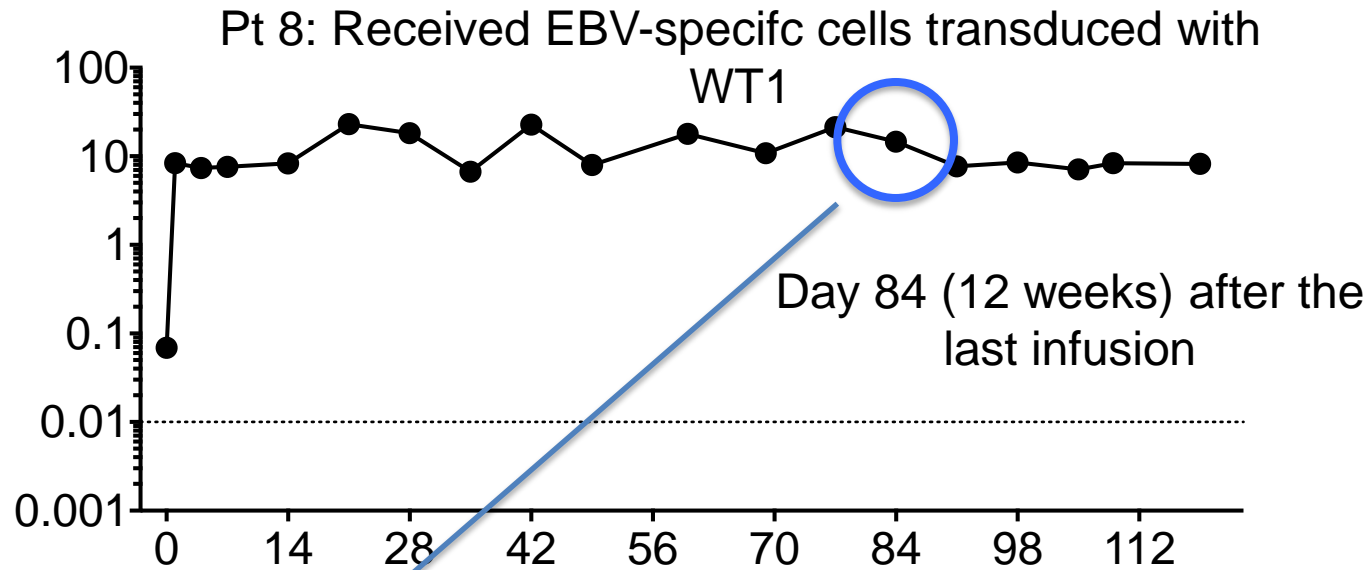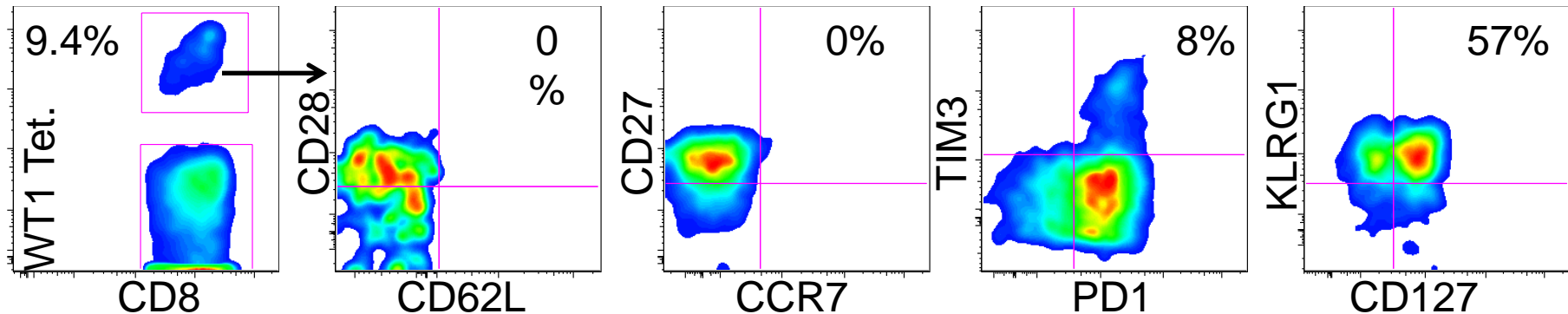
EBV-transduced WT1-specific T-cells ~12 weeks after transfer have a effector phenotype
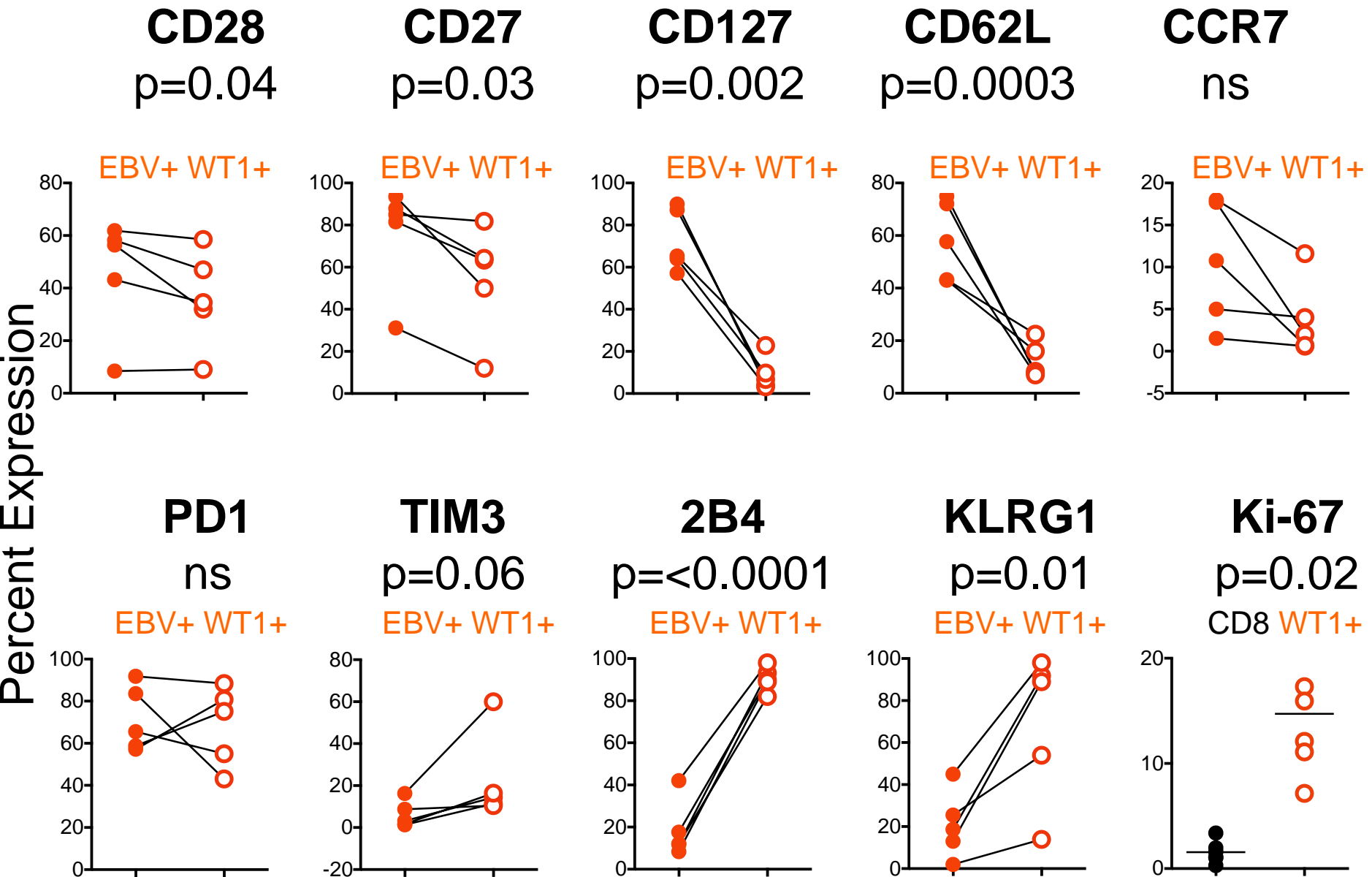
Summary and future directions

- Infusion of up to 10^{10} TCR$_{C4}$ transduced virus-specific cells +/- low-dose s.c. IL-2 is safe and not toxic to tissues expressing low physiologic levels of WT1.
- Transducing a population of substrate cells which contain cells in a resting state with a 'central memory' (T$_{CM}$) phenotype results in prolonged *in vivo* persistence after transfer.

Rationale for targeting Non Small Cell Lung Ca (NSCLC) with WT1-specific cells:

- NSCLC is potentially an immunologically targetable disease (Brahmer H, NEJM 2012; Topalian S, NEJM 2012).
- >96% of NSCLC express WT1 by RT-PCR (Oji K, 2002; Menssen 1995)
- Autologous setting allowing less constraint in the selection of the substrate cells.
- Prospective comparison between cells generated from the naïve pool compared to cells generated from long-lived memory.

Cytoplasmic expression of WT1 in Non Small Cell Lung Cancer

High-risk leukemia: 24% abnormal CD34+ cells in the bone marrow:

Anti-WT1 antibody 6F-H2 (Dako) 40X
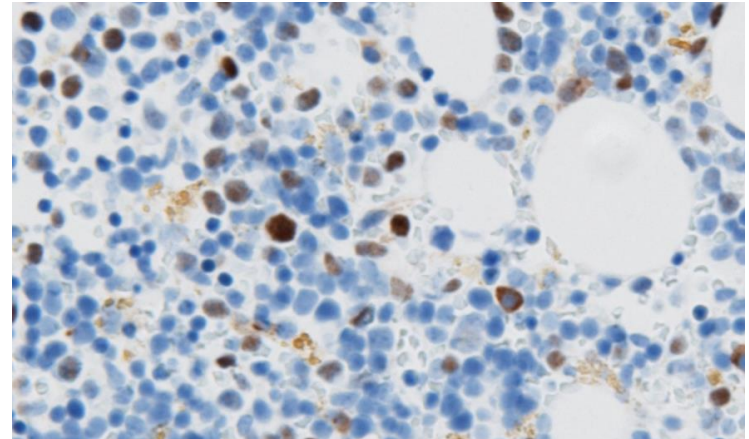
NSCLC:

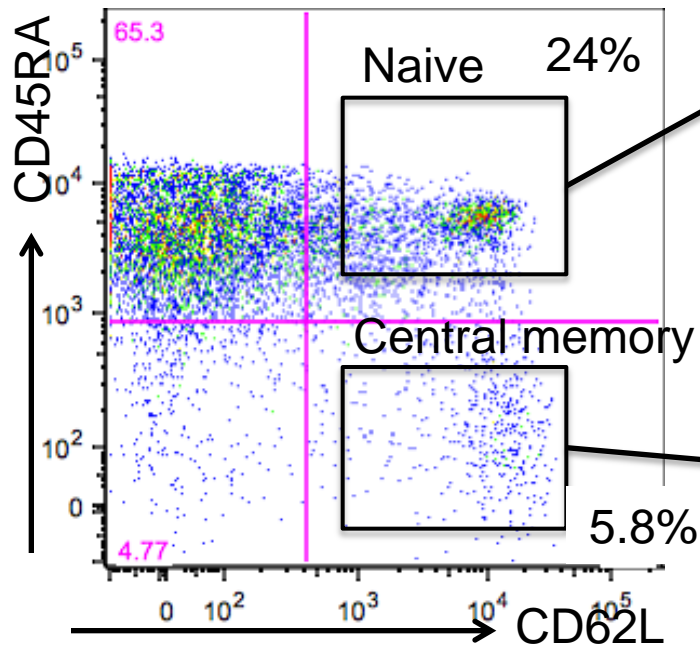
Anti-WT1 antibody 6F-H2 (Dako) 40X
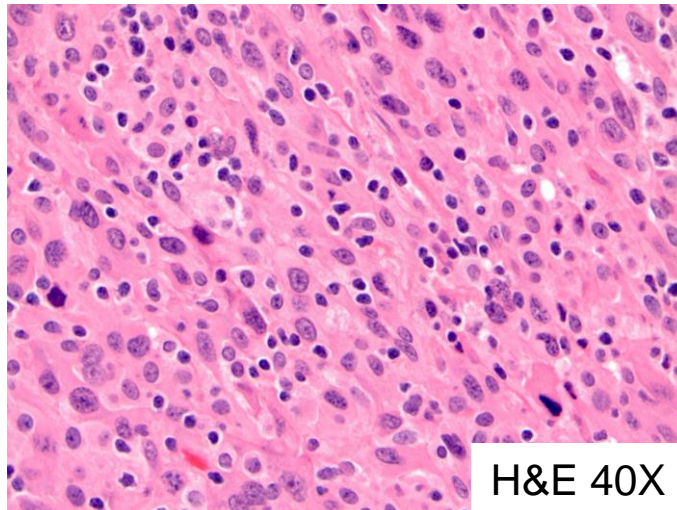
H&E 40X
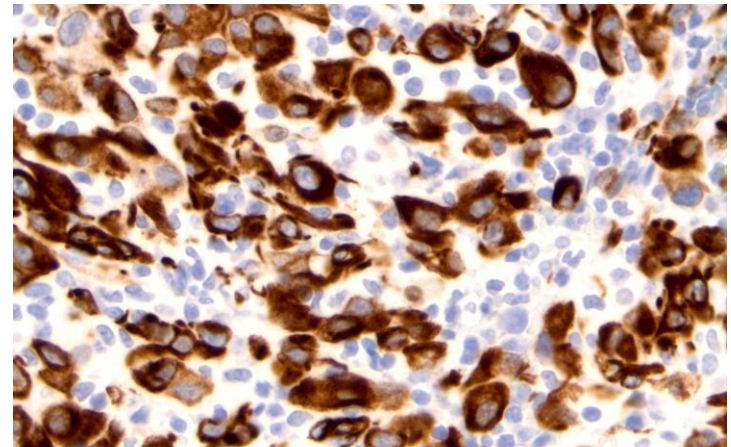
Anti-WT1 antibody 6F-H2 (Dako) 40X

Generation of polyclonal T_{CM}-TCR_{C4} and T_N-TCR_{C4}
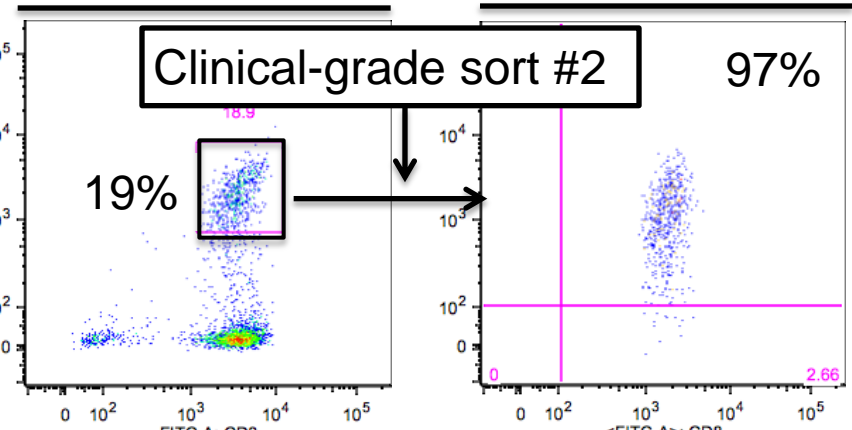
Non Small Cell Lung Patient 1:
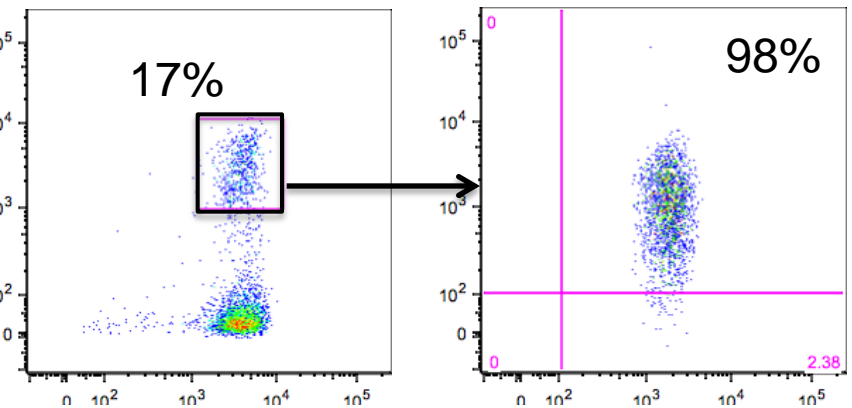
T_N and T_{CM} TCR_{C4}-transduced substrate cells lyse target cells with identical avidity
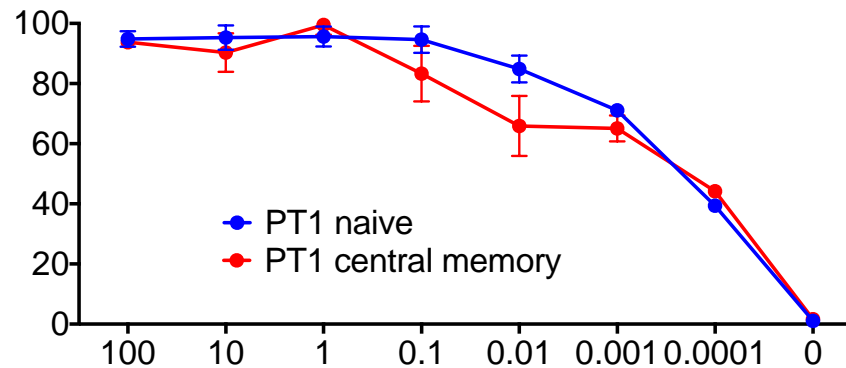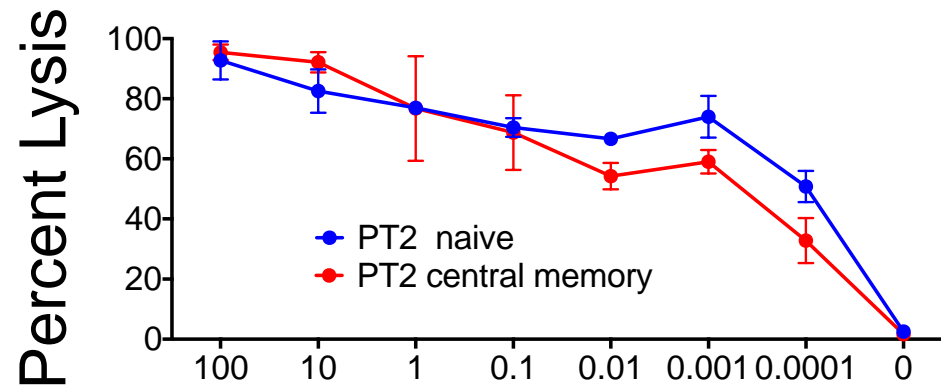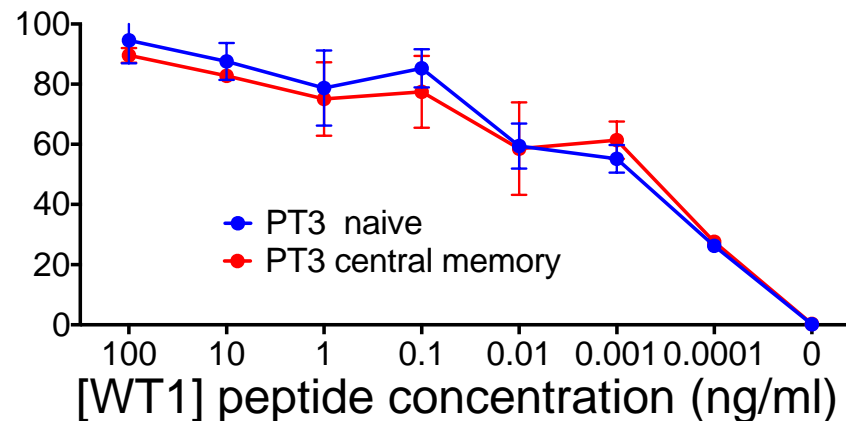
Infusion of TCR_{C4}-transduced T_N and T_{CM} in patients with NSCLC

Arm 1

Arm 2

Acknowledgements