

Phase I/II Study of Adoptive Immunotherapy  
after Allogeneic HCT with Virus-Specific  
CD8<sup>+</sup> T Cells that have been Transduced to  
Express a WT-1-specific T Cell Receptor for  
Patients with High-Risk or Relapsed AML,  
MDS or CML

RAC Review 09/12/12

# Overview

---

- Previous human experience with vaccines and WT1-specific CD8<sup>+</sup> T-cell clones.
- Selection of the high-affinity C4 TCR, vector construct and cell product generation.
  - Pre-clinical WT1 murine model (Dr T. Schmitt).
    - Clinical trial design (PI: Dr M. Bar).

# Background: 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<sup>+</sup> progenitor cells (Inoue K., *Blood* 1997).
- CD8<sup>+</sup> 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.

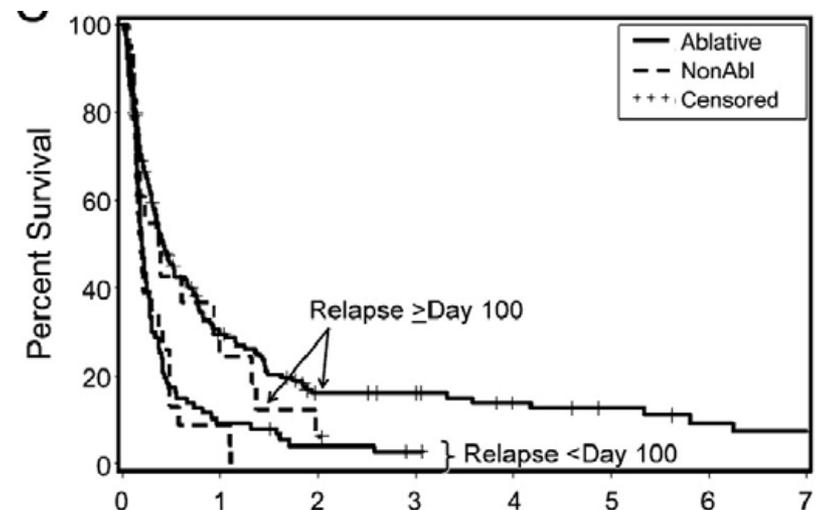
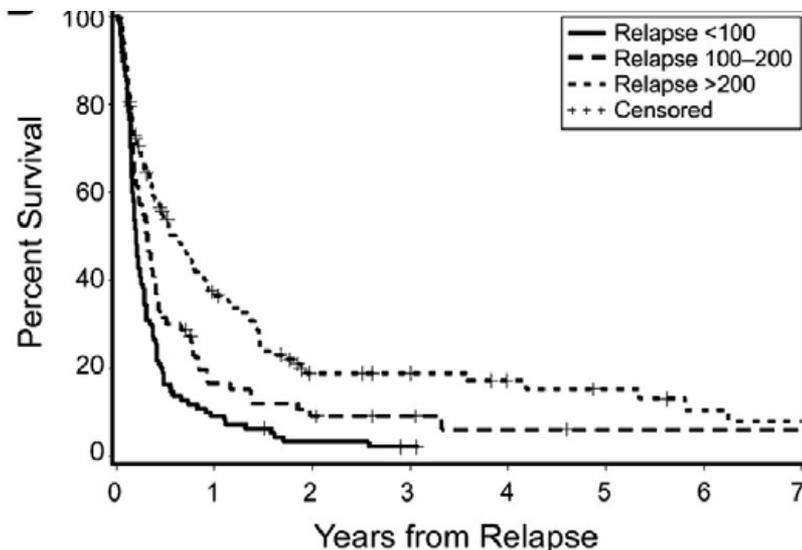
# Background: Prognosis of high-risk leukemias after HCT

---

- Definition of high-risk leukemias:
  - AML/ALL/MDS beyond 1<sup>st</sup> remission.
  - CML beyond chronic phase (CP).
  - Refractory disease at time of HCT.
  - AML with unfavorable cytogenetics (complex, normal with FLT3-ITD mutation)
  - MDS with IPSS score >1.5 and/or unfavorable cytogenetics (complex, Chromosome 7 abnormalities).
- For patients with high-risk leukemias, the probability of post-HCT relapse (>95% fatal) and/or death is >50% within 2 years (Gyurcokza B., JCO 2010; Deeg J., Blood 2002; Gratwohl A., BMT 1996; Radich J., Semin. Hematol. 2010)

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

- Recurrence or persistence of leukemia is the leading cause of death after HCT in patients with high-risk leukemias.
- 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.**



# Previous FHCRC clinical trial with WT1-specific T cells: Eligibility for enrollment and treatment

---

- **Objectives:**
  - Primary: Determine safety and potential toxicities of infusing CD8+ T-cells targeting WT1
  - Secondary #1: *in vivo* persistence, migration to bone marrow (site of leukemic relapse)
  - Secondary #2: evaluate if transferred cells mediate antileukemic activity.
- **Eligibility for enrollment:**
  - High-risk risk disease pre-transplant
  - HLA-A\*0201+
- **Eligibility for treatment:**
  - Relapse post transplant (Relapse group).
  - Patients entering HCT with high risk disease following recovery of hematopoiesis (Prophylactic group).
  - Receiving  $\leq 0.5$ mg/kg/day prednisone equivalents

# Previous clinical trial: Patient characteristics

Patient	Disease	Disease characteristics at HCT	Disease Characteristics at 1st CTL infusion
1	ALL	Chemorefractory, 12% BM blasts.	Detectable disease
2	AML	Hematopoietic aplasia after 2 inductions.	Relapse
6	ALL; BCR/ABL <sup>+</sup>	Molecular relapse BCR-ABL <sup>+</sup> by PCR.	Relapsed, CR induced by Imatinib
10	AML	CR	Relapse, received re-induction chemotherapy
15	AML	Chemorefractory, 3.4% BM blasts entering HCT.	Detectable disease
17	AML	2 <sup>nd</sup> CR.	Relapse, received re-induction chemotherapy
20	AML	3 <sup>rd</sup> relapse	Detectable disease
21	AML	Chemorefractory (4 inductions) 47% blasts	CR
24	AML	Chemorefractory (2 inductions)	Relapse, received re-induction chemotherapy
27	ALL; BCR/ABL <sup>-</sup>	2nd CR after failed 1 <sup>st</sup> HCT	MRD: Clonal B-cell population
28	AML	2nd CR	Relapse, received re-induction chemotherapy

# Previous clinical trial: Adoptive transfer of WT1-specific CTL has **minimal toxicity** and does not mediate injury to tissues expressing physiologic levels of WT1

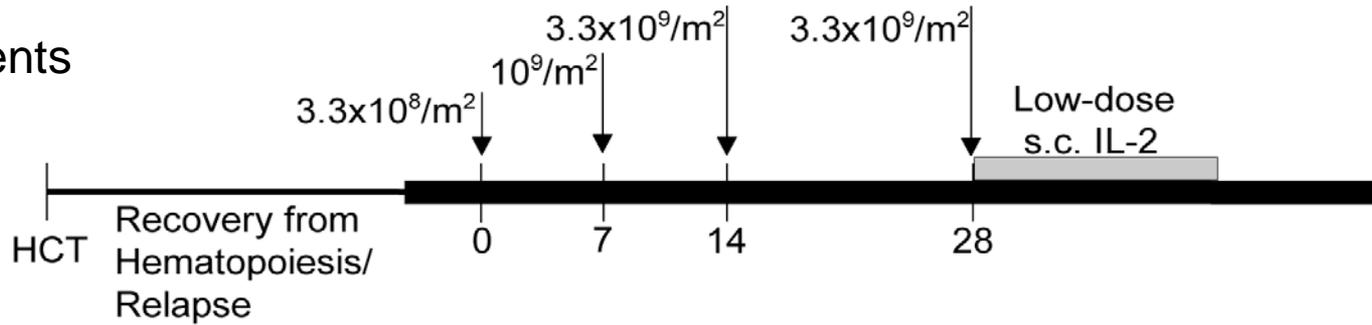
---

- Fevers  $\geq 38^{\circ}\text{C}$  +/- chills resolving within 24 hours: 25%
- Drop in lymphocyte counts <11 days: 77%
- IL-2 injection site reactions: 82%
- NCI CTC v.4.0 related adverse events  $\geq$  grade 3:

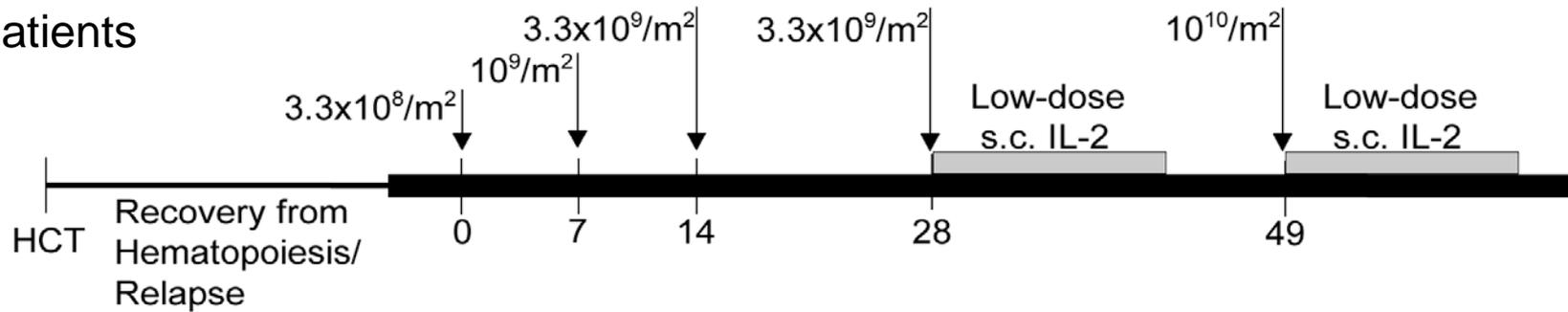
	Events
Total Grade $\geq 3$ Adverse Events	70
Unrelated to CTL infusion	51
Related to CTL infusion	19
Lymphopenia	16
Flu-Like Syndrome	2
Fatigue	1

# Previous clinical trial: Treatment plan

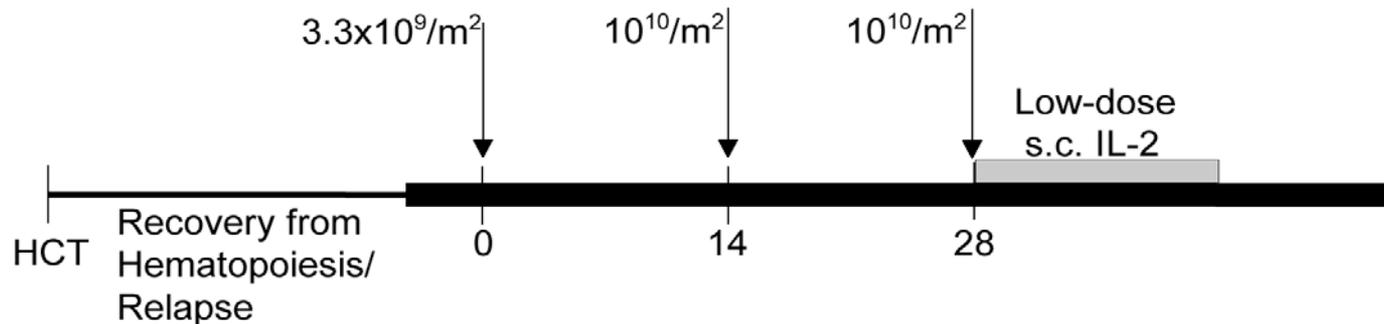
First 2 patients



Second 2 patients

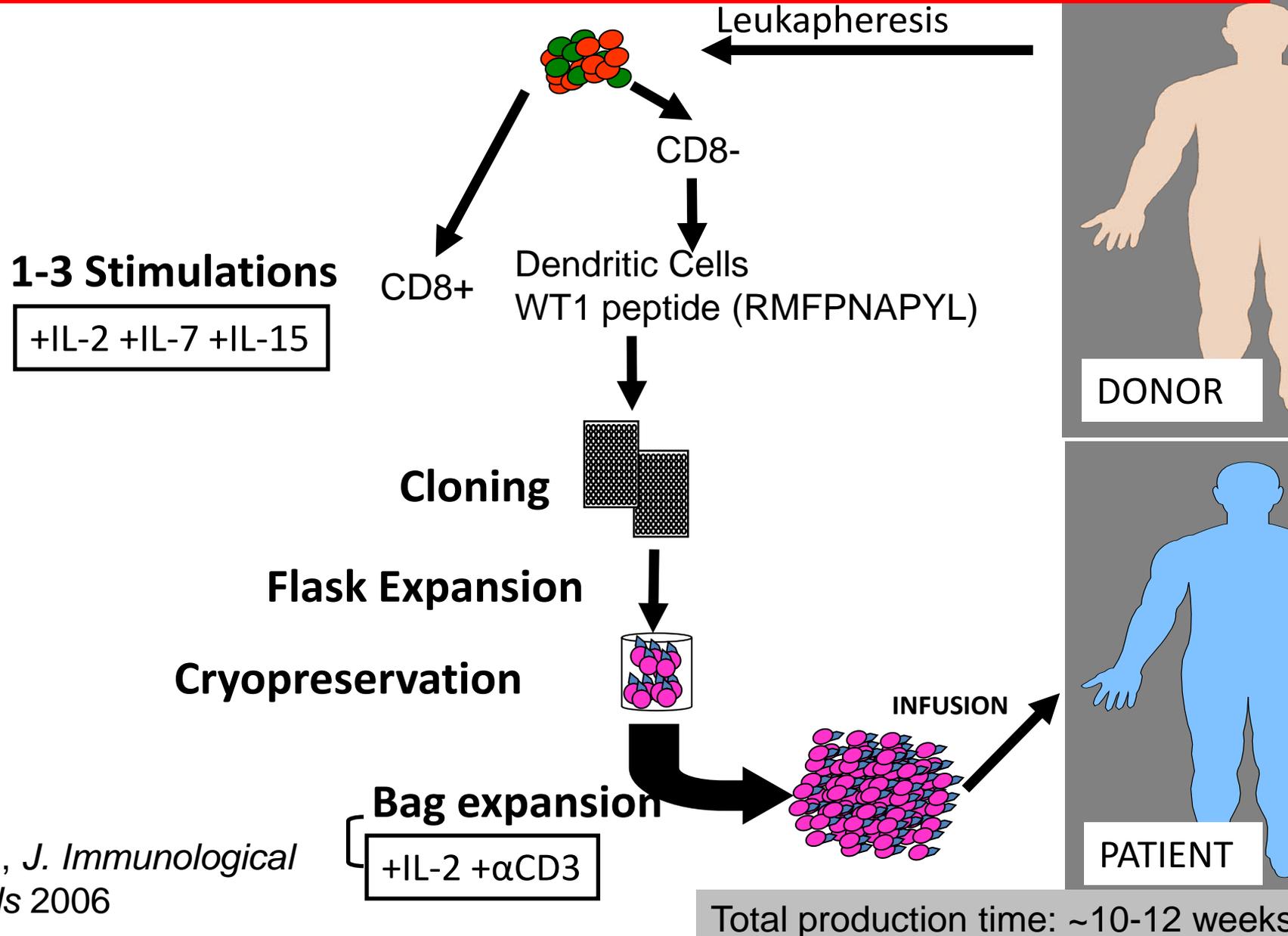


Last 7 patients



# Previous Clinical Trial:

## Generation of WT1-specific clones (first 7 treated pts)



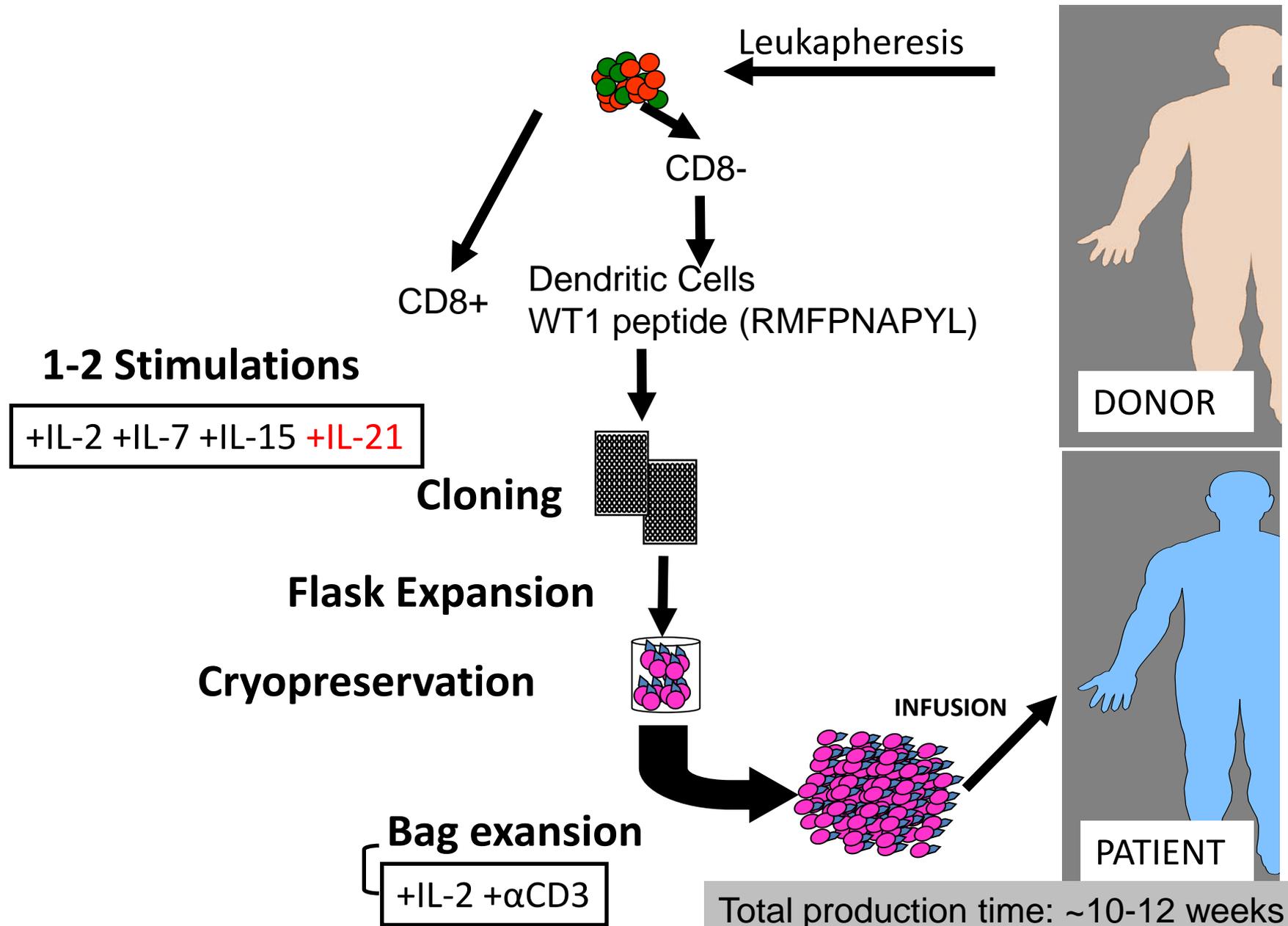
# Rationale for the use of IL-21 during *in vitro* stimulations

---

- Adoptive transfer of less terminally differentiated populations such as central memory CD8<sup>+</sup> populations has been shown to establish prolonged *in vivo* responses (Berger C., *JCI* 2008; Wherry J., *Nat. Immunol* 2003).
- IL-21 promotes expansion of responding T-cells *in vitro* that are less terminally differentiated (Li Y., *J Immunol* 2005).
- Increased *in vivo* persistence observed with murine CD8<sup>+</sup> T-cells derived from the naïve pool when the cells were primed in the presence of the  $\gamma_c$  cytokine IL-21 (Hinrichs C., *Blood* 2008).

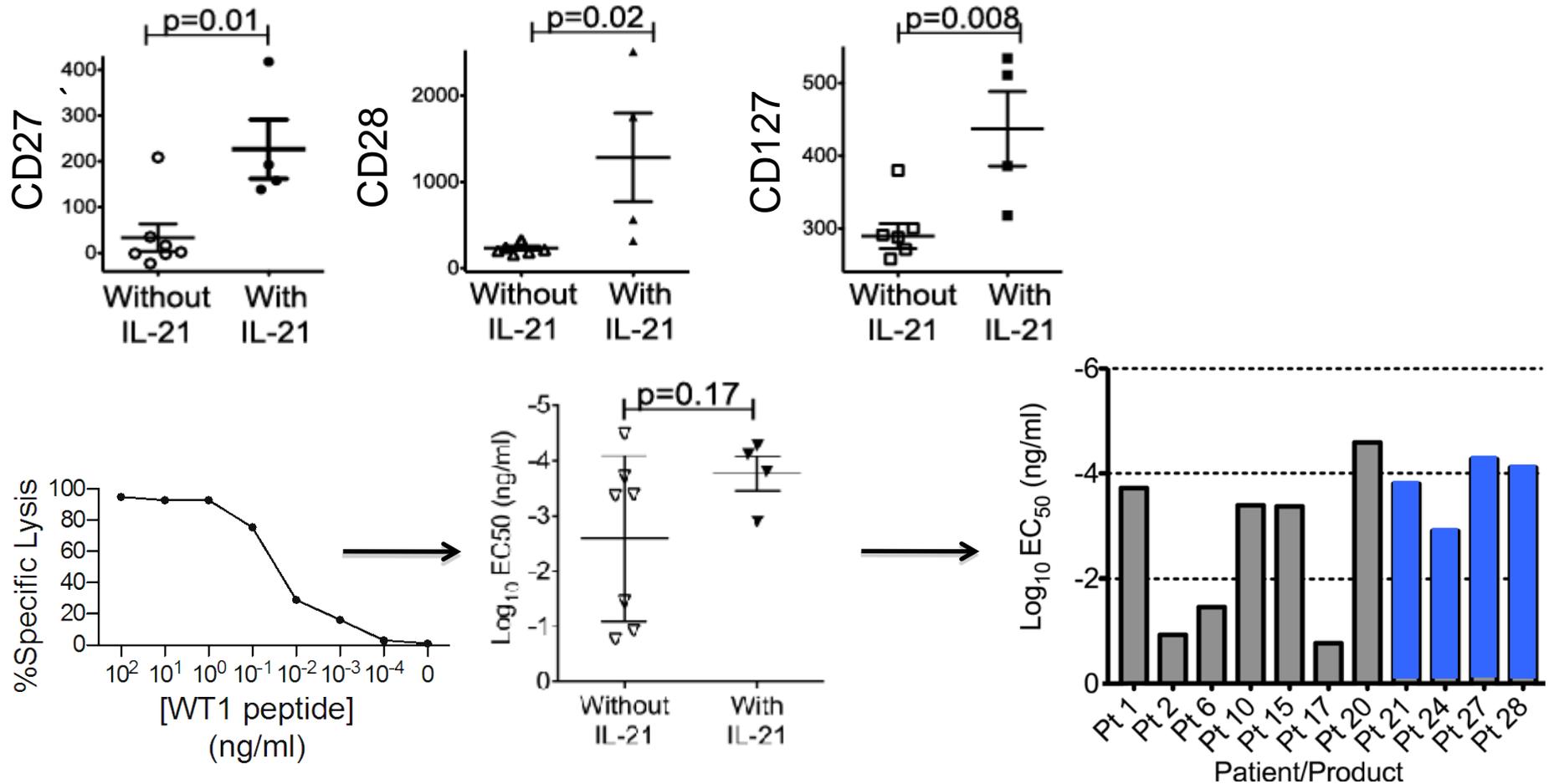
*Therefore, the goal was to develop culture conditions that program the generate T cells with central memory-like characteristics*

# Generation of WT1-specific clones (Last 4 treated pts)



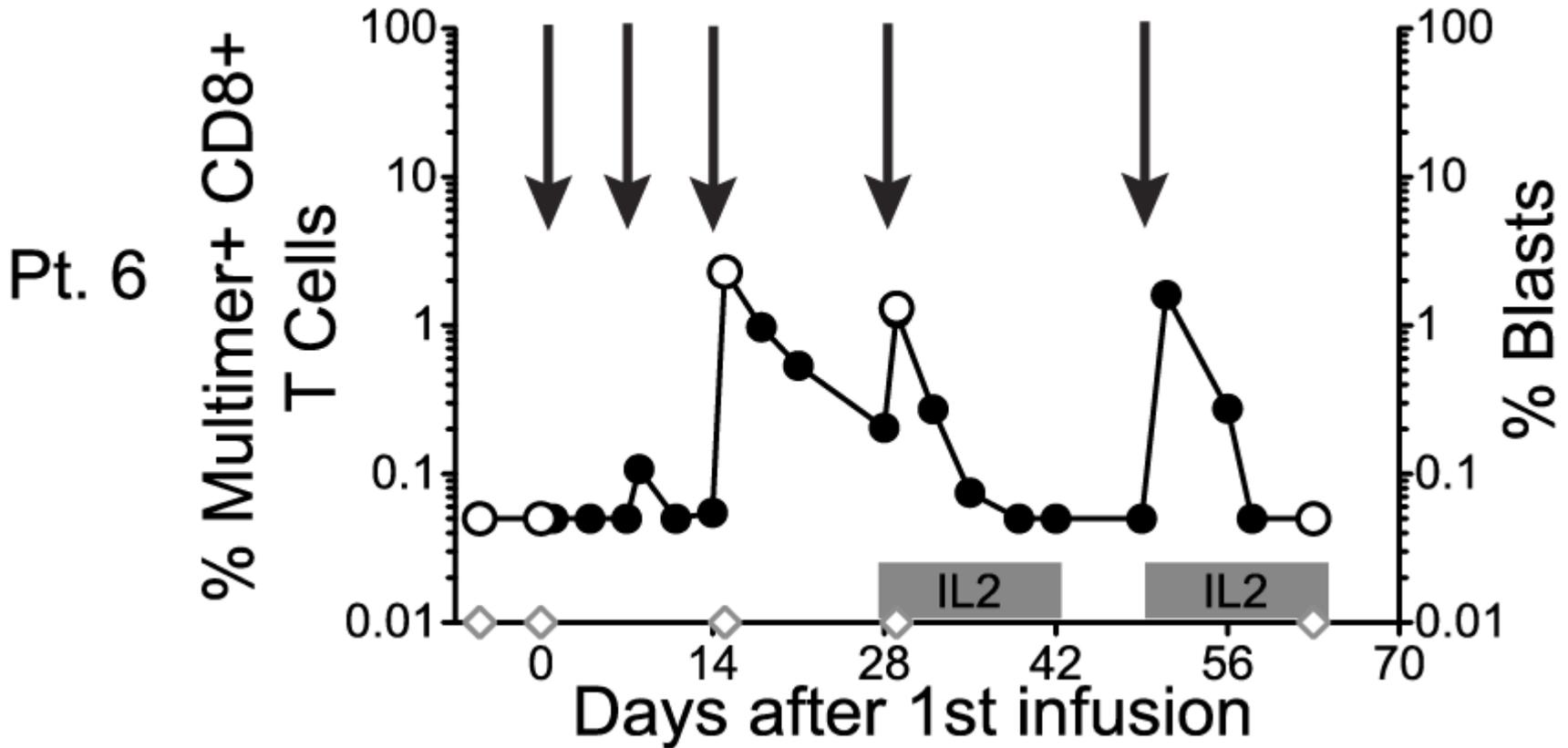
# Previous Clinical Trial: Phenotype and avidity of infused clones primed without or with IL-21

- WT1-specific CD8+ T-cell clones generated in the presence of IL-21 have higher fractions of cells expressing CD27, CD28 and CD127 (associated with central memory [Tcm] characteristics and long-term *in vivo* persistence).
- IL-21 had no significant effect on the avidity of the clones generated.



Previous Clinical Trial: Clones generated in the absence of IL-21 did not persist >14 days even in the absence of detectable disease

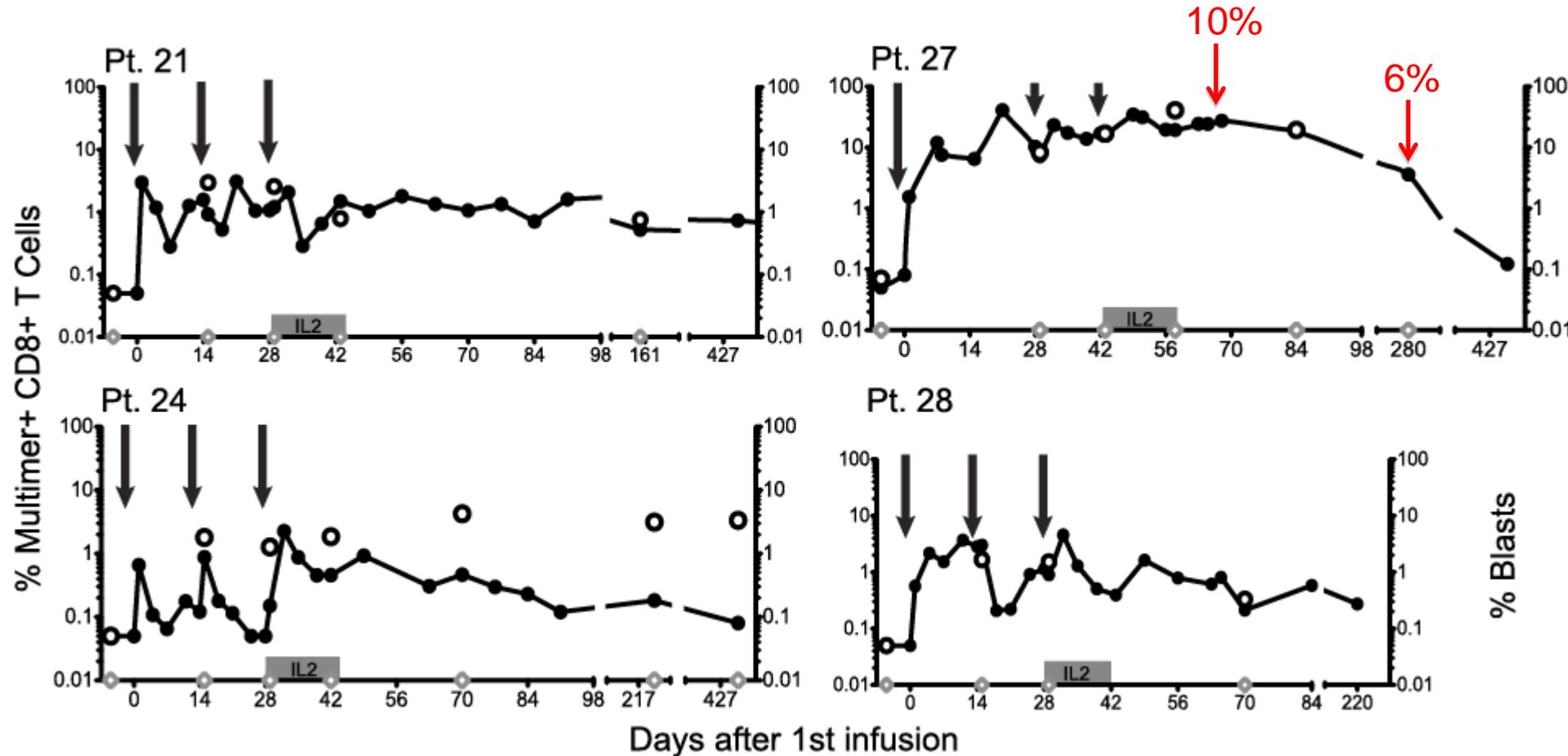
---



Legend

- %Multimer+ CD8+ T Cells in peripheral blood
- %Multimer+ CD8+ T Cells in bone marrow
- ◆ %Blasts in peripheral blood
- ◇ %Blasts in bone marrow

# Previous Clinical Trial: Clones generated with IL-21 persisted long-term in patients with MRD or no detectable disease



## Legend

● %Multimer+ CD8+ T Cells in peripheral blood

○ %Multimer+ CD8+ T Cells in bone marrow

◆ %Blasts in peripheral blood

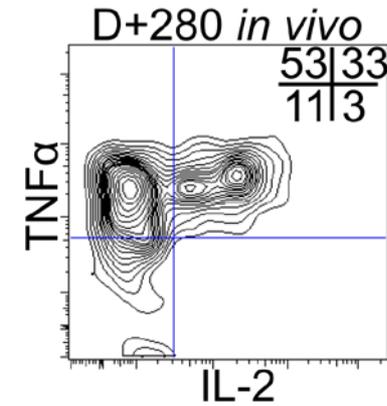
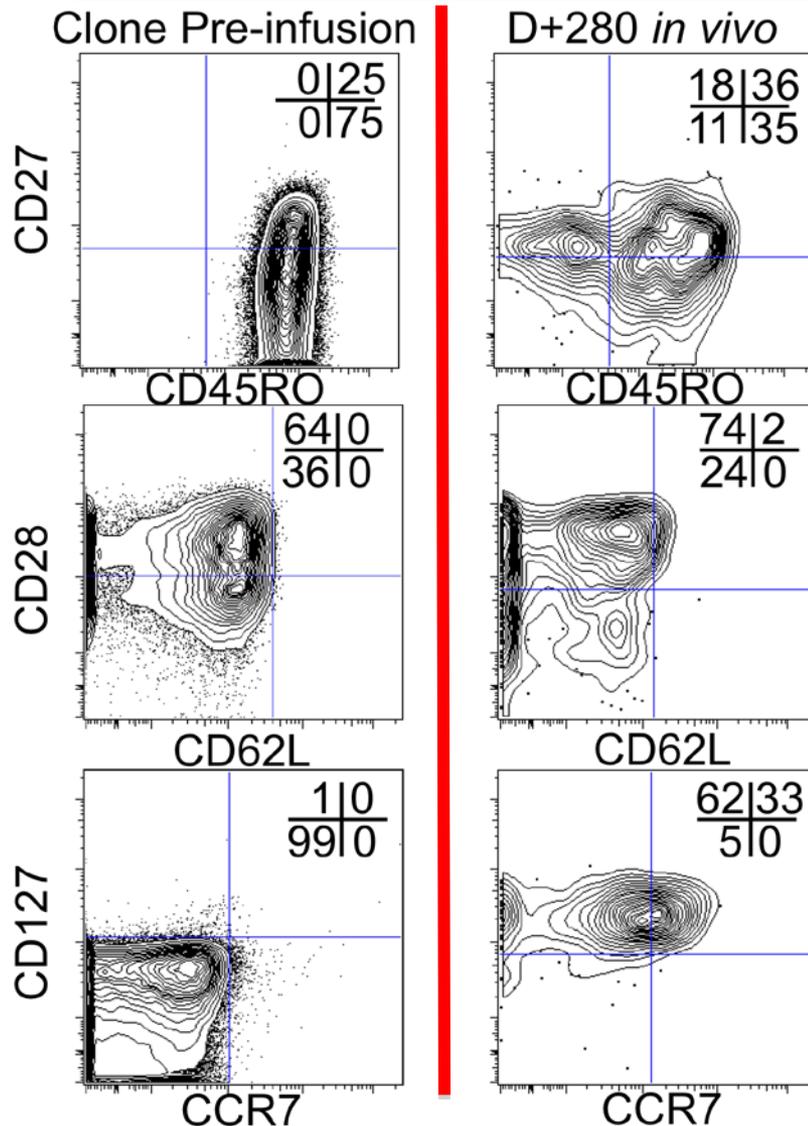
◇ %Blasts in bone marrow

# Previous Clinical Trial: Clones that persisted long-term demonstrated phenotypic and functional characteristics of Tcm

Pt 21

Gated on Multimer+ CD8+ T Cells

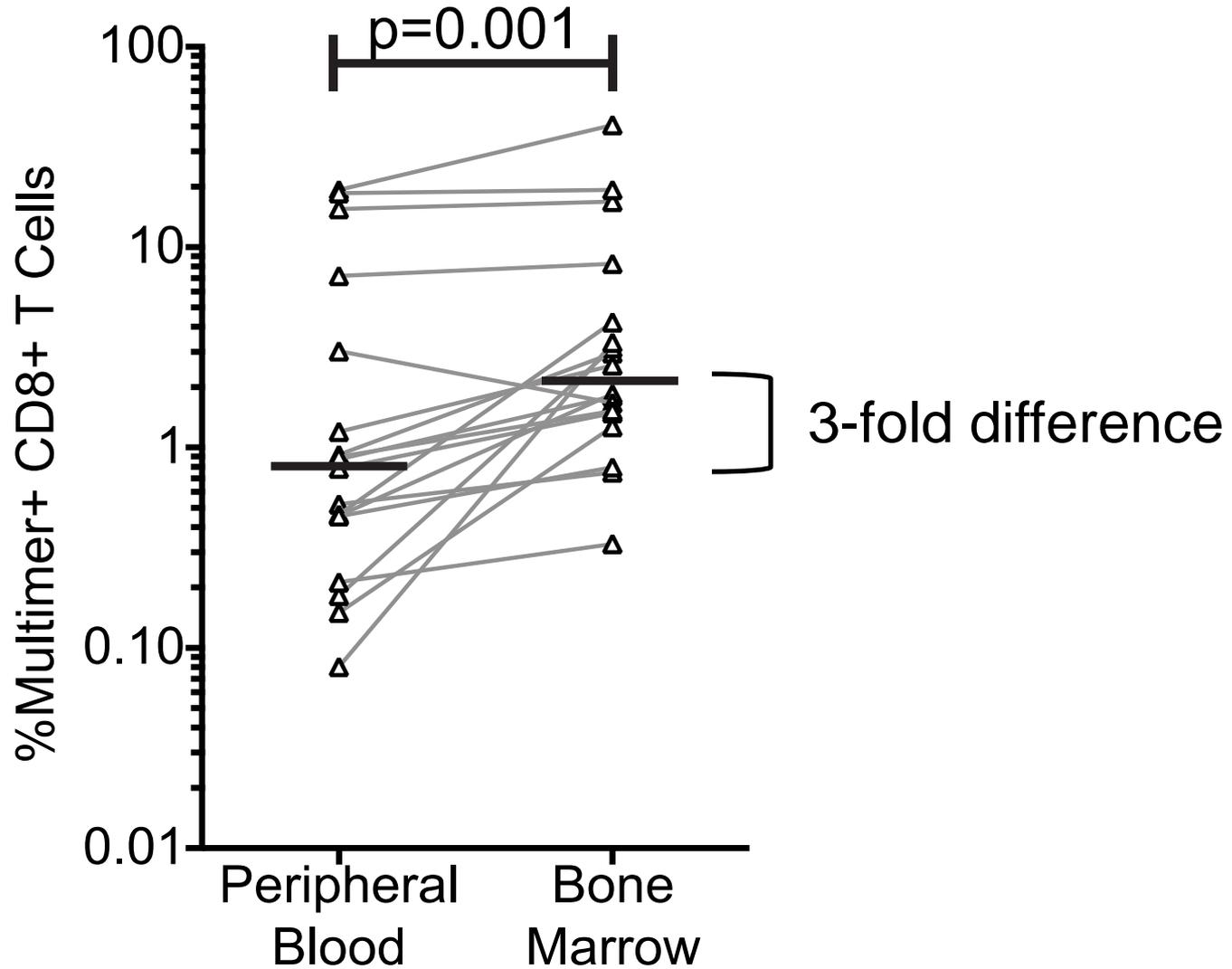
Gated on IFN $\gamma$ + CD8+ T Cells



- Transferred cells acquired/upregulated CD28, CD127, CD62L and CCR7
- Persistent transferred cells were polyfunctional *in vivo*

# Previous Clinical Trial: Clones that persisted long-term preferentially accumulated in the bone marrow

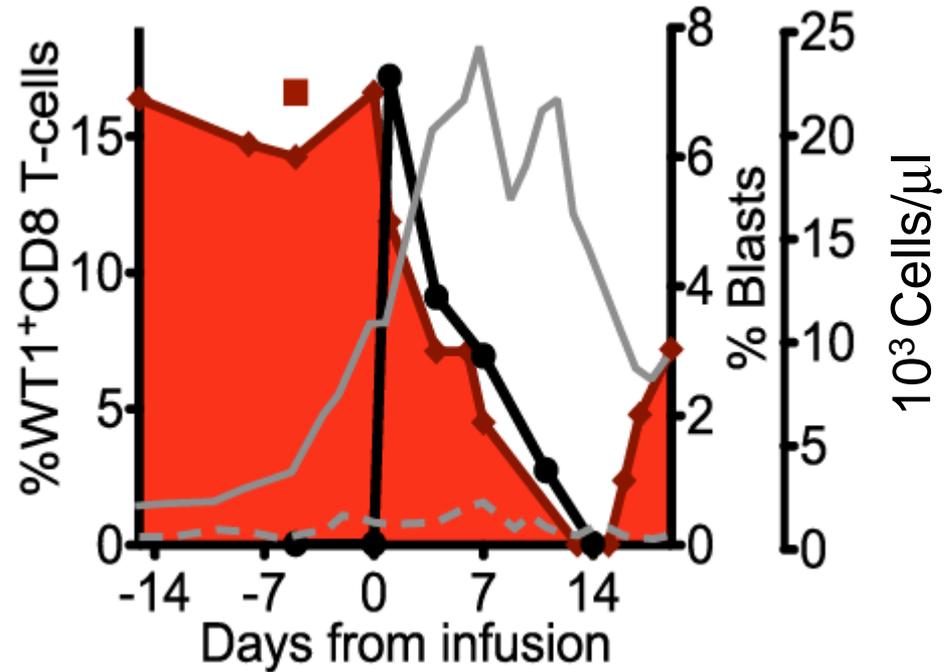
---



# Previous Clinical Trial: Evidence of anti-leukemic activity

---

## Patient 15



# Previous clinical trial: **Direct** and inferred evidence (Disease-Free Survival) of anti-leukemic activity

Patient	Disease	Survival after HCT*	Probability of disease free survival 1 and 2 years post HCT based on patient's prognosis
27	ALL	22 months	<u>2nd transplant</u> : 20% at 1 year, 15% at 2 years (Radich J., JCO 1993). <u>Relapse &lt;100 days after HCT</u> : <10% at 1 year, <3% at 2 years (Mielcarek M., BBMT 2007).
21	AML	35 months	<u>40% blasts entering HCT</u> : <5% at 1 year (Kebriaei P., BMT 2005).
24	AML	33 months	<u>Relapse &lt;100 days after HCT</u> : <10% at 1 year, <3% at 2 years (Mielcarek M., BBMT 2007).
28	AML	38 months	<u>Relapse 100-200 days after HCT</u> : <15% at 1 year, <9% at 2 years (Mielcarek M., BBMT 2007).

\*As of 08/15/2012

# Conclusions/ Rationale for targeting WT1 with a TCR of high affinity

---

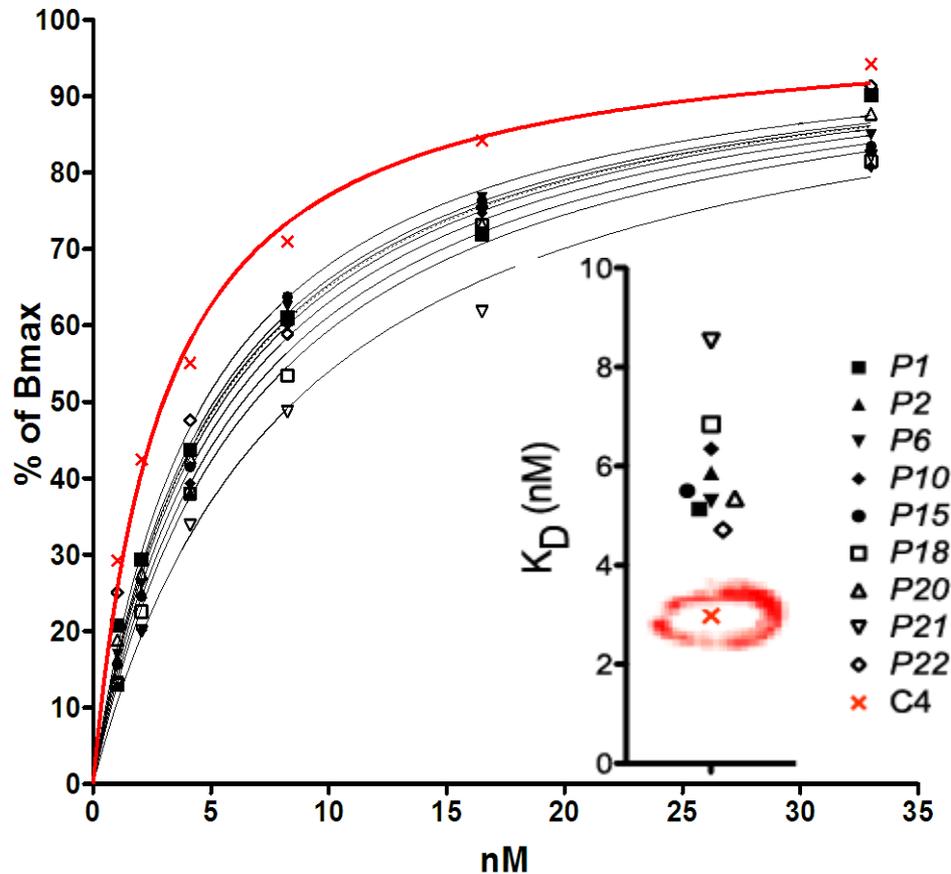
- Adoptive transfer of CTL clones targeting WT1 at doses  $\leq 10^{10}$  cells/m<sup>2</sup> +/- low-dose s.c IL-2 was **safe** with evidence of anti-leukemic activity.
- Priming of WT1-specific cells in the presence of IL-21 conferred cells characteristics associated with long-term memory *in vivo*.
- 4 patients with MRD or no detectable leukemia at time of infusions exhibited long-term T-cell persistence in the blood and bone marrow without toxicity.
- 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<sup>+</sup> target cells**.

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

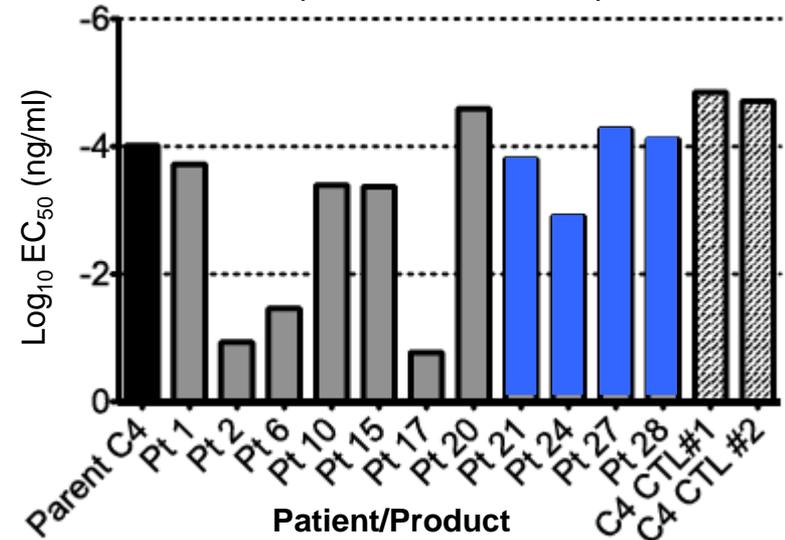
# TCR Selection: Relative avidities of T cells expressing the selected C4 TCR compared to clones administered to patients

WT1 tetramer titration  
(Normalized to Bmax)

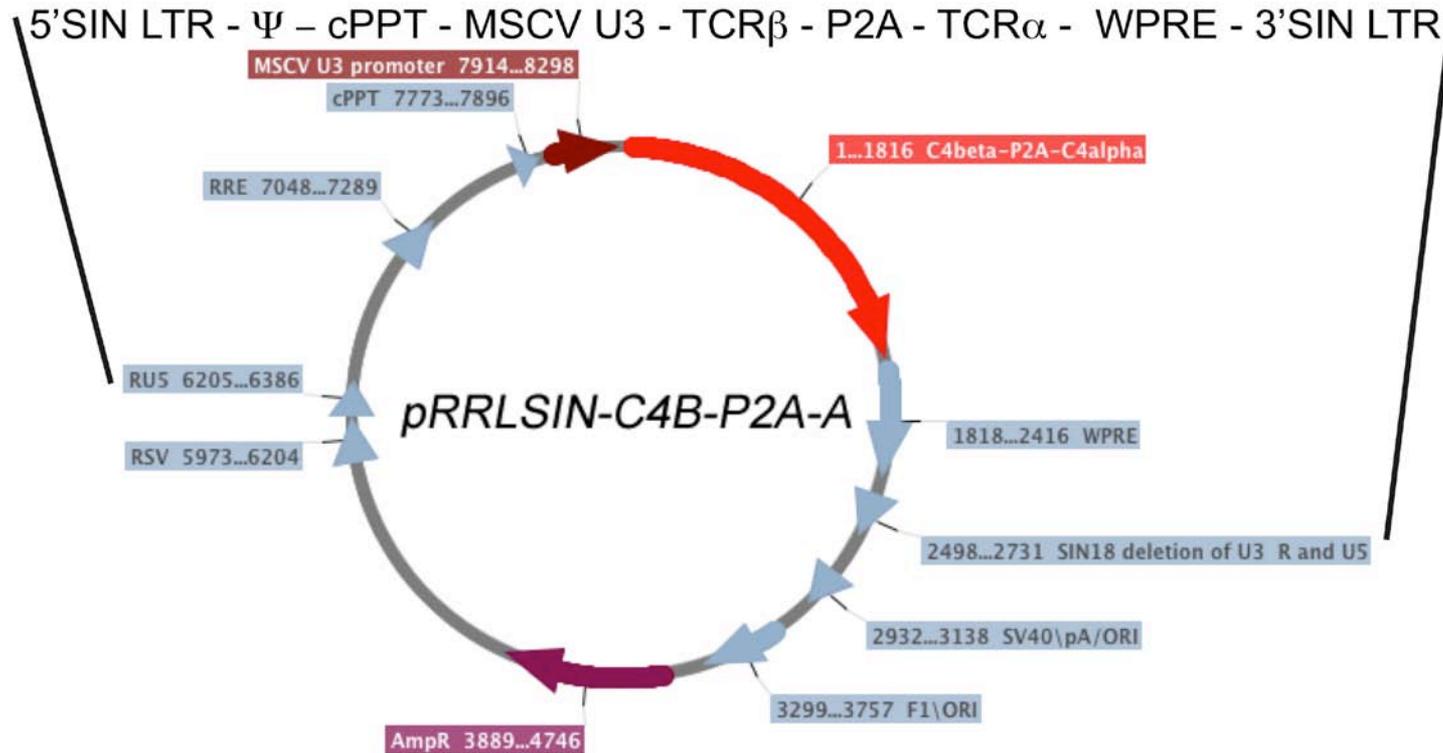
(The  $K_D$  of C4 is 2.98 nM)



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



# ISSUE: Lentiviral SIN vector with strong internal promoter



SIN LTR = Self-inactivating long terminal repeat

Ψ = Packaging signal

cPPT = Central polypurine tract

MSCV = MSCV U3 promoter

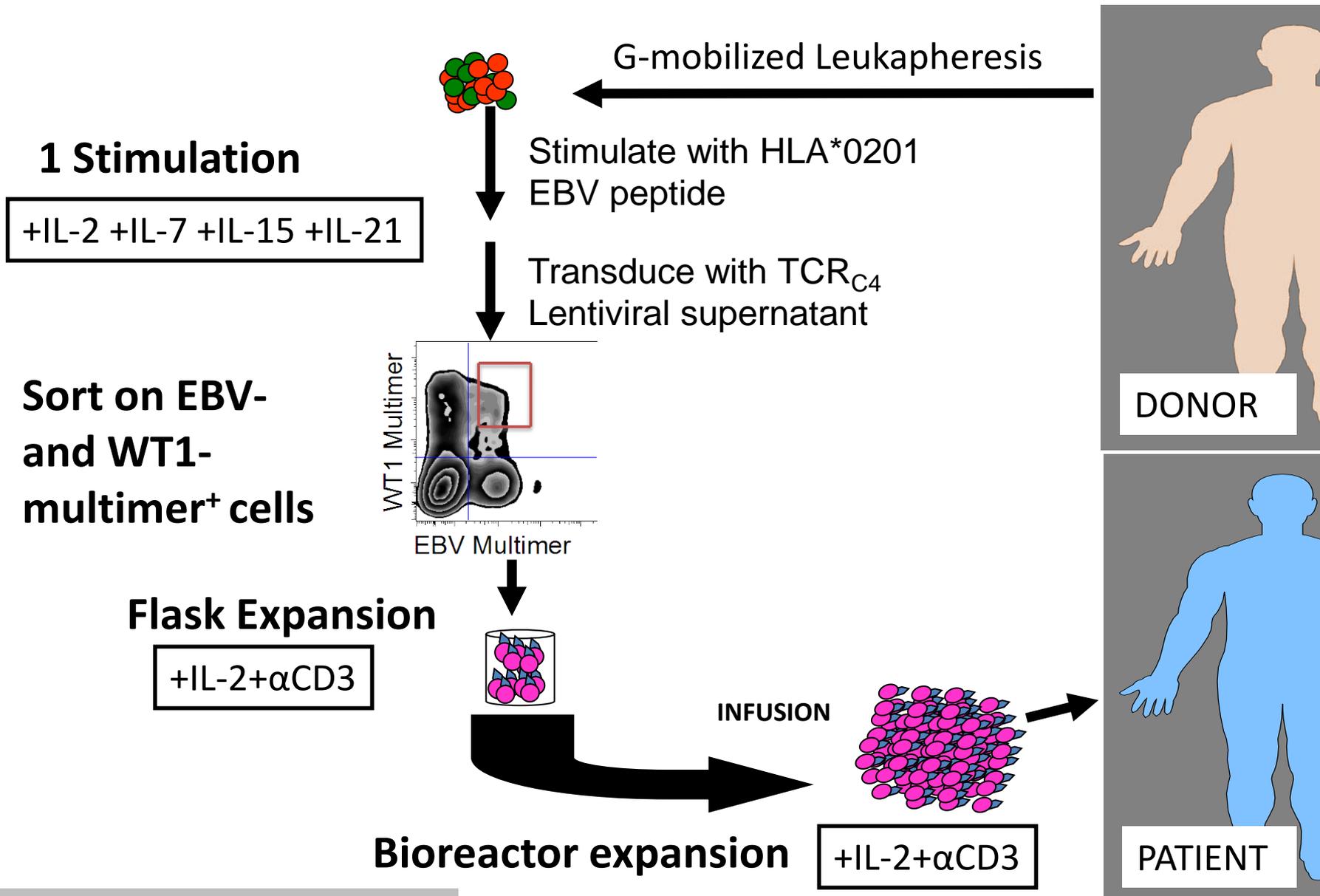
TCRβ = Beta chain of the WT1-specific TCR

P2A= 2A element from the porcine teschovirus

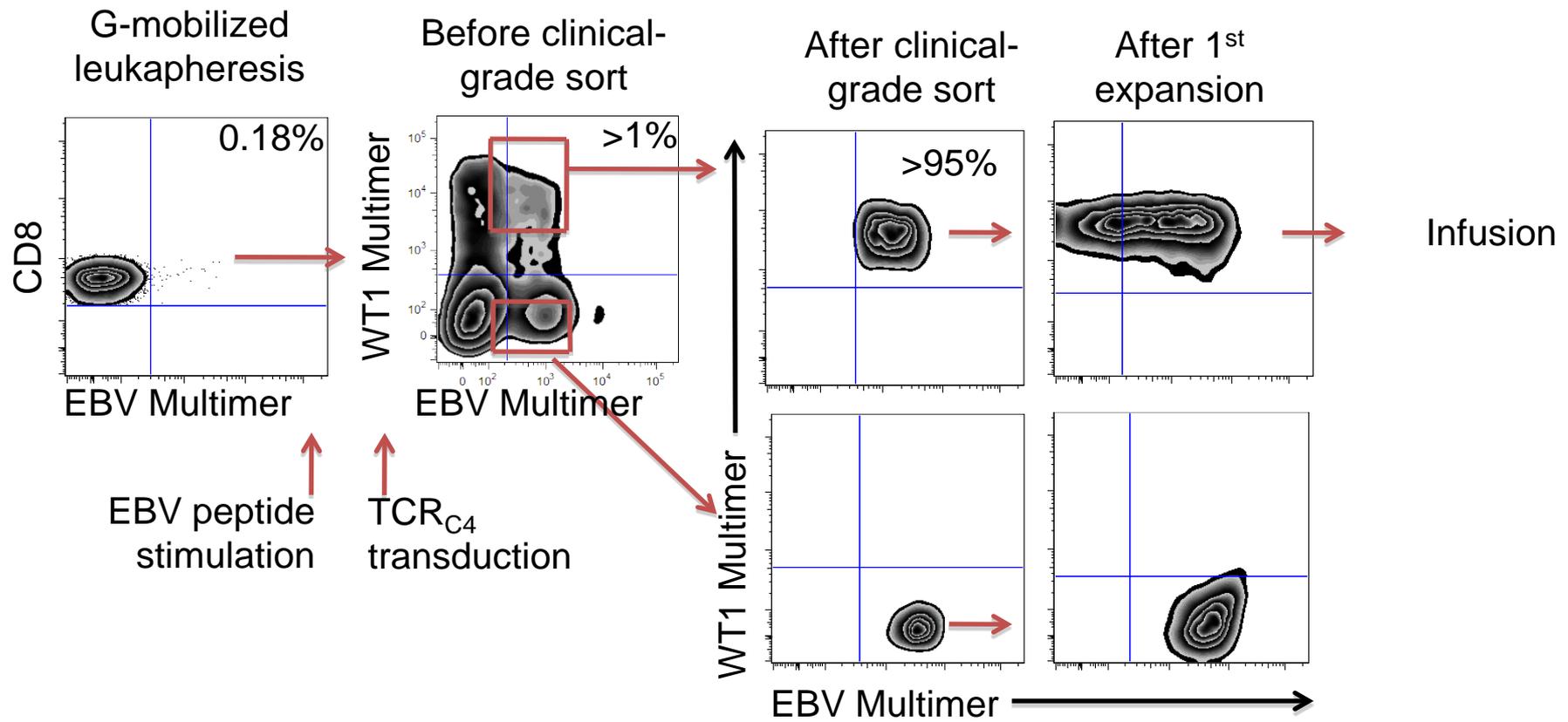
TCRα = Alpha chain of the WT1-specific TCR

WPRE = Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element

# Generation of TCR<sub>C4</sub>-transduced products for infusion



# ISSUE: Generation/release criteria of TCR<sub>C4</sub>-transduced products



**Release criteria:**

Stage I (after sort)  
 Sterility  
 EBV/WT1 > 95%

Stage II (6-10 1<sup>st</sup> expansion)  
 Sterility  
 WT1 > 60%  
 WPRE < 5 copies/cell  
 VSV-G < 10 copies/50µg DNA

Stage III (Infusion)  
 Sterility  
 report EBV/WT1  
 Culture-based RCL

## **ISSUE:** Assessing safety targeting WT1 in pre-clinical murine model

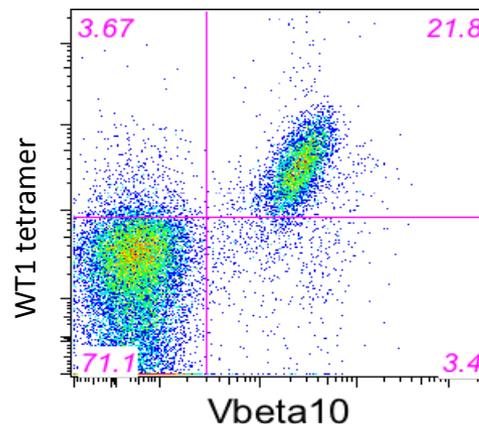
---

- Stauss has already demonstrated that TCRs selected for higher affinity than present in normal repertoires do not recognize or prevent engraftment in NOD/SCID mice of normal CD34<sup>+</sup> HSC that express physiologic levels of WT1 (Gao L., *Blood* 2000; Xue S. *Blood* 2005).
- Since T cells with higher affinity TCRs should more efficiently kill leukemic targets expressing lower levels of antigen, we questioned how much increase in affinity could be attained before recognition of normal targets.
- We generated enhanced affinity TCRs by *in vitro* directed evolution strategies, and evaluated the *in vivo* biology of T cells expressing such TCRS.

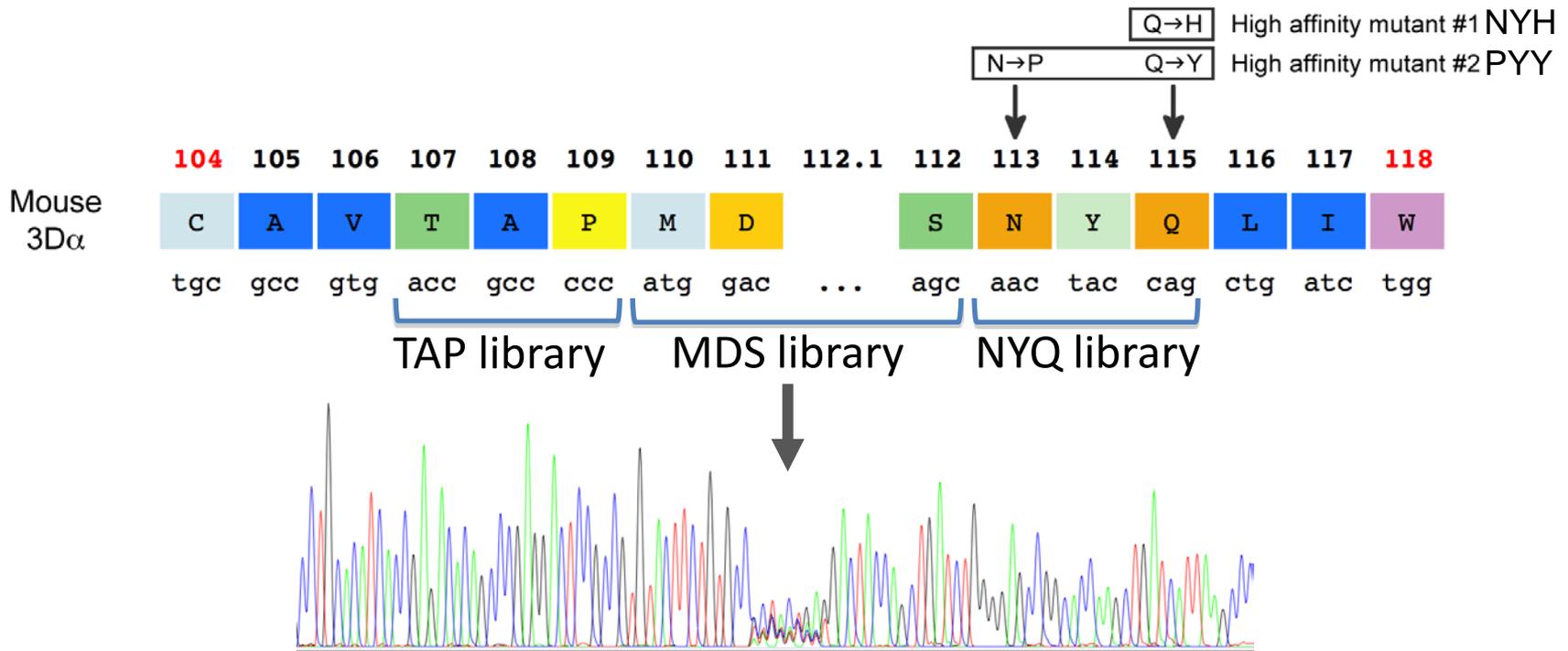
# *ISSUE:* Pre-clinical murine model to assess safety

- Expression of WT1 is similar in mice and humans during fetal development and in adult tissues.
- Mice and humans both recognize the same immunodominant RMFPNAPYL epitope from WT1
  - B6 mice: restricted by H-2D<sup>b</sup>
  - Humans: restricted by HLA A\*0201
- Similar to our human C4 TCR, the high affinity murine 3D TCR was isolated by screening naturally elicited murine H-2D<sup>b</sup>-restricted WT1-specific CD8<sup>+</sup> T-cell clones for relative affinity.

3D TCR



# Pre-clinical WT1 murine model: Generation of high affinity murine WT1-specific TCRs by Directed Saturation Mutagenesis of CDR3 $\alpha$

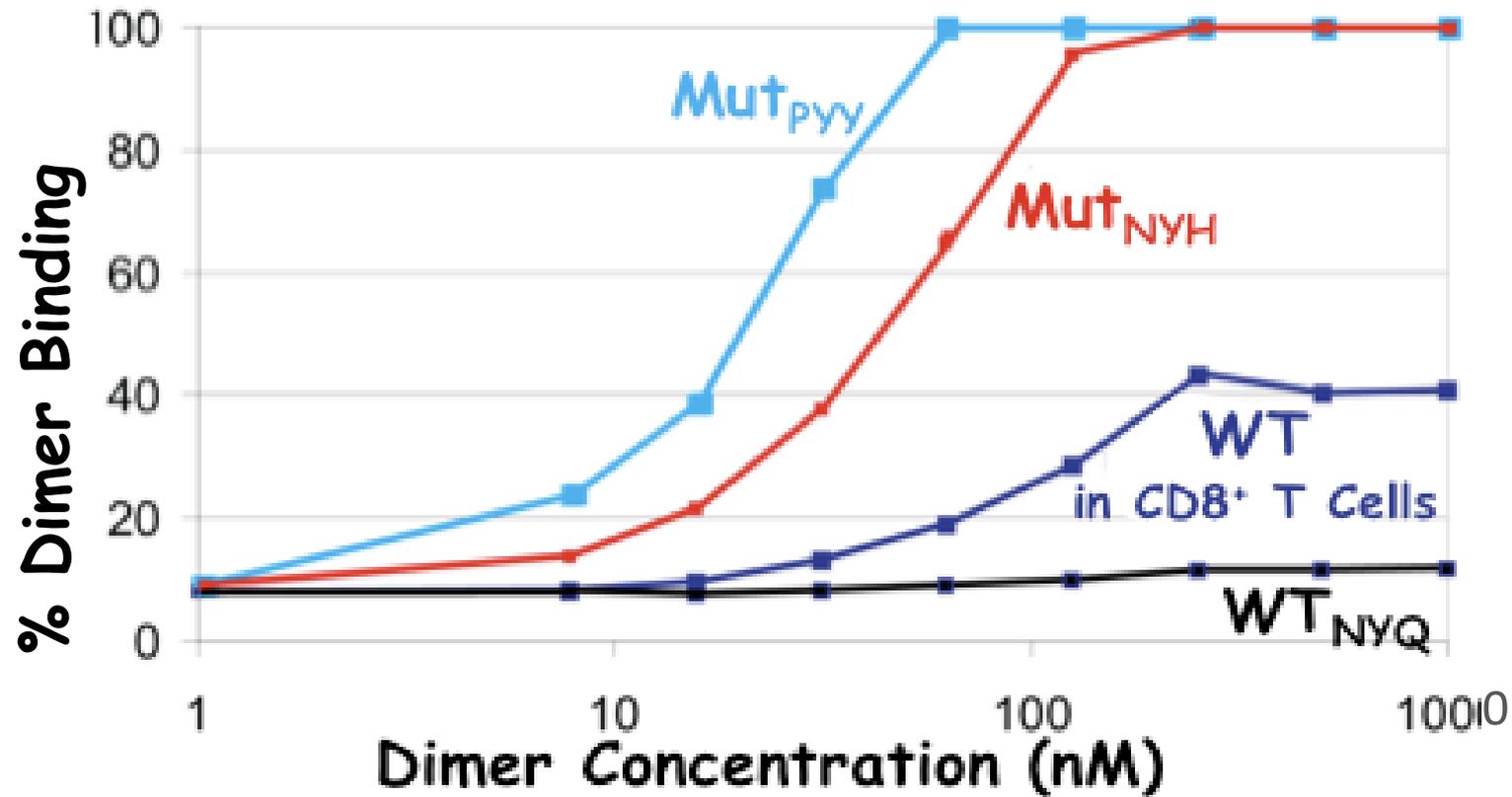


## Results of library screening:

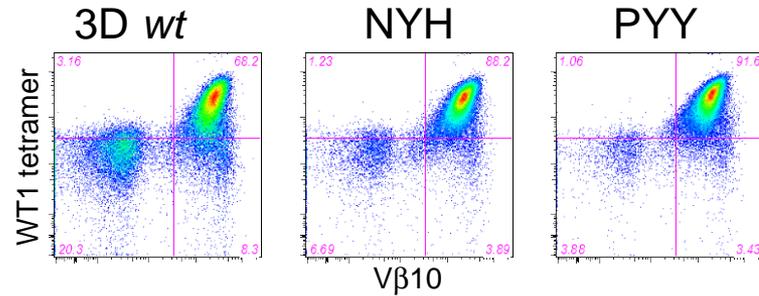
- Each library was transduced into 58<sup>-/-</sup> T cells, which lack TCR- $\alpha$  and  $\beta$ , and are CD8 negative (to screen for CD8 independence)
- Cells with enhanced WT1 tetramer binding could only be isolated from the NYQ library – **Two CD8-independent variants were isolated**

# Pre-clinical WT1 murine model: Affinity of murine WT1-specific TCR mutants generated by directed mutagenesis of the CDR3 $\alpha$

## Equilibrium Binding of Transduced Hybridoma Lines to the WT1/D<sup>b</sup> Dimer



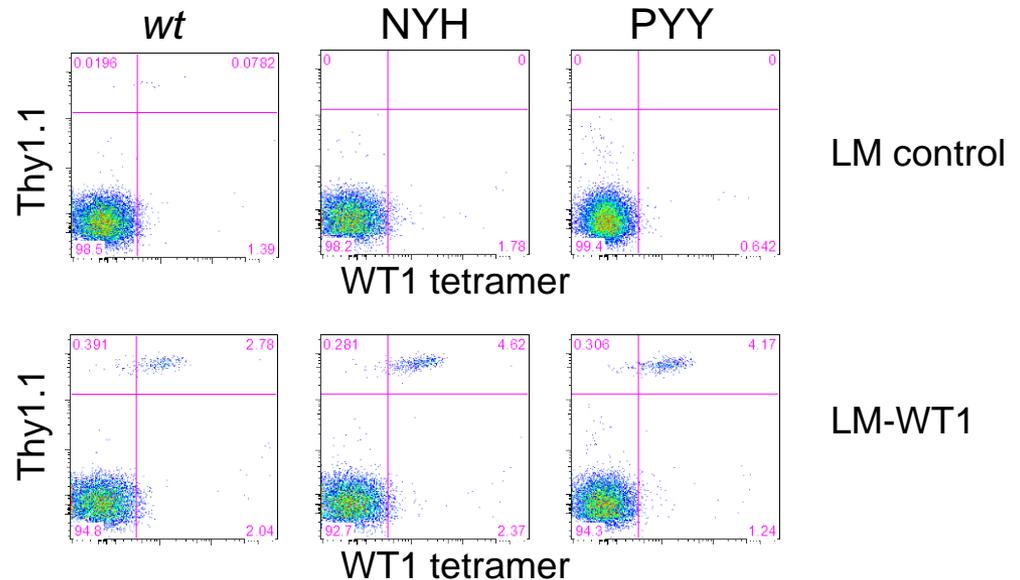
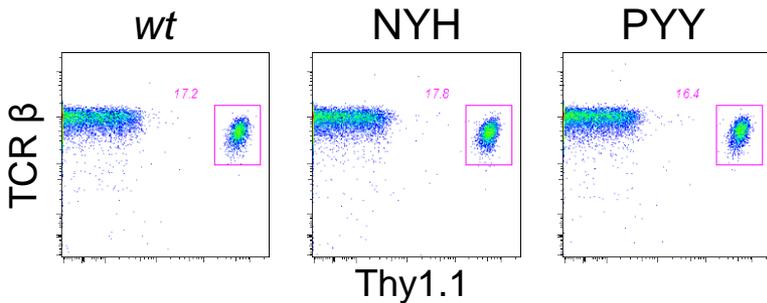
# T cells expressing Mutated High affinity WT1-Specific TCRs Transferred into B6 mice



Transduced T cells were injected into Thy1.2 B6 mice

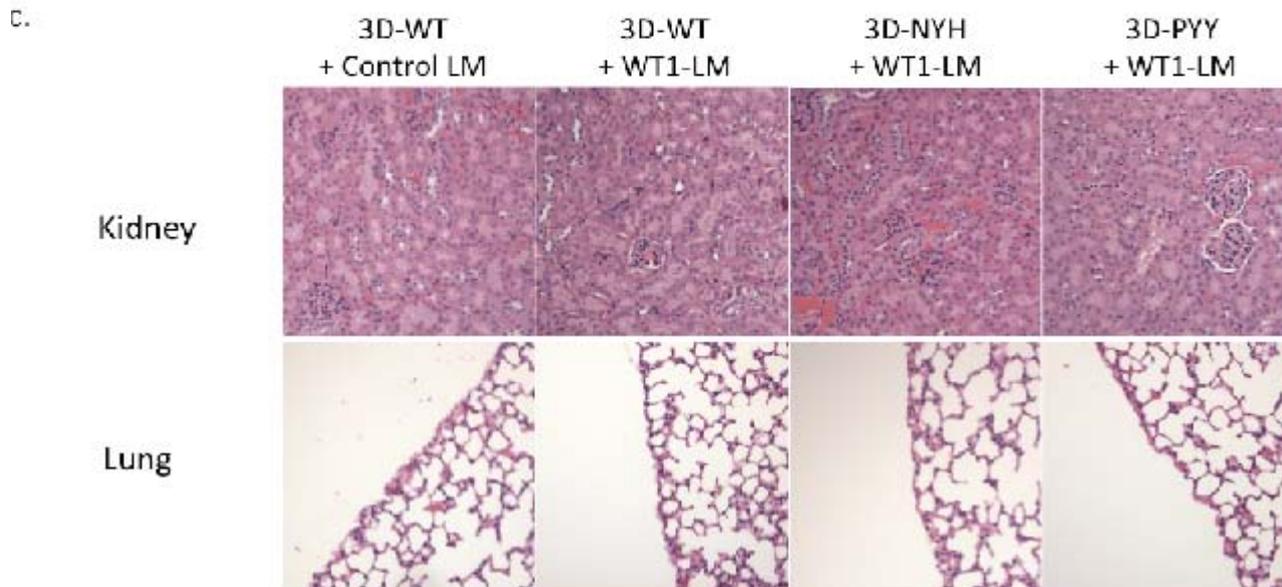
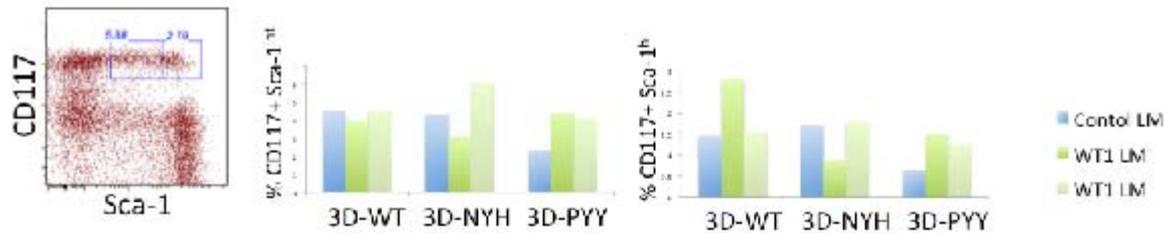
- Mice were challenged 18 days post-T cell transfer with recombinant Listeria expressing WT1
- Blood samples were analyzed following LM boost

Day 7 post-T cell transfer:  
Gated on CD8<sup>+</sup> T cells



# Pre-clinical WT1 murine model: Analysis of tissues expressing WT1 for T cell-mediated damage

- No tissue toxicities observed in mice 2-3 weeks following LM boost.
- No depletion of hematopoietic stem cells.
- No damage to WT1-expressing organs (lung/kidneys)
- Mice have remained healthy for >4months with persisting functional T cells



# Clinical protocol 1206-1169: P.I. Dr. Merav Bar

## Objectives

---

### Primary Objectives

- Determine the safety and potential toxicities associated with treating patients with high risk or relapsed AML, MDS, and CML after allogeneic HCT by adoptive transfer of virus-specific CD8 T cells genetically-modified to express a high affinity WT1-specific TCR.
- Determine the anti-leukemic activity associated with treating patients with relapsed AML, MDS, and CML after allogeneic HCT by adoptive transfer of virus-specific CD8 T cells genetically-modified to express a high affinity WT1-specific TCR.

### Secondary objectives

- Determine the *in vivo* persistence of transferred T cells and ability to migrate to and accumulate in bone marrow.
- Determine the maintenance of TCR expression and function of transduced T cells.

# ***ISSUE:* Patient and Donor selection**

---

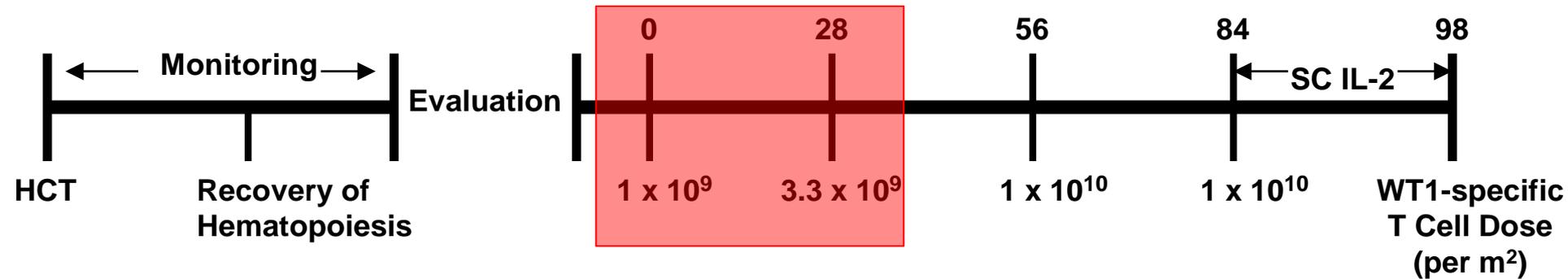
## **Patient eligibility for enrollment**

1. Patients must express HLA-A\*0201
2. Patients undergoing matched allogeneic HCT for high-risk leukemias.
3. **Patients must be  $\geq 50\text{kg}$** , as patients with lower weight would be incapable of providing high volume and frequent blood samples for monitoring and analysis
4. Patients  $>18$  years old must be able to give informed consent. **The parent or legal representative will be asked to consent for patients younger than 18 year old.**

# **ISSUE:** Safety Strategy for Arm 1 Treatment Plan

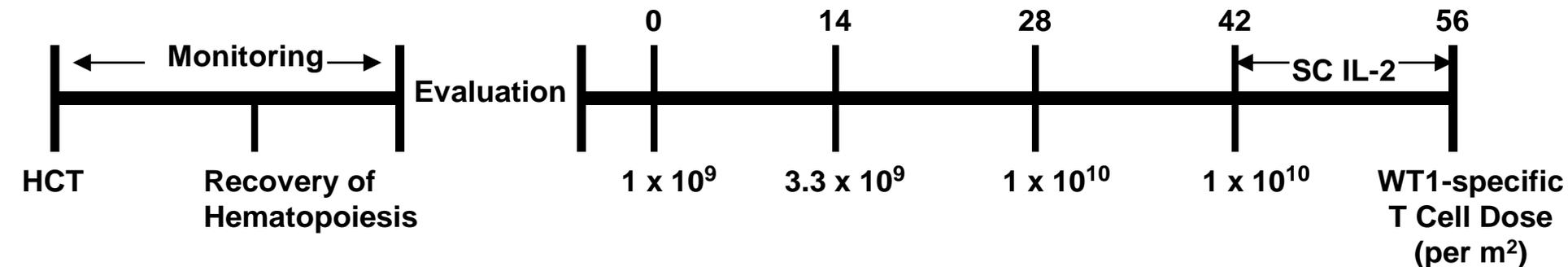
## Arm 1 (Prevention of relapse), Stage 1

Day of Treatment



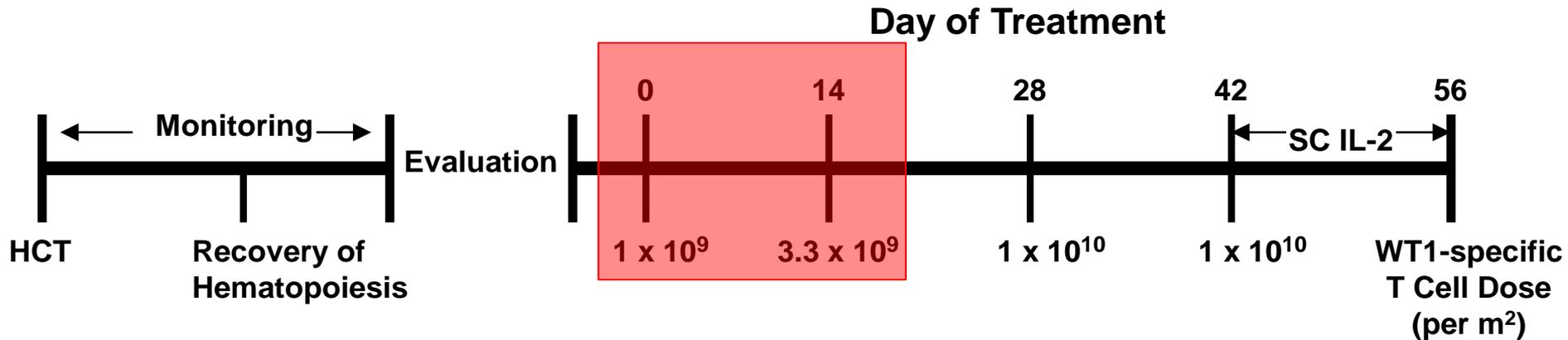
## Arm 1, Stage 2

Day of Treatment

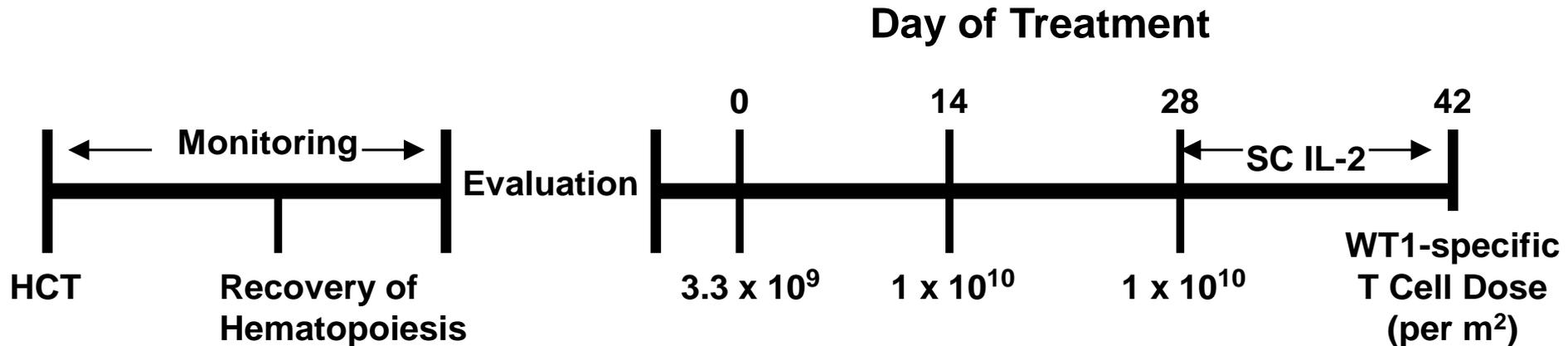


# Treatment Plan – Arm 2

## Arm 2 (Relapsed patients), Stage 1



## Arm 2, Stage 2



# ***ISSUE:* Plan to address dose-limiting toxicities observed in Stage 1**

---

## **Arm 1, Stage 1; Arm 2, Stage 1**

T cells will not be administered if cell frequency  $\geq 3\%$  of total CD8+ T-cells.

### **First 3 patients:**

- If  $\leq 1$  patient experiences a dose-limiting toxicity → transition to Stage 2
- If  $> 1$  patient experiences a dose-limiting toxicity → additional 3 patients

### **6 patients:**

- If  $\leq 2$  patients experiences a dose-limiting toxicity → transition to Stage 2
- If  $> 2$  patients experiences a dose-limiting toxicity → the maximum dose to be used on Stage 2 will be the dose that caused toxicity in  $\leq 2$  patients.
- If  $> 2$  patients experienced toxicity with the lowest dose administered → will not proceed to Stage 2 of the study, and DSMB will meet.

## **Arm 1, Stage 2**

Acceptable dose-limiting toxicity rate on arm 1, stage 2 will be 20%

-selected to match adverse event rate for new therapies in HCT patients

## **Arm 2, Stage 2**

Acceptable dose-limiting toxicity rate on arm 2, stage 2 will be 30%

-selected based on underlying toxicities and  $> 90\%$  mortality rate after relapse

## ***ISSUE:*** Modification of the consent forms

---

All modifications suggested by the reviewers were incorporated in the consent forms.

# Acknowledgements

---

## Philip D. Greenberg

Merav Bar

Heather Sloan

Gunnar Ragnarsson

Natalie Duerkopp

Colette Chaney

Lisa Rolczynski

Hieu Nguyen

Ilana Roberts

T.M. Schmitt

Galina Pogosov

William Ho

Jerry Radich

**FRED HUTCHINSON**  
**CANCER RESEARCH CENTER**

---