Building a Better T Cell for Targeting Tumors

Phil Greenberg

27th Annual Meeting

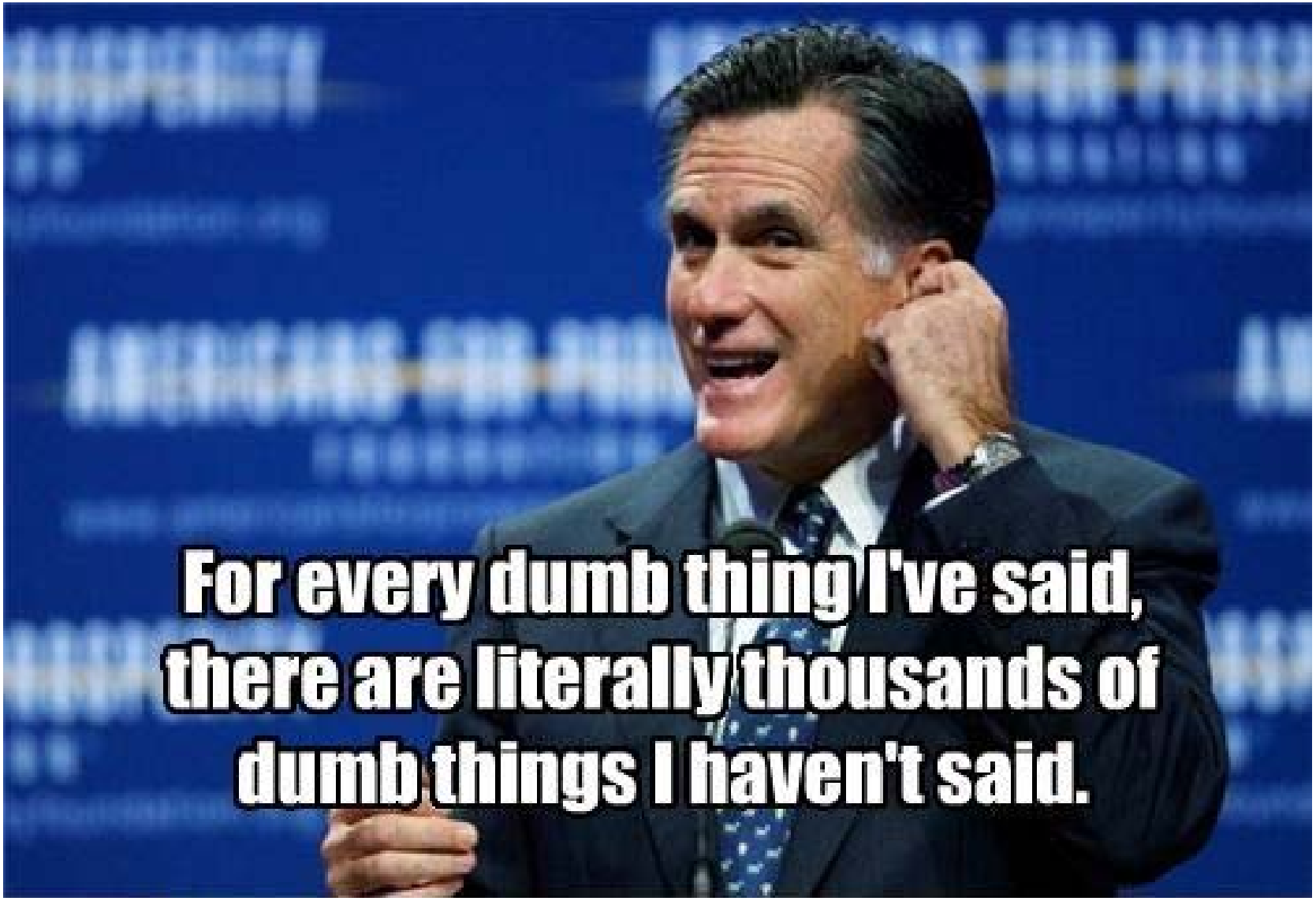
October 26-28, 2012
North Bethesda, MD

Disclosure

I have no financial relationships to disclose.

In other words, I am uninteresting, but available.

Disclaimer



"I'm Phil Greenberg, and I approve this message"

The Antigen:
T cell therapy needs good targets

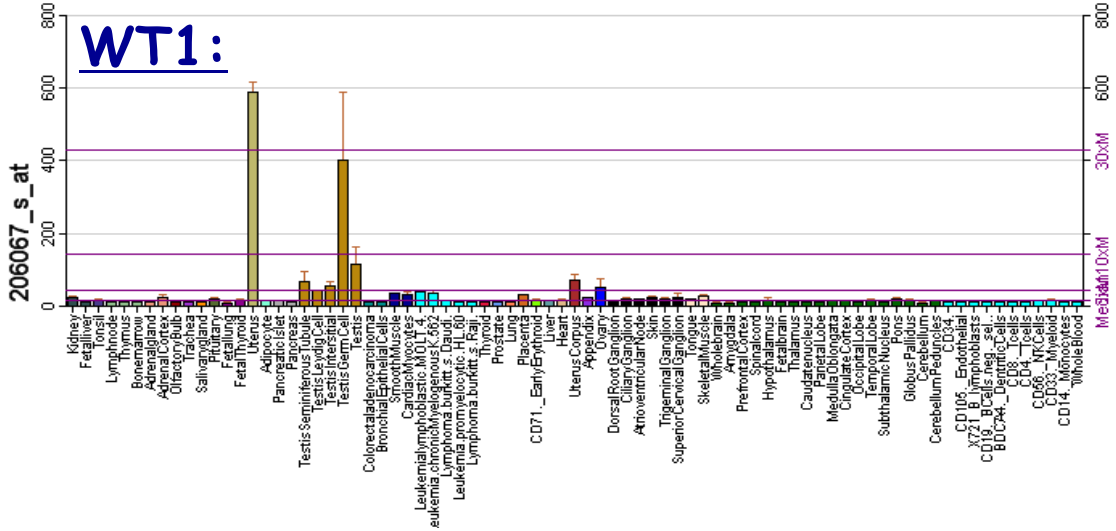
The search for targetable tumor antigens: Identifying proteins that can be safely/effectively targeted

Leukemia as a prototype:

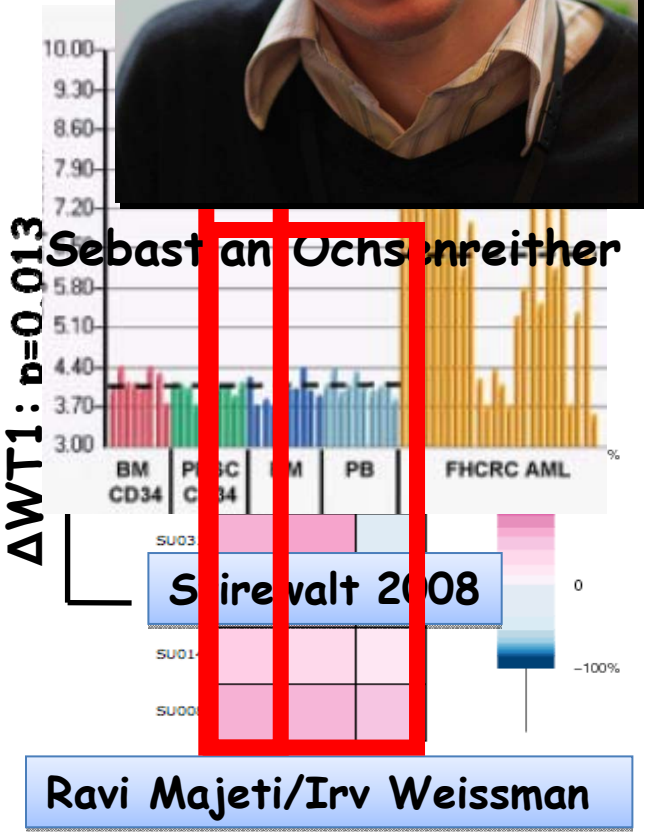
1) Identify candidates: Analyze mRNA expression to find genes over-expressed in leukemic cells and associated with malignant phenotype

2) Can targeting eradicate disease? Purify leukemic stem cells (LSC) and hematopoietic stem cells (HSC) to ascertain if the gene (WT1) is preferentially over-expressed in LSC

3) Will this be safe to target? Analyze normal tissues for expression to identify risks



Sebastian Ochsenreither



Shirevalt 2008

Ravi Majeti/Irv Weissman

The T Cell: Establishing Therapeutic Responses to WT1 in Patients

Clinical Trial targeting WT1 with T cells derived from the normal repertoire

Phase I Clinical Trial of WT1-specific CD8 T Cell Clones: Patients undergoing allogeneic HSCT for high risk ALL, AML, MDS and CML (~50% Relapse Rate within 2 years)

Primary Obj

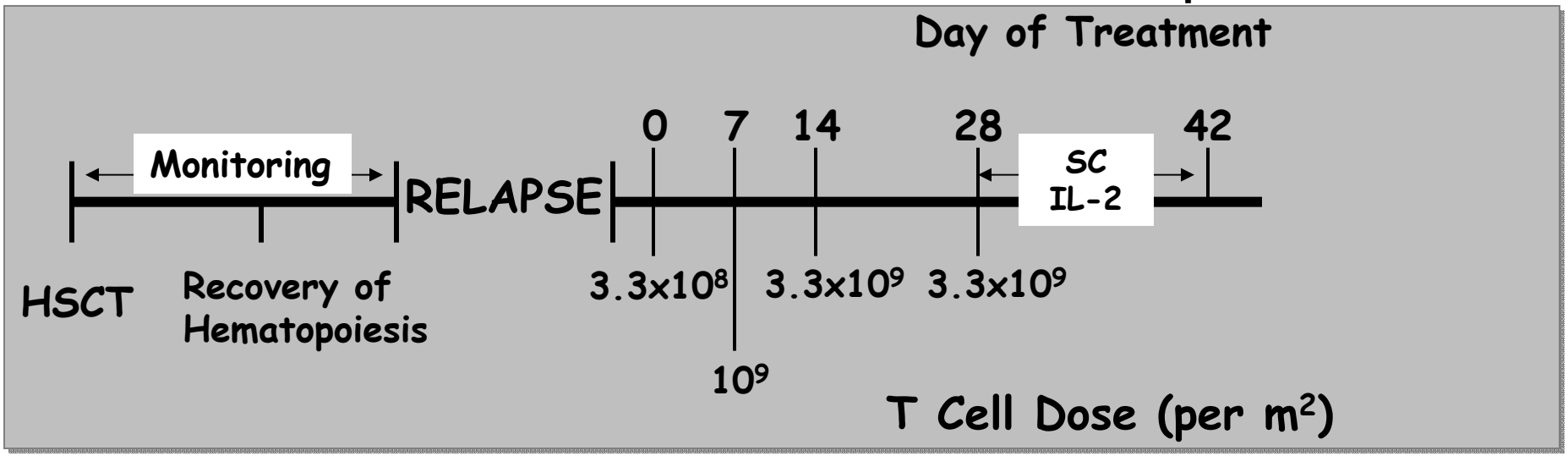
- To deterr y and potential iated with infusing d L clones specific patients

Secondary C

- To deterr o persistence of cells and assess mi bone marrow
- To deterr ely transferred T cells mediate antileukemic activity

Gunnar Ragnarsson

Aude Chapuis



Clinical Trial targeting WT1 with T cells derived from the normal repertoire

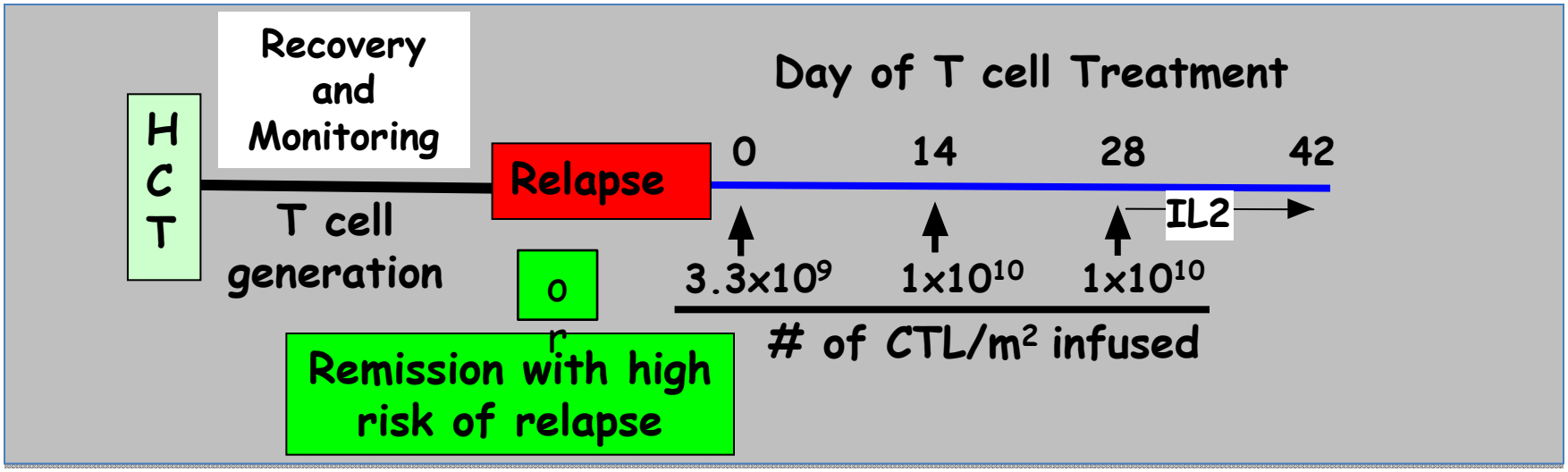
Phase I Clinical Trial of WT1-specific CD8 T Cell Clones: Patients undergoing allogeneic HSCT for high risk ALL, AML, MDS and CML (~50% Relapse Rate within 2 years)

Primary Objective:

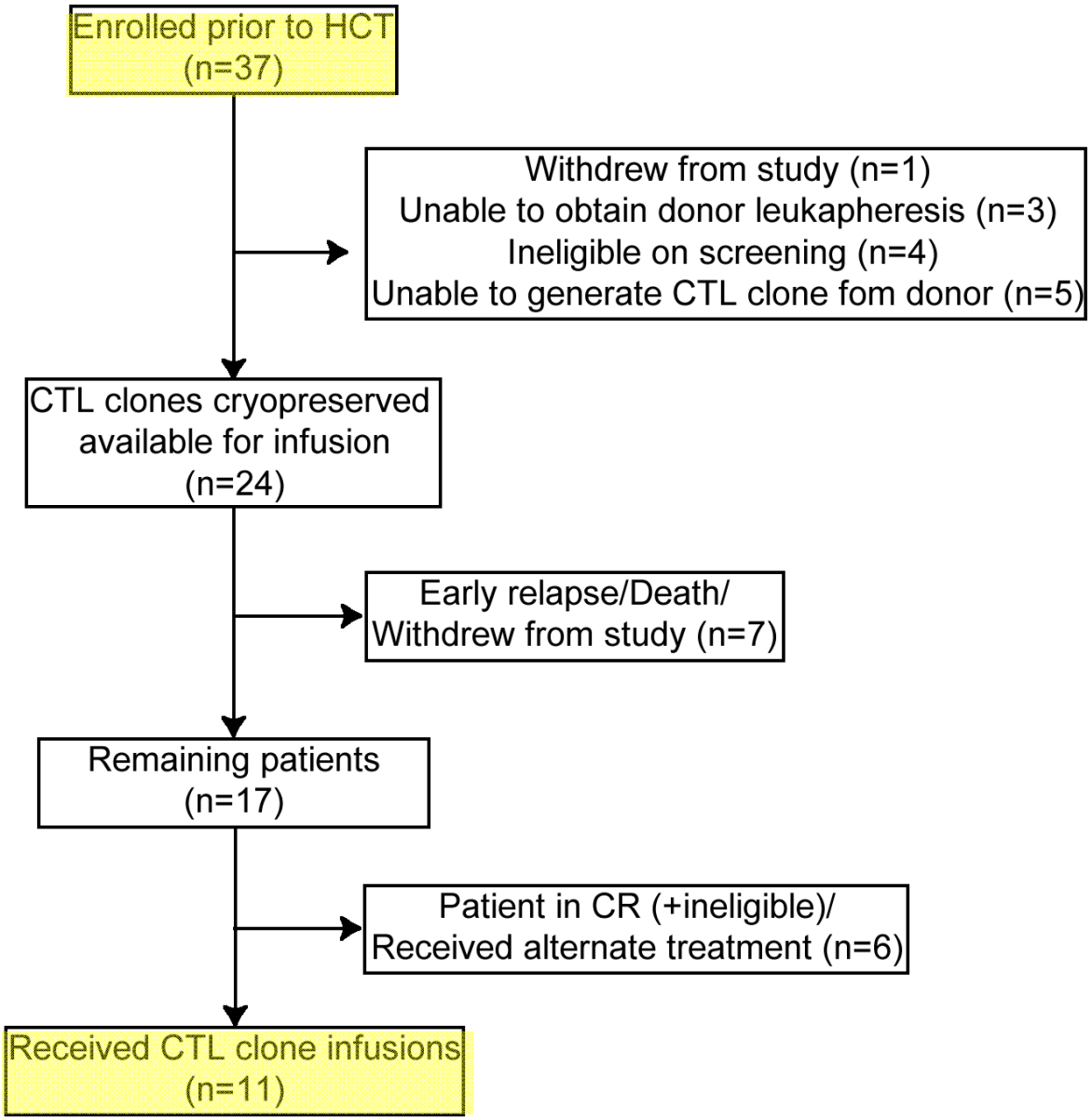
- To determine the safety and potential toxicities associated with infusing donor CD8+ CTL clones specific for WT1 into patients

Secondary Objectives:

- To determine the *in vivo* persistence of transferred T cells and assess migration to the bone marrow
- To determine if adoptively transferred WT1-specific T cells mediate antileukemic activity

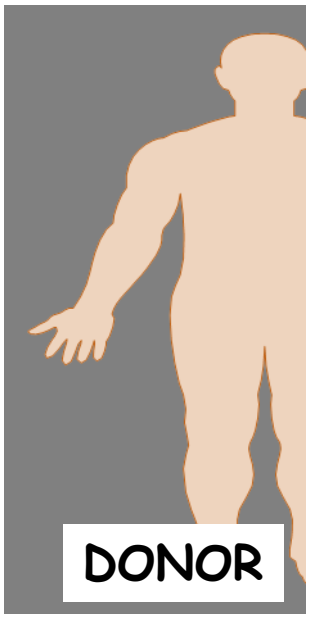


Clinical Trial: Enrollment/Status



Evolving methodology for generation of WT1-specific clones (Last 4 treated pts)

Cassian Yee



Leukapheresis

CD8-

CD8+

1-2 Stimulations

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

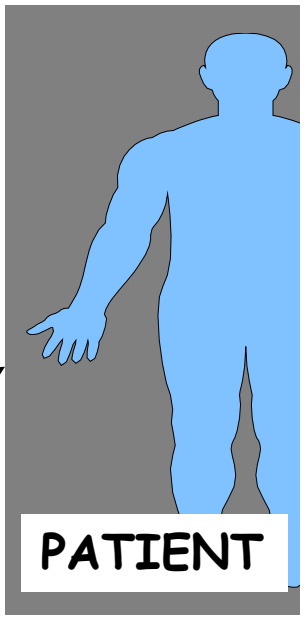
Dendritic Cells + WT1 peptide (RMFPNAPYL)

Cloning

Flask Expansion

Cryopreservation

INFUSION



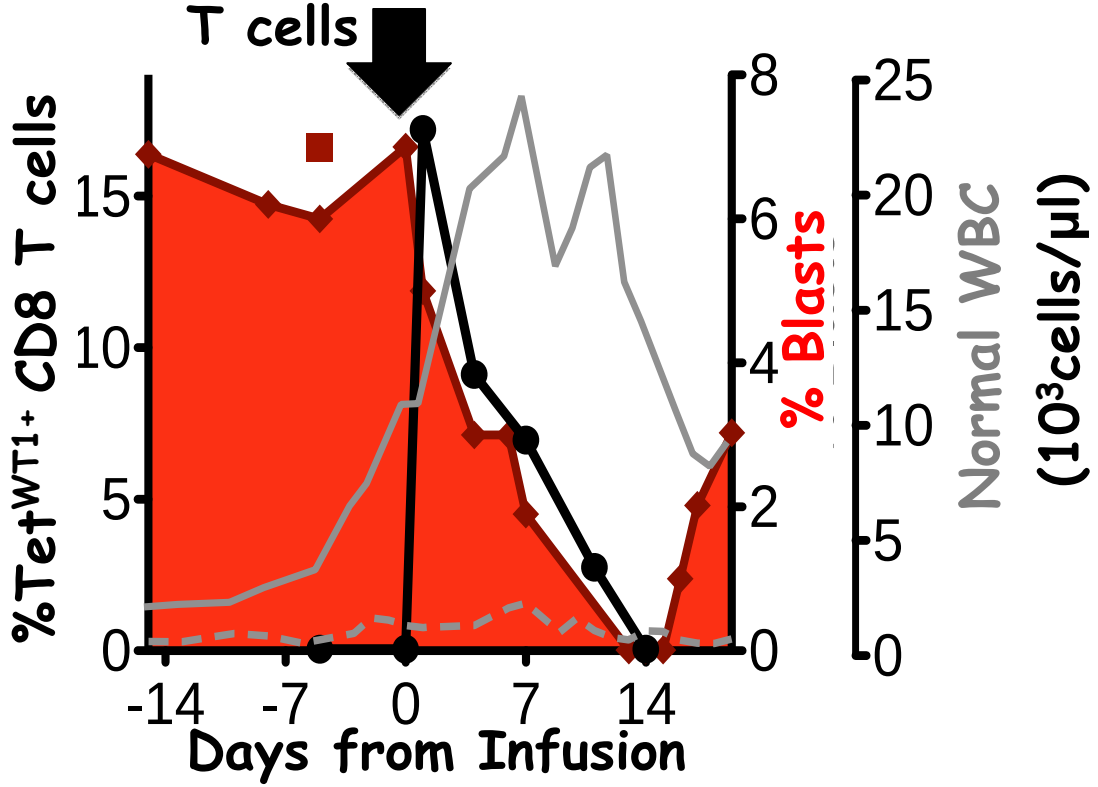
Bag exansion

+IL-2 + αCD3

Total production time: ~10-12 weeks

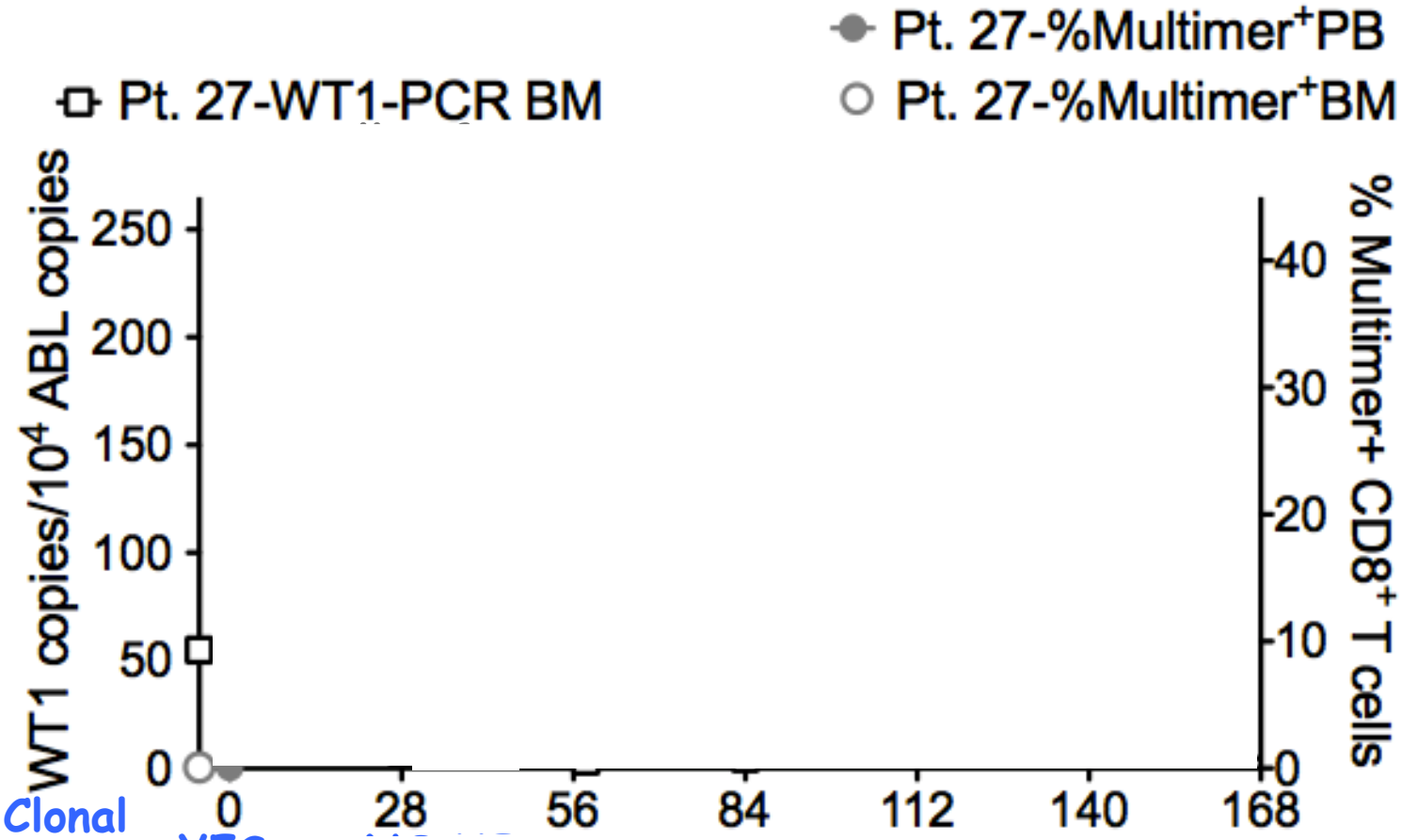
Reduction of Leukemic Burden in Patient With Advanced Progressive Drug-Resistant AML

Patient # 15



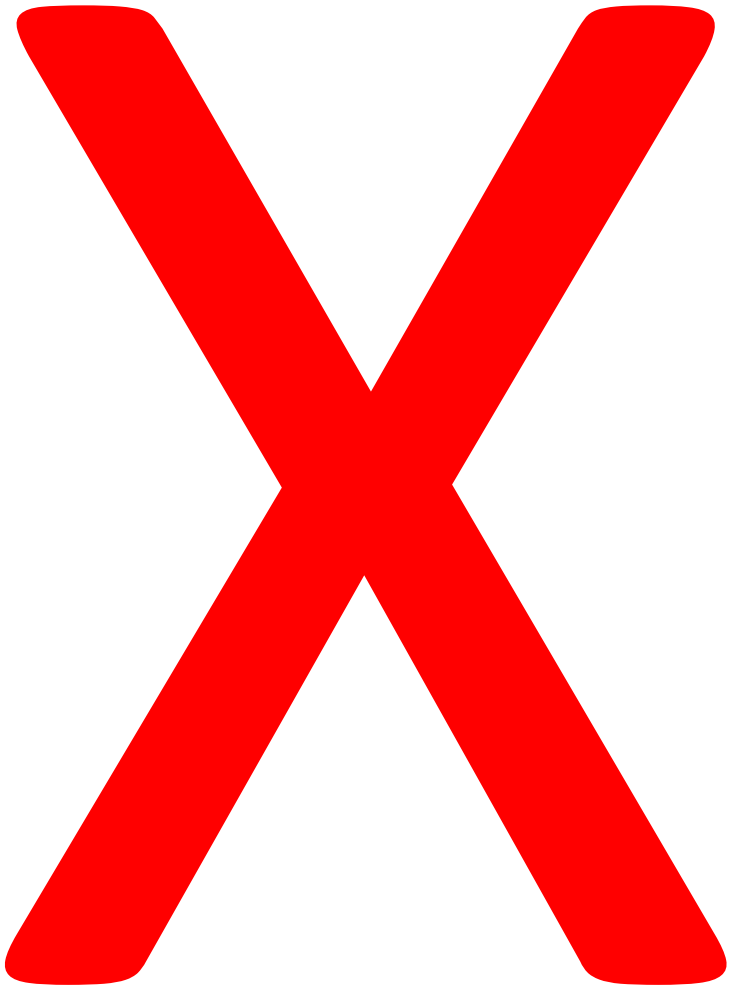
- Patient developed a fatal transplant/chemotherapy-related toxicity, unrelated to the activity of the infused T cells, was ineligible to receive subsequent T cell infusions, and died of progressive leukemia
- PROBLEM: If treat heavily pre-treated patients with late stage advanced leukemia, infused T cells usually quickly deleted and consequences of disease often precludes completing T cell therapy

Reduction of Leukemic Burden And Persistence of Transferred T cells in Patient Treated With Minimal Disease (by PCR and flow cytometry)



Detection of Clonal B cells in BM?

YES



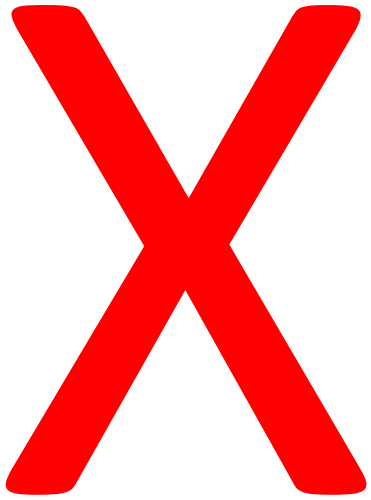
SUMMARY OF PATIENT OUTCOMES

37 patients entered

-11 treated (*PROBLEM:Many entered never eligible/receive treatment*)

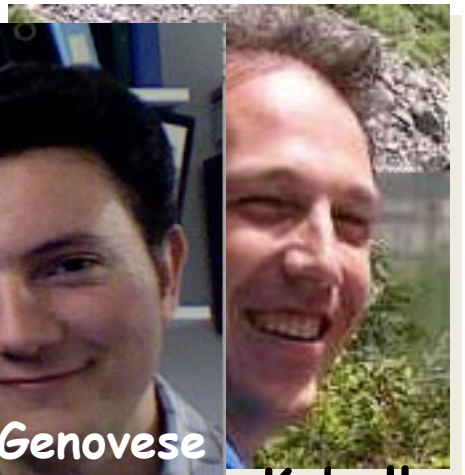
-2/7 with detectable disease exhibited reduction in leukemic burden (*PROBLEMS: Cell persistence, T cell avidity for patient leukemia)*

-4/4 patients with MRD or no detectable disease but a very poor prognosis predicting early relapse remain disease free at >1 year

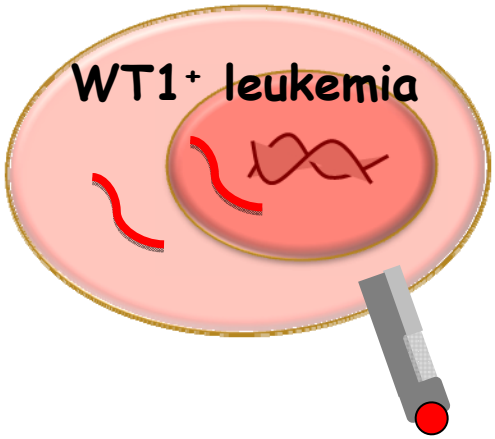


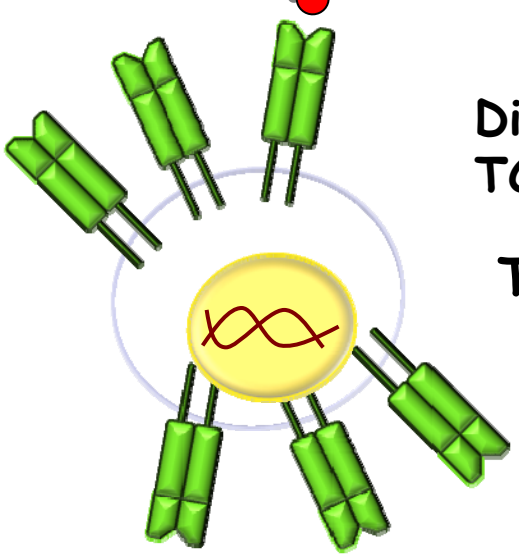
The TCR: Making Higher Avidity Responses Available In "Better Quality" T Cells

Improving Expression of WT1-Specific TCR Genes and Overcoming the Problem of Mis-matched Pairing



Kuball

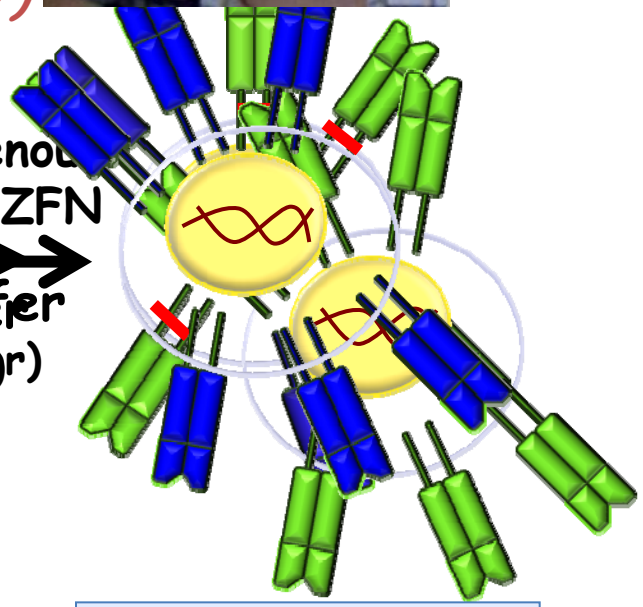




WT1-specific CD8 T cell

Disruption of endogenous TCR chain genes by ZFN

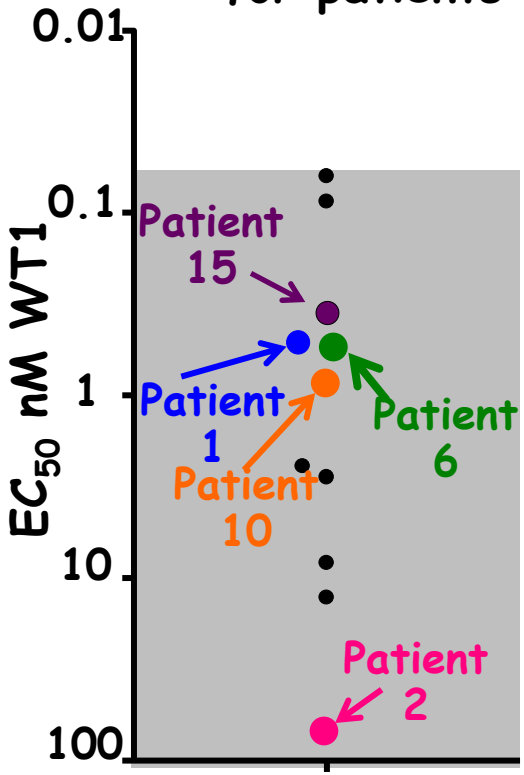
TCR gene transfer (via lentiviral vector)



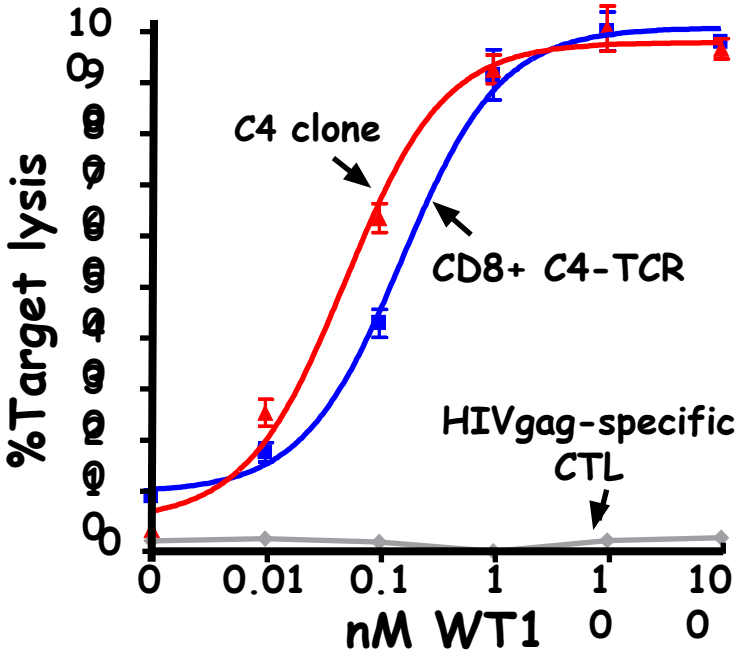
Nat Med (2012)

Identification of a high avidity CD8 T cell clone as a source for WT1-specific TCR gene transfer

Avidity of T cell clones generated for patients

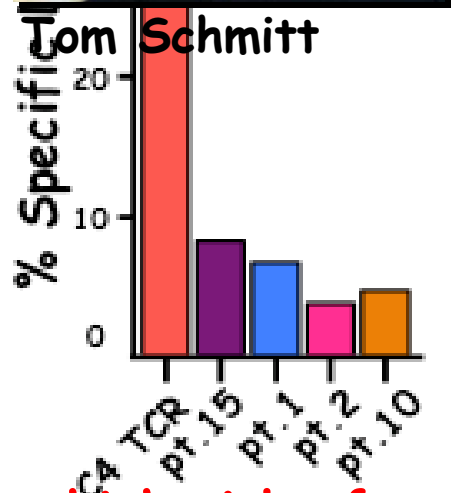


Avidity of CD8 T cells transduced with the codon-optimized, Cys-mutated TCR from the C4 clone



Lysis of patient leukemia cell ce ad D8 T nes nts

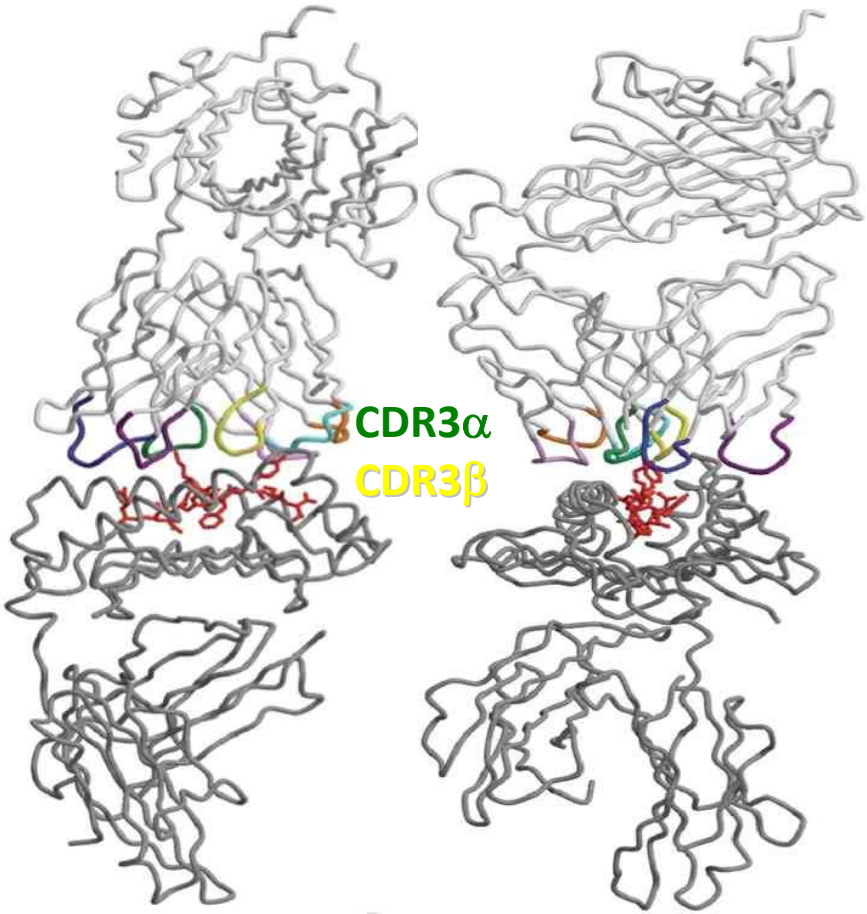
Tom Schmitt

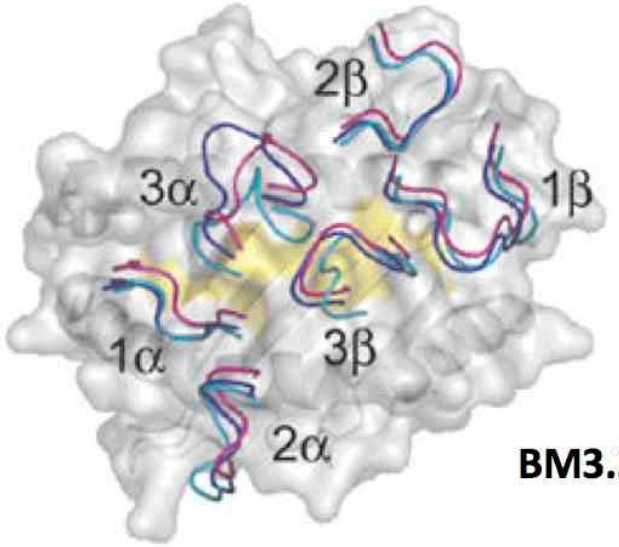


IND approved/Trial pending: Therapy of patients at high risk of relapse with CD8 T cells tranduced with the C4-TCR

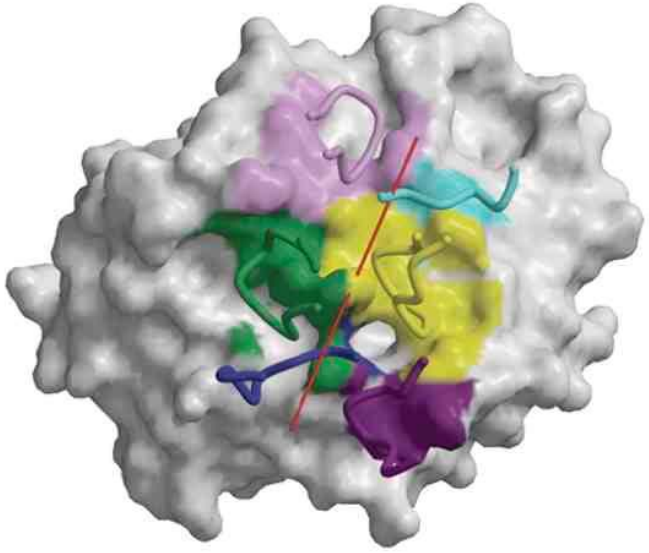
Engineering better TCRs: What needs changing?

TCR sitting on peptide/Class I complex





BM3.3 TCR

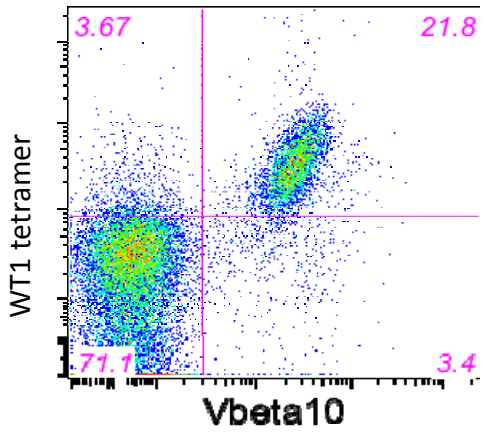


1G4/HLA-A2/ESO9C

FROM: Rudolph, et al (2006), *Ann Rev Immunol*
Mazza, et al (2007) *EMBO*
Garcia, et al (2009) *Nat Immunol*

Pre-clinical murine model to assess safety

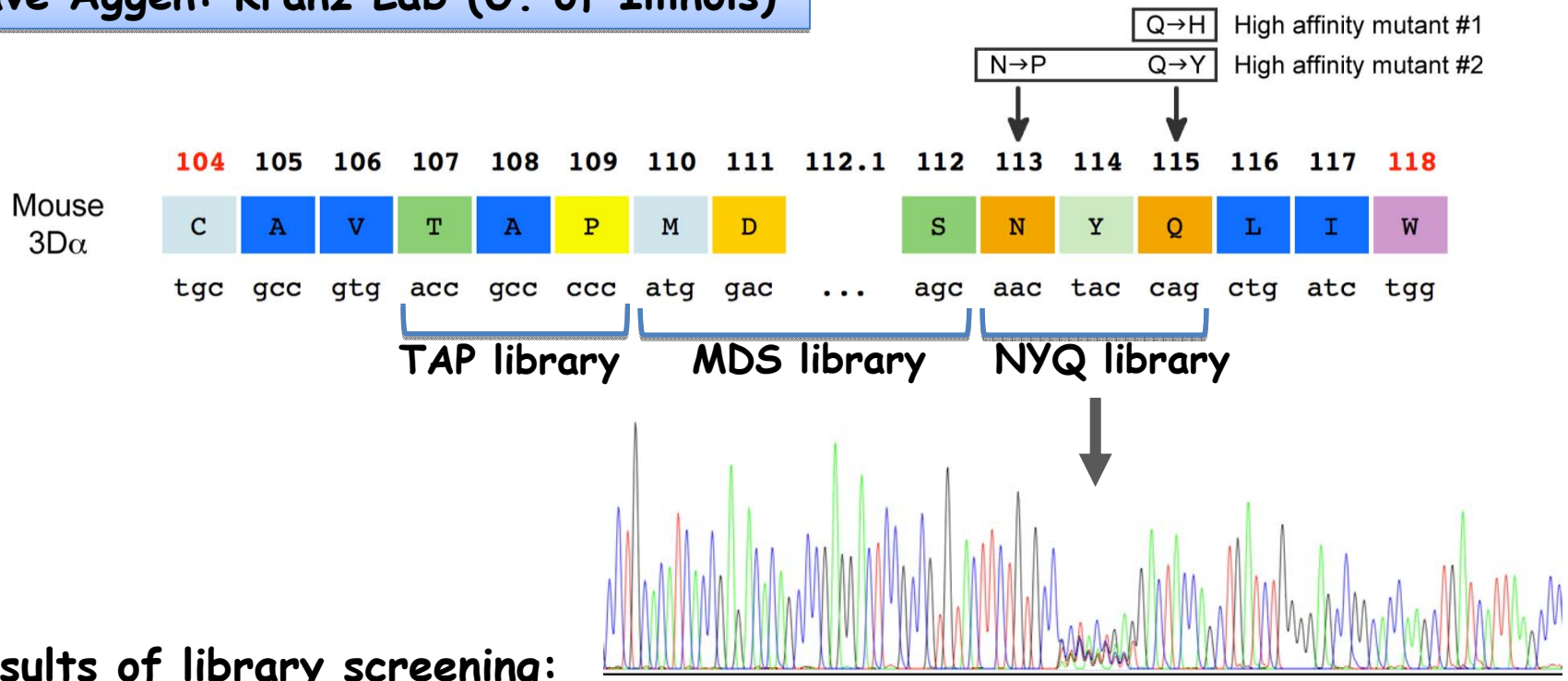
- Expression of WT1 is similar in mice and humans during fetal development, in adult tissues, and in tumors
- Mice and humans recognize the same immuno-dominant RMFPNAPYL epitope from WT1
  - B6 mice: restricted by H-2D^{b}
  - Humans: restricted by HLA A*0201
- Similar to our human C4-TCR, the high affinity murine 3D-TCR was isolated by screening naturally elicited murine H-2D^{b}-restricted WT1-specific CD8^{+} T cell clones for the highest avidity $_{\text{3D TCR}}$



Tom Schmitt

Mouse model: Generation/testing of high affinity variants of CDR3α by saturation mutagenesis

Dave Aggen: Kranz Lab (U. of Illinois)



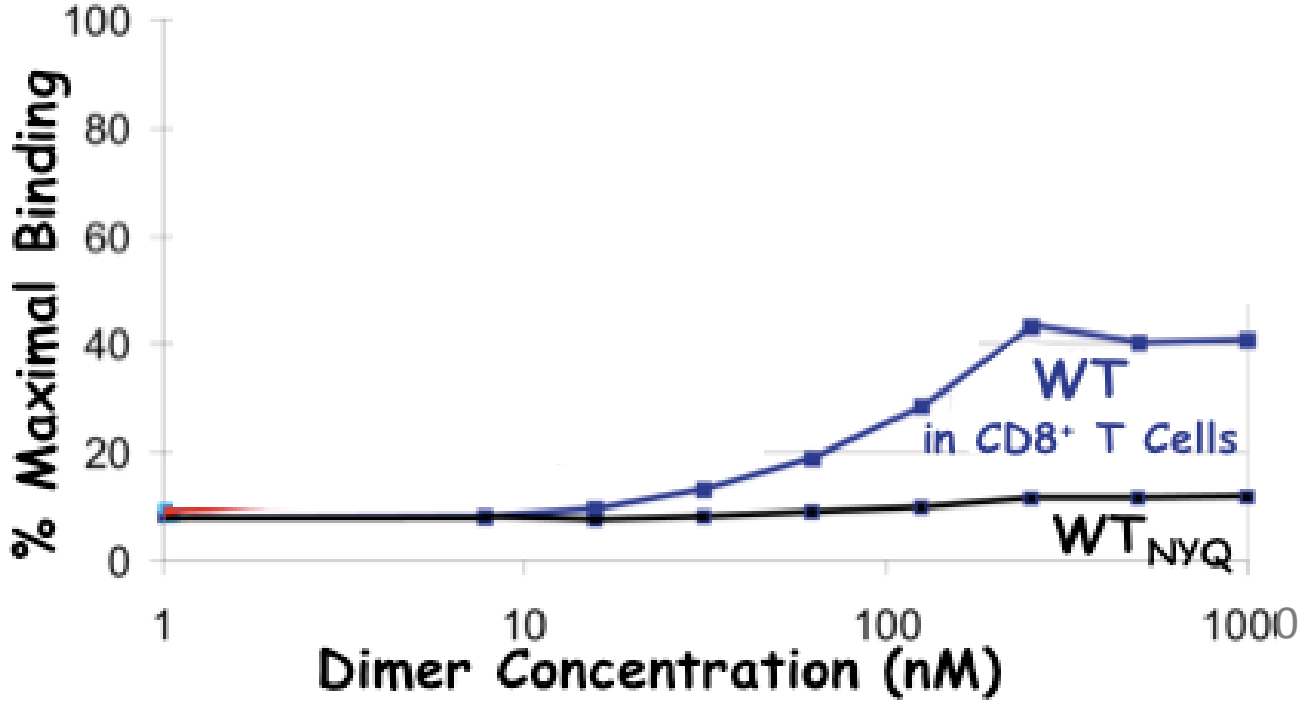
<u>Results of library screening:</u>

- The diverse libraries were transduced into $58^{-/-}$ hybridoma T cells already transduced with 3D-Vβ, which lack endogenous TCR-α and -β and are $CD8^{-/-}$ (to screen for CD8 independence)
- CD8-independent WT1 $tetramer^{+}$ cells could only be isolated from the NYQ library – The 2 most promising variants were isolated for further analysis

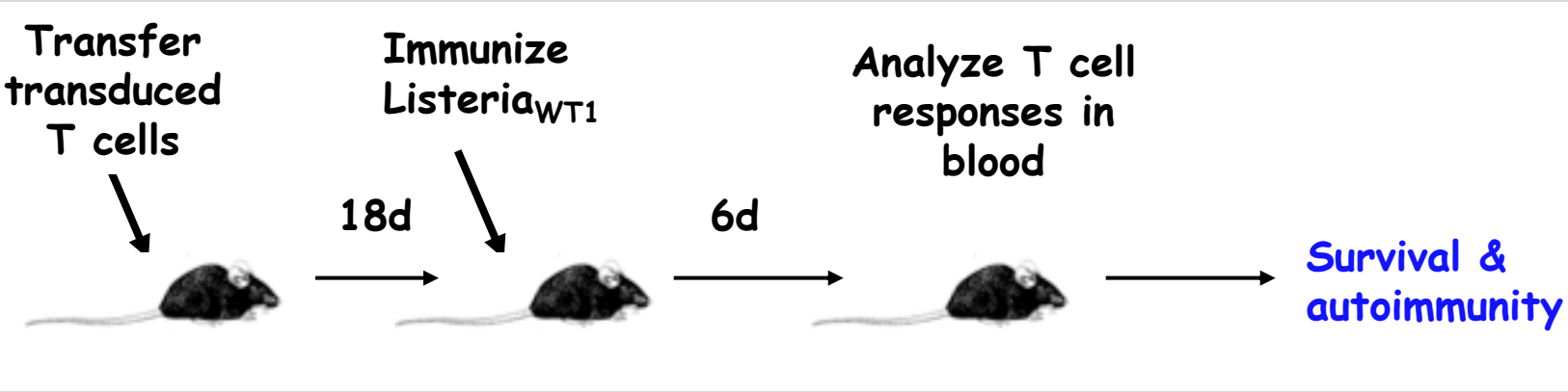
Two higher affinity TCRs were identified from directed mutagenesis of the CDR3 region of the 3D TCRα chain

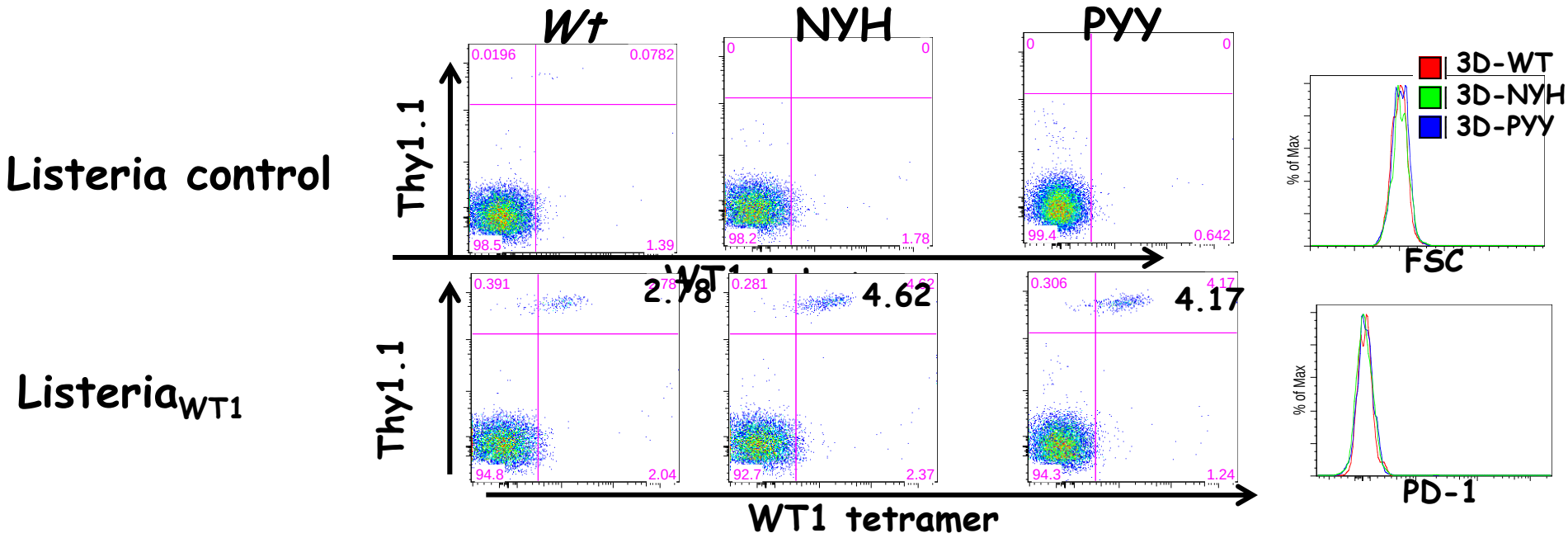
Dave Aggen: Kranz Lab (U. of Illinois)

EXPT: Equilibrium Binding of WT-1/D^b multimer by transduced $58^{-/-}$ hybridoma cells lacking CD8 expression that have been transduced with selected TCRs

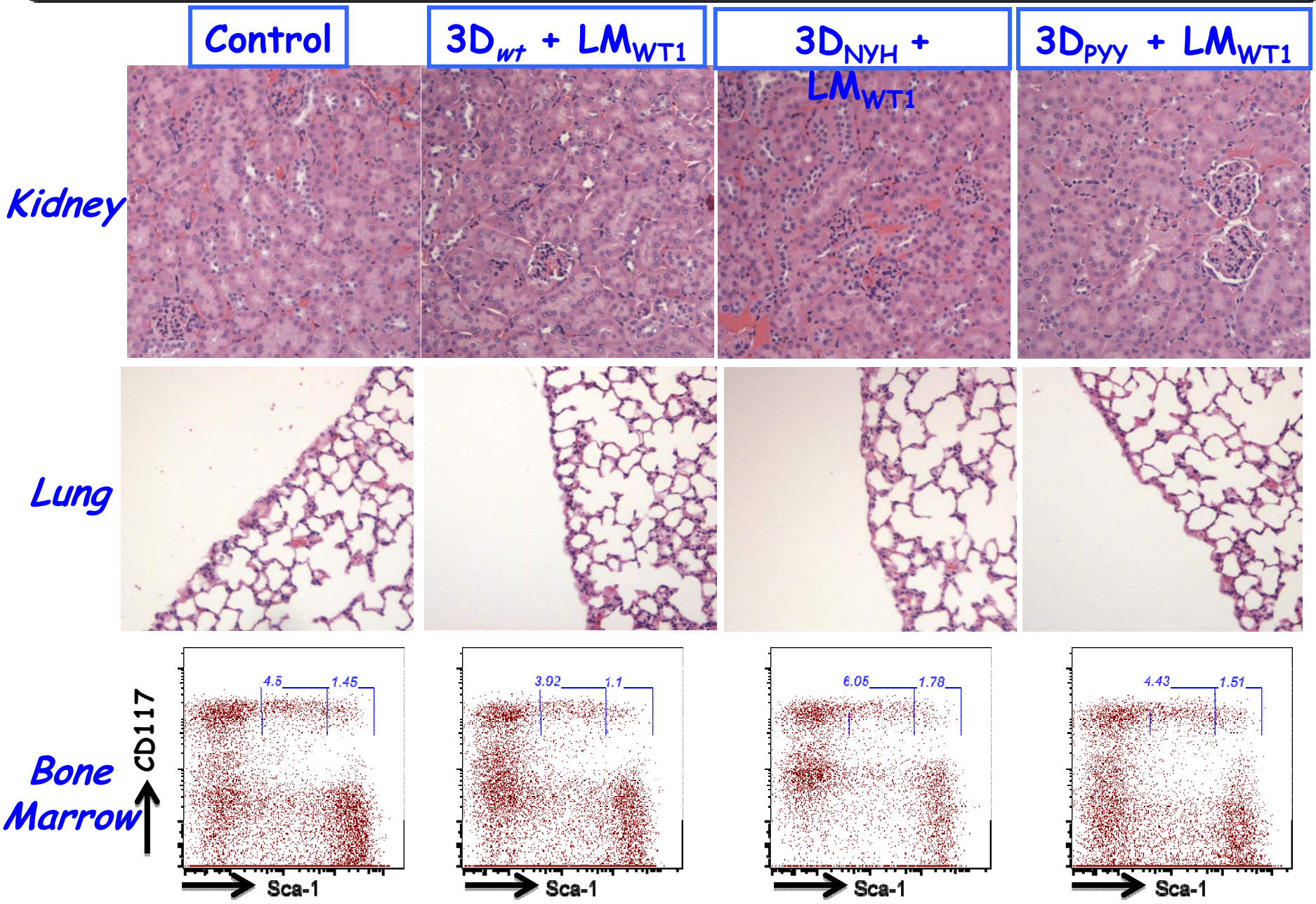


Transferred CD8 T cells expressing high affinity WT1-specific TCRs respond normally to immunization, and do not mediate detectable autoimmune injury

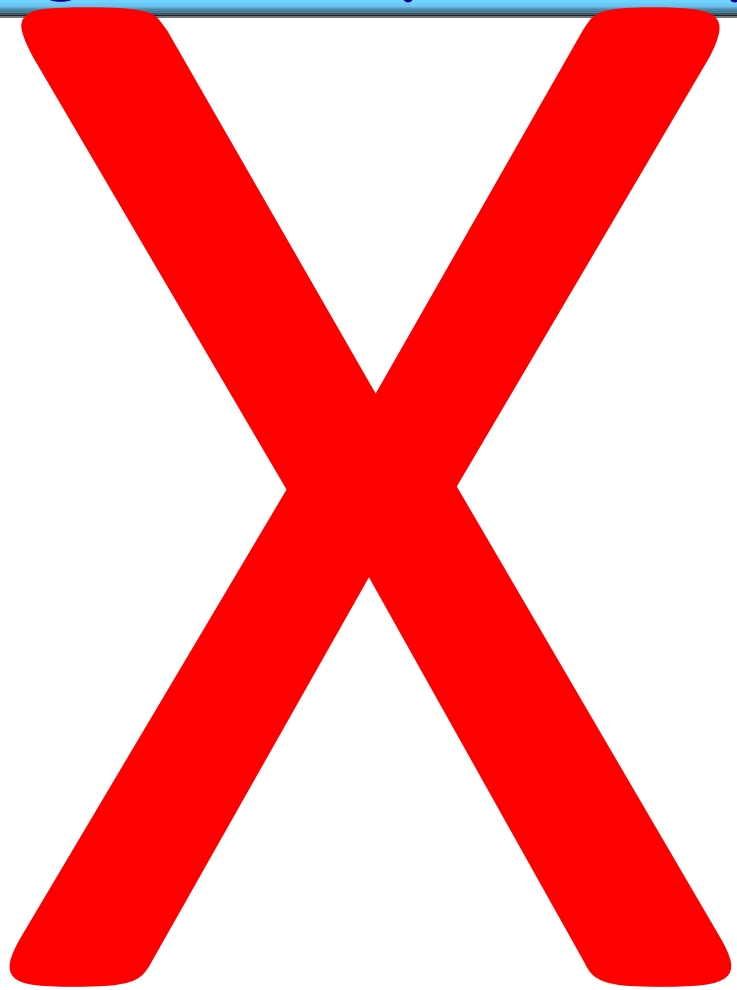




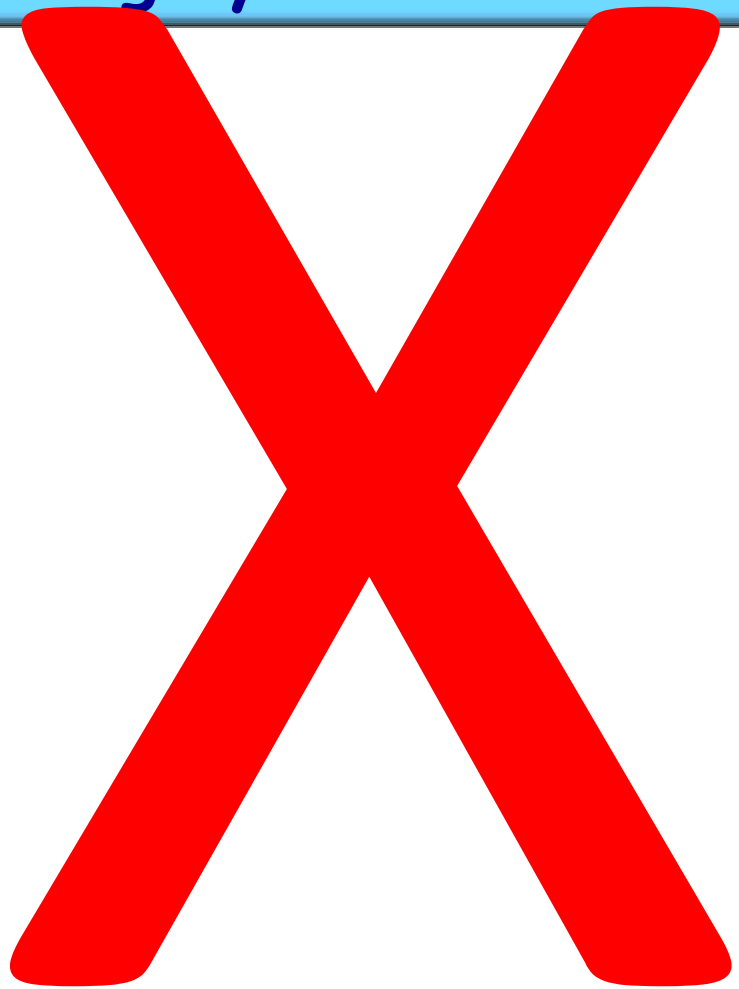
Transferred CD8 T cells expressing high affinity WT1-specific TCRs do not mediate toxicity to the normal tissues expressing WT1



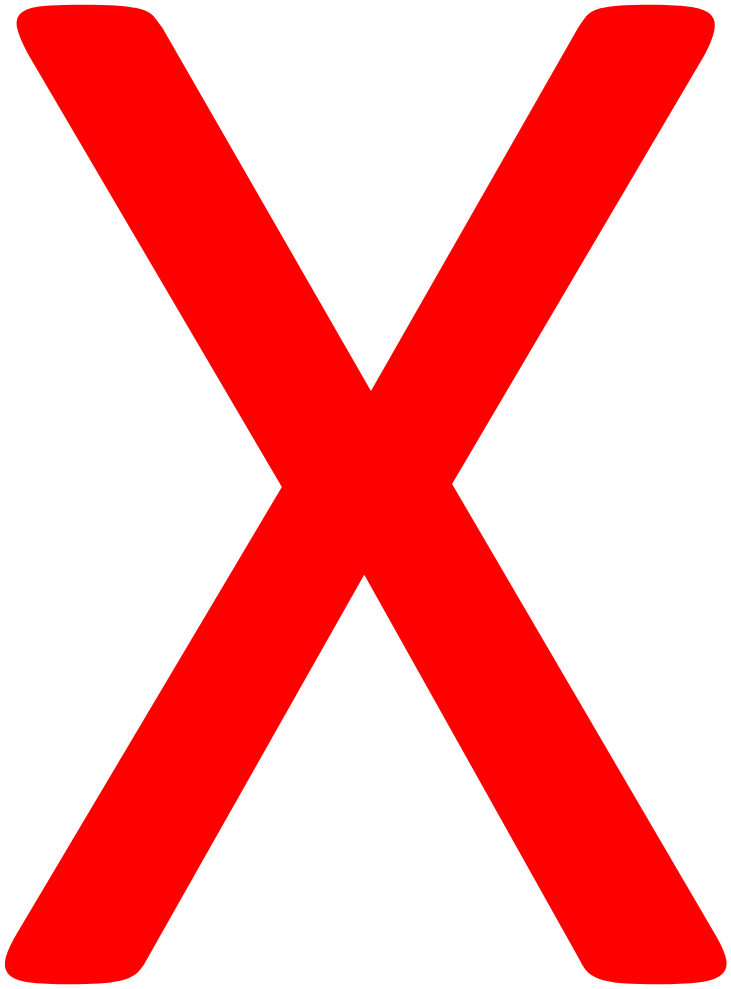
Where are these T cells in normal hosts:
Why can't we elicit with vaccines such T cells expressing high affinity WT1-specific TCRs ?



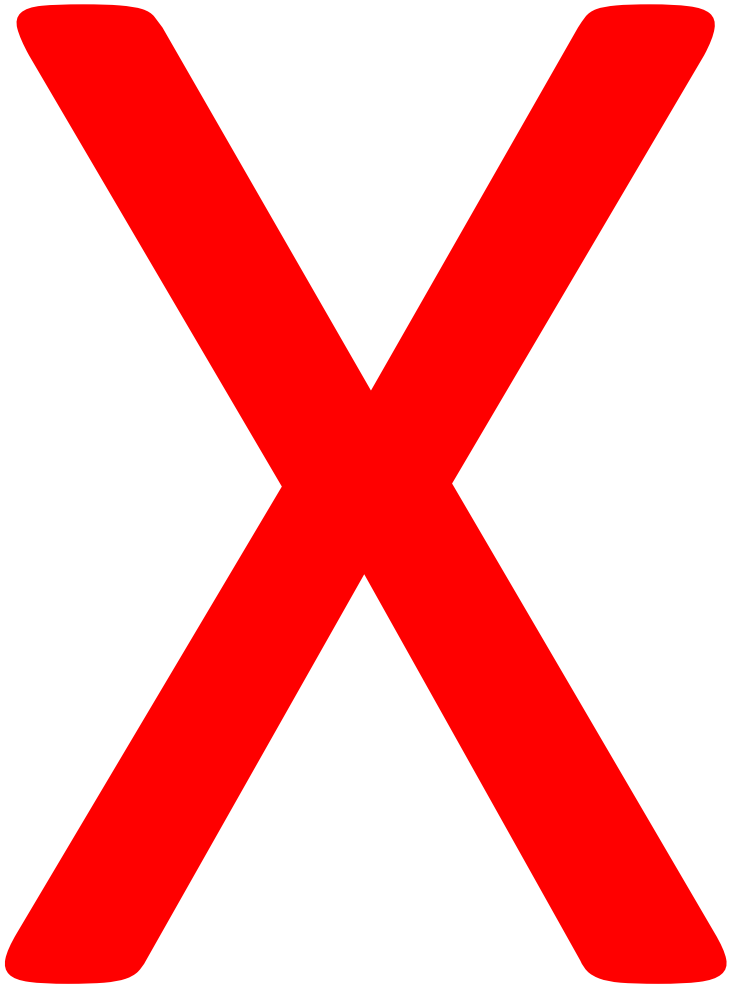
Analysis of Human A2-restricted WT1-specific TCRs targeting the same WT1-epitope restricted to D^b revealed highly similar CDR3-α sequences



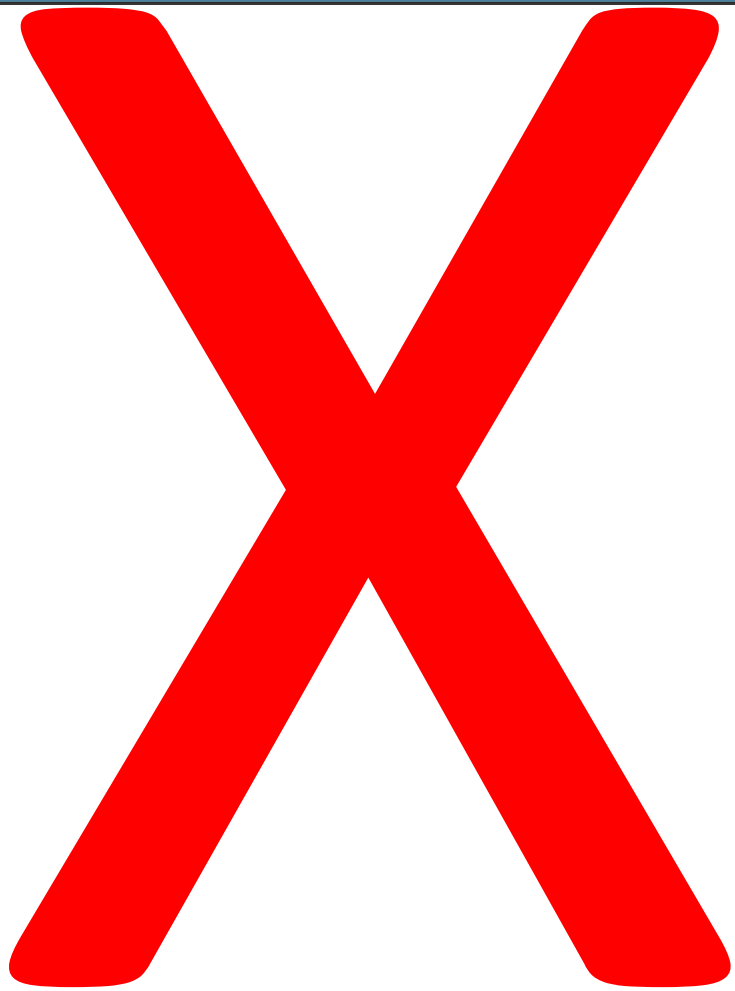
Generating Better TCRs: Developing a system for capturing more diverse high affinity TCRs



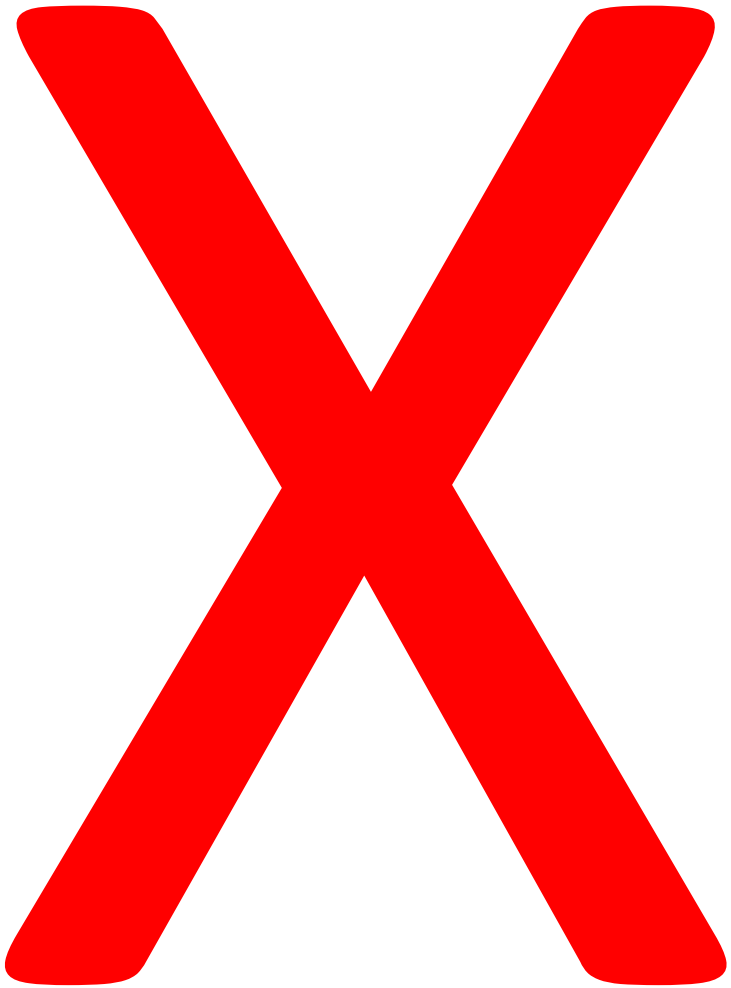
Strategy for generating high affinity TCRβ chains by positive selection



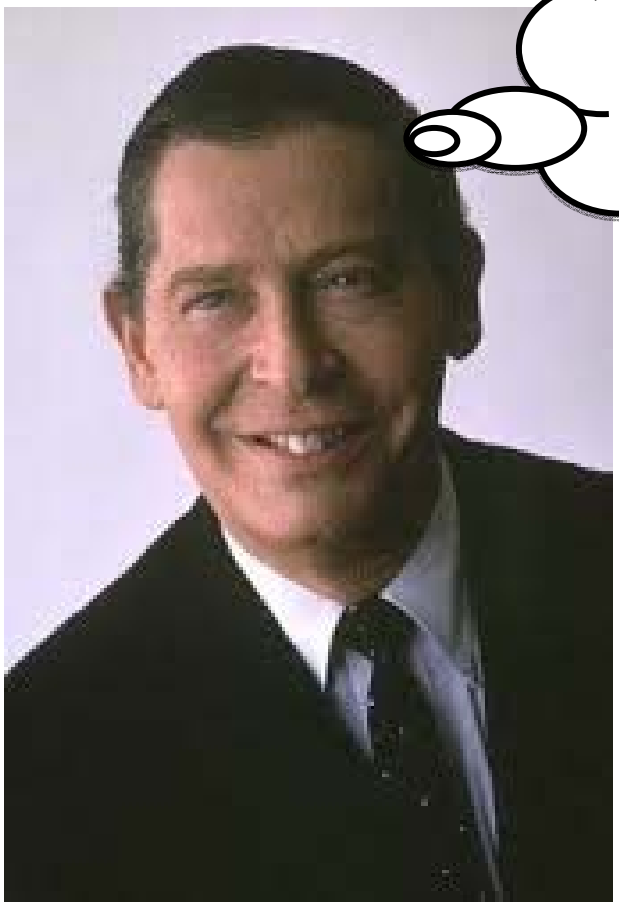
Sequencing of alternative TCRβ chains selected from the full endogenous repertoire that form high affinity WT1-specific TCRs by pairing during in *vitro* thymic selection with the mutated 3D α-chains



Making higher affinity WT1-specific TCRs: Selecting alternative rearranged β-chains



Reminder: Vote early and often



People who don't know why America is called the Land of Promise should be here during an election campaign

Milton Berle

Identifying New Leukemia Targets

Sebastian Ochsenreither
Matthias Wölfl
- Ravi Majeti- Stanford
- Irv Weissman- Stanford
- Derek Stirewalt- FHCRC
- Jerry Radich- FHCRC

TCR Transfer/Modification

Tom Schmitt
Jürgen Kuball
Michelle Dossett
- David Kranz- U of Illinois
- Dave Aggen- U of Illinois
- Sarah Richman- U of Illinois
- Roland Strong- FHCRC
- Elena Provasi- H.S.Raffaele
- Pietro Genovese- H.S.Raffaele
- Chiara Bonini- H.S.Raffaele
- Luigi Naldini- H.S.Raffaele
- Philip Gregory- Sangamo

T Cell Therapy of Leukemia

Gunnar Ragnarrson
Aude Chapuis
Cassian Yee
Merav Bar
Bill Ho
Hieu Nguyen
Natalie Duerkopp
Matthias Wölfl
Jeff Pufnock

Tolerance to Candidate Antigens & T Cell Genetic Modification

Andrea Schietinger
Ryan Teague
Junko Morimoto
- Günther Hämmerling-Heidelberg

Cassie Chou
Joe Blattman
Ingunn Stromnes
Carla Fowler
Xiaoxia Tan