Building a Better T Cell for Targeting Tumors

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Disclosure

I have no financial relationships to disclose.

In other words, I am uninteresting, but available.
For every dumb thing I've said, there are literally thousands of dumb things I haven't said.

“I'm Phil Greenberg, and I approve this message”
The Antigen:
T cell therapy needs good targets
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

WT1:

Sebastian Ochsner et al

Stirewalt 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 Objectives:
- To determine the safety and potential toxicity associated with infusing donor CD8 T cell 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

Monitoring

RELAPSE

0 7 14 28 42

SC IL-2

T Cell Dose (per m²)

HSCT

Recovery of Hematopoiesis

3.3x10⁸ 3.3x10⁹ 3.3x10⁹ 10⁹
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

Enrolled prior to HCT (n=37)

- Withdrew from study (n=1)
- Unable to obtain donor leukapheresis (n=3)
- Ineligible on screening (n=4)
- Unable to generate CTL clone from donor (n=5)

CTL clones cryopreserved available for infusion (n=24)

- Early relapse/Death/Withdrew from study (n=7)

Remaining patients (n=17)

- Patient in CR (+ineligible)/Received alternate treatment (n=6)

Received CTL clone infusions (n=11)
Evolving methodology for generation of WT1-specific clones (Last 4 treated pts)

1-2 Stimulations
+IL-2 +IL-7 +IL-15 +IL-21

CD8+

CD8- Dendritic Cells + WT1 peptide (RMFPNAPYL)

Cloning

Flask Expansion

Cryopreservation

Bag expansion
+IL-2 +αCD3

Leukapheresis

Total production time: ~10-12 weeks

Cassian Yee

DONOR

INFUSION

PATIENT
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?

Days after first T cell infusion
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

WT1+ leukemia

Disruption of endogenous TCR chain genes by ZFN

TCR gene transfer
(via lentiviral vector)

WT1-specific CD8 T cell

Elena Provasi
(Bonini Lab)

Pietro Genovese
(Naldini Lab)

Jürgen Kuball

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 lines by CD8 T cells administered to patients

IND approved/Trial pending: Therapy of patients at high risk of relapse with CD8 T cells transduced with the C4-TCR
Engineering better TCRs: What needs changing?

TCR sitting on peptide/Class I complex


BM3.3 TCR

1G4/HLA-A2/ESO9C
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\textsuperscript{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\textsuperscript{b}-restricted WT1-specific CD8\textsuperscript{+} T cell clones for the highest avidity 3D TCR
Mouse model: Generation/testing of high affinity variants of CDR3α by saturation mutagenesis

Results of library screening:

- 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.

**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 Db 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 TCR\(\beta\) 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 \(\alpha\)-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 Wölfli
   - Ravi Majeti - Stanford
   - Irv Weissman - Stanford
   - Derek Stirewalt - FHCRC
   - Jerry Radich - FHCRC

**T Cell Therapy of Leukemia**
Gunnar Ragnarsson
Aude Chapuis
Cassian Yee
Merav Bar
Bill Ho
Hieu Nguyen
Natalie Duerkopp
Matthias Wölfli
Jeff Pufnock

**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

**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