Phase Ia/Ib Trial of Anti-PSMA Designer T Cells in Advanced Prostate Cancer after Nonmyeloablative Conditioning

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History of Biotherapies

HUMORAL

SPECIFICITY
AFFINITY
ADAPTABILITY

CELLULAR

LAK
(IL2)
TIL

BIFUNCTIONAL Abs

TUMOR VACCINES

Ab2 IMMUNIZATION

CHIMERIC IgTCR

CYTOTOXICITY
SELF RENEW
ACCESS

Renal Cell
Melanoma

Lymphoma
Leukemia
Melanoma
Colorectal
TCR

Gene-Modified TCR

Anti-Cancer T Cell
Gene Therapy
Prostate Specific Membrane Antigen (PSMA)

- Surface membrane glycoprotein 100,000 Daltons
- Normal prostate epithelium and prostatic vasculature
- Elevated expression in metastatic lesions and hormone refractory disease
- High clinical relevance:
  - 25,000 deaths per year from PSMA+ prostate tumors

- Antibody (3D8) from G. Murphy and A. Boynton
Clinical Retroviral Vector

- Single gene sFv-CD8α hinge-TCRζ construct
- No prokaryotic selection marker
- No internal regulatory elements
Expression

DESIGNER T CELLS ARE EFFICIENTLY GENERATED AND EXPRESS HIGH LEVELS OF IgTCR
Tumor Cell Killing by T Cells
Activation: Cytotoxicity

ANTI-PSMA DESIGNER T CELLS KILL PSMA+ TUMOR CELLS

![Graph showing tumor cell kill]
Animal model:

PSMA(-)

Selective *in vivo* Tumor Suppression by Anti-PSMA Designer T Cells

PSMA(+)  
55% (5/9) tumor free
Conclusion

- Anti-PSMA designer T cells prepared
- Ready for use in prostate cancer

- But......
Clinical Data: 1st Generation (heterologous system)

Phase I Study of Anti-CEA Designer T Cells in Adenocarcinoma ("1st generation")

FDA BB IND 7301
Hypotheses

- IgTCR will redirect modified T cells to recognize CEA+ tumor in an MHC-independent manner.
- Recognition of tumor cells will induce proliferation, IL2 secretion (CD4+) and cytotoxicity (CD8+) by “designer T cells” in vivo.
- A self-sustaining immune response will cause tumor regression in vivo.
Clinical Summary

- Number of doses administered (24): 17 -IL2, 7 +IL2
- Patients treated (7): 5 colorectal, 2 breast; 5 -IL2, 2 +IL2
- Doses sizes administered
  - $1 \times 10^9$, $3 \times 10^9$, $1 \times 10^{10}$, $3 \times 10^{10}$, $1 \times 10^{11}$ cells
- Drug Toxicity ("Probably related" or "Definitely related")
  - No grade III toxicity, one grade IV toxicity
    (grade II fever $\gg$ grade IV SVT)
  - No delayed grade III, IV toxicity
  - Positive for low grade fevers, mild GI symptoms ($<\text{grade III}$)
- Response
  - Partial, transient (1 patient -IL2)
  - Minor, transient (1 patient +IL2)
CEA Profile on Patient GT

- CEA (NG/ML)
- Day of Treatment

**T Cells**

- Increasing pain
- Pain resolved
Conclusions: 1st Gen Phase I Study

- Adequate safety
- Proof-of-principle biologic response
- *But*... Time-limited efficacy profile
  - Don’t want PSMA targeting to have same fate…

- WHY DIDN’T IT *CURE??*
  - Laboratory correlate studies >>>>>>>>>>
Immunology 101
T Cell Activation

Antigen Presenting Cell

MHC

TCR

B7

CD28

“1”

T Cell

“2”

- Gene expression
  - Cytokines (IL-2, 4, IFN-γ, etc)
  - Surface molecules (CD25, CD40L, etc)
- Cytotoxicity
- Proliferation
Designer T Cells – First Generation

- IgTCR – *chimeric immunoglobulin – T cell receptor*

**Advantage:** IgTCR provides Signal 1: adequate T cell cytotoxicity.

**Disadvantage:** Lacking Signal 2, undergoes Activation-Induced Cell Death (AICD) after killing target cells. [HYPOTHESIS]
Comparing Signal 1 with Signal 1+2

Signal 1-only = AICD
Signal 1+2 = Proliferation
Proliferation = Increased tumor cell killing

--> EFFICACY HAMPERED BY PROLIFERATION DEFECT
Strategy

Bypass co-stimulation:

Auto-Transplant: Engraft designer T cells via lympho-expansive capacities of the body after lympho-depletion treatments
TIL -- Melanoma

But:
Responses not durable
Only melanoma, limited numbers
Technically challenging, antigen(s) unknown
Not reproducible in other studies

NMA – Melanoma TILs

Tumor Harvest

Non-myeloablative (NMA) Conditioning

Hematologic Recovery

Tumor Response

6/13 major responses

Dudley et al Science 2002;298:850
Prostate Cancer

T Cell Harvest

Ex vivo gene therapy

Anti-PSMA designer T cells

Non-myeloablative (NMA) Conditioning

Hematologic Recovery

Tumor Response

“...we’ll make more!”
-Prof J Leno

Junghans, proposal
Phase I Study of NMA Autologous Transplantation with Anti-PSMA Designer T Cells in Prostate Cancer
Treatment Schema

T cell collection ------ G-CSF ------ PSC collection ------- chemotherapy ---------
-45*          -20*          -16*       -7       -1 0 +28

Study Day

CTX 30 or 60 mg/kg d-7, d-6
Fludarabine 25 mg/m2 d-5 to d-1
## Phase I Study Enrollment Plan

**T Cell Dose, Number of Cells**

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**Monitoring**

- Safety
- Designer T cell persistence/expansion
  - in blood
  - In tumor
- Tumor response
Summary

- Uses in vivo expansion capabilities *independent* of costimulation
  - requires chemotherapy, but should be well tolerated
- All technologies available now
- FDA IND filing (11/09/04)
  - no clinical hold issues
  - minor product hold issues
- IRB/IBC approvals
- NGVL vector production pending
- Funding for trial pending
- Potential clinical start date 1/06
Regulatory Implications

- Safety of retroviral gene therapy
  - Replication competent retrovirus
  - Oncogenesis (insertional mutagenesis)

- Occurrence of T cell leukemias
  - 3/12 children treated for X-SCID
  - Autologous stem cells, RV vector, gamma-c gene
  - Mean 3 years after treatment/engraftment
  - 2 leuks with activation of LMO2; 1 with something else (TBD, but not LMO2)

- Uncertain relevance to other studies
Probability of Hit #1: LMO2 targeting in X-scid

*LMO2* targeting suggests either that there is a “physical hotspot” of integration at this locus, or more likely, that random, activating, *LMO2* integrants are selected simply by the growth advantage conferred on them. The chance of integration of any active gene is assumed to be 1/10.5 (a rough estimate of a random hit within 10 kbp among the estimated transcriptionally active 1 x 10.9 base pairs). It is likely that each patient received at least 1 to 10 *LMO2*-targeted cells, because the patients received 1 x 10.6 or more transduced T lymphocyte precursors (estimating that at least 1% of the total number of injected transduced cells—92 x 10.6 and 133 x 10.6 for patients P4 and P5, respectively—could give rise to T cells).

Hacein-Bey-Abina et al, 2003
More Than 1 Hit

LMO2++ mice, 100% of stem cells express only 10-70% --> leukemia (T-ALL), clonal lag time of 1 year.

Neale et al., Blood 86:3060;
Larson et al., Oncogene 9:3675.

This strongly suggests that additional factors leading to secondary genomic alterations were required for the development of the leukemia-like stage of lymphoproliferation in these patients.

Progression to Leukemia

100%  30%
HIT #1  HIT #2  LEUKEMIA
Ex vivo  In vivo
# Cells modified  # Cells expanded
- only for <10^5  - unlimited
Lineage independent  Lineage dependent
Effect of Cell Dose on 1st Hit

- Only chance to make real difference is $n < 0.1 \times (1/p)$
- Above this, all preps have same fraction of cells with first hit ($= p$).
- Final number at risk for 2nd hit = $pN$, where $N$ is final # of expanded cells.
  - Child $10^{-5} \times 10^{11} = 10^6$ (4)
  - Adult $10^{-5} \times 10^{12} = 10^7$ (5)

$P(1^{st}) = 1 - (1-p)^n$

$p = 10^{-5}$ of hitting a site
Comparing Gene Therapy Settings
2nd Hit

- **X-scid**
  - Insertional events saturating
  - Stem cells
    - Recombination may be part of risk
  - Infant/child
    - T-ALL childhood disease

- **Cancer**
  - Insertional events saturating
  - Mature T cells
    - Recombination complete
  - Adult
    - T-ALL rare
T Cell Ontogeny

T cell progenitors → TN stage → ISP stage → DP stage → SP stage → Mature T cells

- β gene rearr.
- γ, δ gene rearr.
- preTCR expr.
- α gene rearr.
- α:β TCR expr.
- cell division
  - hi
  - low

Duration
- n 14d
- 3-4d
- 7-14d

Thymocyte fraction
- 3%
- 80%
- 15%

Site
- Subcapsular Zone
- Cortex
- Medulla
- Periphery

TCR/CD3int → hi
HSAhi → lo
PNAhi → lo
Patient Survival with Treatment

![Graph showing patient survival with treatment. The graph indicates a comparison between no treatment and treatment with leukocytes. The x-axis represents years (0, 2, 6, 10), the y-axis represents percent survival, and the z-axis represents the presence or absence of leukocytes (-leuk, +leuk). The graph visually demonstrates a higher survival rate with treatment (+leuk) compared to no treatment (-leuk).]
Patient Survival with Treatment

![Survival graph showing comparison between no treatment and treatment with designer T cells over 10 years. The graph indicates a higher survival rate with treatment.]
Suicide Gene

Suicide gene drawbacks

- No data to support need
  - 100 adults treated with modified T cells, no leukemias
  - If cures obtained with T cell engraftment, can readdress risk
- Immunogenic
- Efficacy is major hurdle presently
- Occupies important site for gene co-expression
- Complicates vector
  - Difficult to omit “safety feature” once introduced
  - Increases delay to test efficacy
- May not work
Solutions

- Potential for leukemia in cancer protocol unknown
  - Data needed
  - Some cured patients *may* die from leukemia
    - Acceptable if treatment prolongs life for most (e.g., allo-BMT)
    - Focus on means
      - To prevent leukemia
      - To treat leukemia

- Cost to delay treatment while awaiting solutions
  - Each month delay = cost of life of 3000 patient deaths from prostate ca

- Best option: treat now
  - obtain data
  - solutions applied only if needed
THE END
IL2 (High Dose) and T Cell Numbers

- IL2 d1-d5
- T cells rebound in periphery after IL2 stop
- Gradually reduce in number to baseline
- Engraftment = stable
Tumor Infiltrating Lymphocytes (TIL)


- Surgery Branch, National Cancer Institute, Bethesda, MD 20892.

Lymphocytes extracted from freshly resected melanomas can be expanded in vitro and can often mediate specific lysis of autologous tumor cells but not allogeneic tumor or autologous normal cells. We treated 20 patients with metastatic melanoma by means of adoptive transfer of these tumor-infiltrating lymphocytes and interleukin-2, after the patients had received a single intravenous dose of cyclophosphamide. Objective regression of the cancer was observed in 9 of 15 patients (60 percent) who had not previously been treated with interleukin-2 and in 2 of 5 patients (40 percent) in whom previous therapy with interleukin-2 had failed. Regression of cancer occurred in the lungs, liver, bone, skin, and subcutaneous sites and lasted from 2 to more than 13 months. Toxic effects of interleukin-2 occurred, although the treatment course was short (five days); these side effects were reversible. It appears that in patients with metastatic melanoma, this experimental treatment regimen can produce higher response rates than those achieved with interleukin-2 administered alone or with lymphokine-activated killer cells. It is too early to determine whether this new form of immunotherapy can improve survival, but further trials seem warranted.

- TIL (11/20) = 55%
Interventions

- Phlebotomy/Apheresis
- Isolate patient’s peripheral blood mononuclear cells (PBMC)
- Activate/transduce with IgTCR
- Expand in IL2
- Harvest cells equal to dose; Infuse
- Monitor for Toxicity/Response
Costimulation For T-Cell Activation

**T Cell Activation**

- Signal 1
- Signal 1 + 2

- Anergy

**Naïve T Cells**

- Gene expression
  - Cytokines (IL-2, 4, IFN-γ, etc)
  - Surface molecules (CD25, CD69, CD40L, etc)
- Effector function (T help, Cytotoxicity)
- Proliferation
- Apoptosis (AICD – Activation induced cell death)

**T Cell Re-activation**

- Signal 1
- Signal 1 + 2

- Cytotoxicity, AICD-Apoptosis
- Cytotoxicity, Cytokine release, Proliferation

**Activated T Cells**
Cancer Regression and Autoimmunity in Patients After Clonal Repopulation with Antitumor Lymphocytes

Mark E. Dudley,¹ John R. Wunderlich,¹ Paul F. Robbins,¹ James C. Yang,¹ Patrick Hwu,¹ Douglas J. Schwartzentruber,¹ Suzanne L. Topalian,¹ Richard Sherry,¹ Nicholas P. Restifo,¹ Amy M. Hubicki,¹ Michael R. Robinson,² Mark Raffeld,³ Paul Duray,³ Claudia A. Seipp,¹ Linda Rogers-Freezer,¹ Kathleen E. Morton,¹ Sharon A. Mavroukakis,¹ Donald E. White,¹ Steven A. Rosenberg¹*
Informed Consent

Assignment

Low Dose: Blood Draw

Moderate Dose: Leukopheresis

High Dose: Leukopheresis

Collect T Cells: 1-2 months before treatment

Collect Cells: 2-3 weeks before treatment

Chemo 1 week before treatment

Number of T Cells

Leukopheresis Collect Cells For Immune Support

Cytoxan 2 Days Inpatient

Fludarabine 5 Days Outpatient

Day 0:
Inpatient For 1-2 Days
Receive T cells
Start IL2:
IL2 for 4 wks

High Dose Patients will receive Bone Marrow Biopsy on Days 2 and 14 during treatment.
Animal model:

**Tumor Free Mice after Anti-PSMA Designer T Cell Therapy**

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<tr>
<td>T</td>
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<td>T-IgTCR</td>
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Treatment Plan

- T cell harvest, Designer T cell preparation
- NMA conditioning (CTX, Fludarabine)
- Designer T cell infusion (x1)
  - interleukin 2 co-administration (outpatient)
- Monitoring
  - Safety
  - Designer T cell persistence/expansion in blood
  - Tumor response