P1-27

T CELL GENE THERAPY TO ERADICATE DISSEMINATED BREAST CANCERS

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ABSTRACT (P1-27)

Background: We created "designer T cells" (dTc) by retroviral gene therapy to express chimeric immunoglobulin-T cell receptors (IgTCR) with specificity for carcinoembryonic antigen (CEA). CEA is the 2nd most prevalent tumor antigen on breast cancers, on 30-60% of metastatic cases, accounting for 12-25,000 breast cancer deaths per year. Our previous Phase I trial with 1st generation (1st gen) dTc showed proof-of-principle "biologic responses", but of limited duration. Lab correlates showed modified T cells repeatedly kill tumor targets over 4-7 days but then undergo activation-induced cell death (AICD). We created 2nd gen dTc that incorporate CD28 co-stimulation (signal 2) into the IgTCR (IgCD28TCR), suppressing AICD and promoting T cell proliferation on tumor contact with superior tumor responses *in vivo* (Emtage et al. Clin Cancer Res 2008;14:8112). A Phase I clinical trial was approved under FDA BB-IND 10791. A dose escalation was performed without co-administration of IL2 and was well-tolerated. The importance of IL2 is assessed in the present setting in a Phase Ib/Pilot. Following this, methods are proposed in which to further optimize the power of this modality to eradicate breast cancers. The research plan includes a recursive set of studies that link the laboratory and clinic, assessing the role of cytokines, administration method and anti-apoptotic molecules.

Methods: Patient T cells are modified ex vivo, expanded and then administered by intravenous infusion. At the current time, a Phase Ib/Pilot study is being conducted at our maximum practical dose (MPD; 10^11 cells), with randomization between –IL2 and +IL2 by continuous outpatient infusion. Patients undergo tumor biopsy at day 2 and day 14 to assess for initial dTc infiltration and subsequent expansion to judge the value of IL2 co-administration. Patients are monitored for safety, pharmacokinetics and response. Laboratory studies explore the use of additional signals to improve the dTc expansions and to overcome the time-limited production of IL2 by dTc after repeated tumor contact. We also investigated methods to allow co-expression of chimeric receptors containing homologous domains that are normally deleted in retroviral replication.

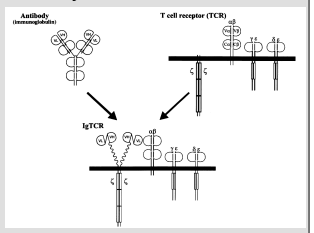
Results: We proved that addition of LFA1 to deliver signal 3 into the chimeric receptor significantly enhances the quantity of IL2 production and dTc expansions, but did not achieve the primary goal of avoiding IL2 exhaustion with recurrent T cell stimulation. Degenerate codon nucleotides introduced into repeated domains suppressed deletion during retroviral transduction, enabling the co-expression of more than one signaling molecule in a retrovirus. For the clinical trial, harmonization of the Phase Ib/Pilot IRB-approved protocol with the Army HSRRB was completed. One subject has been enrolled and treated who randomized to the +IL2 arm. Cells were infused over 30 min without complication. Blood clearance was rapid. Results will be updated to include new patients at conference time.

Conclusions: A new approach to adoptive immune therapy in metastatic breast cancer has been devised. Laboratory studies are supporting the development of still more advanced dTc versions and methods of administration. Patients with breast cancer are being actively recruited on the clinical trial. **For patient referrals, please contact Dr Junghans' office by telephone: (401) 456-2507 or email: RDavies@rwmc.org.**

DESIGNER T CELL (dTc) BASICS

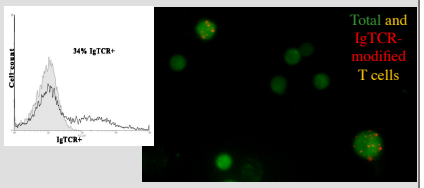
IgTCR = Fusion of Antibody and TCR Zeta Signaling Chain

Also: CAR = Chimeric Antigen Receptor

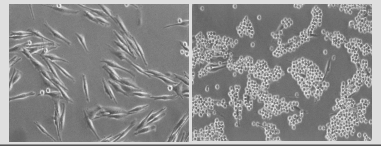


Clinical Retroviral Vector

DESIGNER T CELLS ARE EFFICIENTLY GENERATED & REDIRECT T CELLS TO TUMOR



Tumor + Normal T cells *Tumor + Designer T cells*



BREAST CANCER TARGET ANTIGEN

Antigen	Tumor Incidence	Ag+ Tumor Deaths/yr	Prior ClinTrial?	Advanced CIR?	IND Filed?	Study Active?
Mucin (muc-1)	80%	35,000	No	*Yes*	No	No
CEA	***30-60%***	***12-25,000***	*Yes*	*Yes*	*Yes*	*Yes*
Her2/neu	20-30%	6-12,000	No	No	No	No
Folate binding prot	10-20%	3-6,000	*Yes*	No	No	No

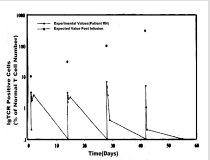
Carcinoembryonic antigen (CEA) in breast cancer

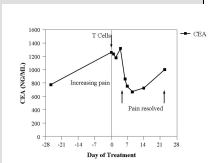
- Expression
  - High on tumor, low on normal tissues
  - Topological sequestration (lumenal surface of bowel)
- High clinical relevance:
  - On 30-60% of metastatic breast cancers
    - **More common on breast tumors than Her2**
    - 12-24,000 deaths/yr from CEA+ breast cancers
  - Also: colorectal, pancreas, lung, others

PRIOR CLINICAL

PHASE I CLINICAL TRIAL ANTI-CEA DESIGNER T CELLS (BB-IND 7301)

TWO LESSONS:
1. RAPID SYSTEMIC LOSS OF DESIGNER T CELLS
2. TRANSIENT ANTI-TUMOR EFFECTS

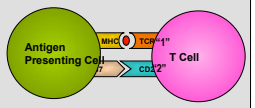




T CELL BIOLOGY

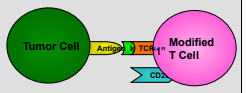
"T cells evolved to kill virus-infected cells."

T Cell Activation =
2 Signals!
TCR = Signal 1
CD28 = Signal 2



Designer T Cells
1st Generation =
Signal 1 only!

Can lead to Activation-Induced Cell Death (AICD)



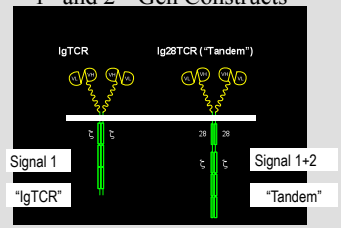
MIPCEA

HYPOTHESES

1. Failure to sustain initial tumor response with prolonged tumor destruction can be attributed to signal deficiency of 1st gen dTc
2. Incorporation of CD28 into the CAR will avoid AICD, promote dTc proliferation on contact with tumor, and lead to sustained anti-tumor responses.

CAR RE-DESIGN

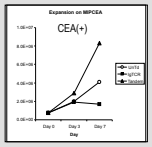
1st and 2nd Gen Constructs



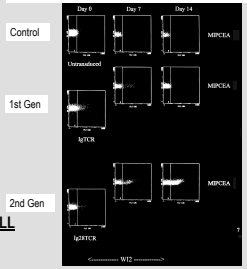
PRECLINICAL DATA

1. IMPROVED PROLIFERATION

2nd Gen dTc Resist AICD and Proliferate on Tumor Contact



2nd Gen dTc are Selectively Expanded

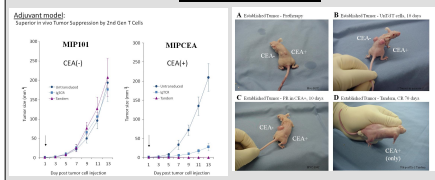


ALSO (not shown):

2. IMPROVED TUMOR CELL KILLING

3. IMPROVED CYTOKINE PRODUCTION

4. IN VIVO ASSAYS SHOW TUMOR CURES



OTHER DATA IMPLY IL2 CRITICAL TO CURE

SELF PRODUCTION OF INTERLEUKIN 2 INSUFFICIENT

Improved IL2 secretion but exhausted on repeat tumor contact.

With established tumor:
1. Only animals +IL2 had response
2. Only with +IL2 was superiority of 2nd gen evident

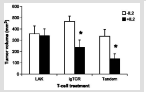


Table 1. Frequency of complete responses increases with more signals

	LAK 1 signal	IgTCR 1 signal	Tandem 2 signals
Complete response	0	2	6
Total complete responses	16	18	8

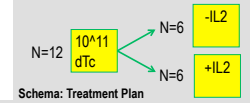
NOTE: More than +IL2 experiments of Fig. 4 plus animals from two further pilot tests were scored for response at 19 d, with regressed tumors termed as complete responses. "p" for trend" shows complete responses with more signals significant at P = 0.0060.

IMPLIES: NEED TO SUPPLEMENT HUMAN THERAPIES +IL2 ?

CLINICAL TRIAL DESIGN

Phase Ib/Pilot Trial of 2nd Generation Anti-CEA Designer T Cells in Metastatic Breast Cancer; BB-IND 10791*

ELIGIBILITY
Stage IV or inoperable
PS 0-2
CEA+ (by tumor IHC unless serum CEA elevated)
Measureable disease



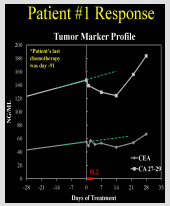
Schema: Treatment Plan

* (Prior Phase Ia with doses of 10^9 and 10^10 dTc (-IL2) showed safety and minor responses)

Hypotheses: High dose 2nd gen dTc will suppress breast cancer; IL2 supplementation is required to realize full benefit of therapy.
Objectives: Safety/tolerance, optimal biologic dose (-IL2/+IL2), tumor response, pharmacokinetics/pharmacodynamics

Patients randomized to –IL2 or +IL2 (28d civi low dose outpatient IL2; 75 KIU/kg/d), 6 pts per arm.
Tumor Bx for initial trafficking (day 2) and then persistence/ amplification (day 10) of dTc in tumor.

IL2 ◀ 20 IU/ml



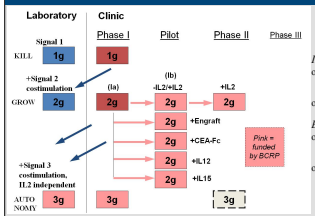
Pt #1, randomized to +IL2.
Two limiting factors: Dose only ~30% of target, and IL2 was d/c after 3d due to poor patient tolerance.
Response of 20-25% in serum markers, nadir at d14, then increasing.

Indicates "biologic response" = immune targeting. But transient and shallow. With full target dose and continued IL2, response much better?

Pt #2 dose prepared. But had rapid progression and was not treated.
Pt #3 (new Pt #2 replacement) dose currently being prepared.

PLAN: Patient recruitments; 10 more to complete Phase Ib/Pilot

FUTURE DIRECTIONS



Hypotheses for Benefit of Adjunctive Measures

In vivo
- Engraftment : to promote sustained high dTc levels for improved clinical response
- CEA-Fc: to activate and expand systemic dTc levels

Ex vivo
- IL12: to induce Th1/Tc1 shift to improve dTc potency ("quality")
- IL15: with IL2, to improve dTc expansions for better target dose achievement ("quantity")

SUMMARY

1. *Phase Ib/Pilot tests value of +IL2 with dTc:*
   3 doses prepared or in preparation;
   1 pt treated with encouraging features
2. *Continue patient enrollments*
3. *Lab studies to improve IL2 independence*
4. *Future trials to test improvements*

Goal: 100% tumor reduction = Eradication!

Thanks to Dr Susan Love/Avon Army of Women and Rhode Island Breast Cancer Coalition for recruiting assistance

PATIENT REFERRALS
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