Strategies to Enhance Dendritic Cell-Mediated Antitumor Immunity

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Objective

*Therapeutic Immunity*

Vaccine Potency = **Magnitude** x **Quality** x **Persistence**

**Threshold of ‘Therapeutic’ Immunity**

**Vaccine Development**

**Vaccine with therapeutic impact**

**Vaccine with no or minimal therapeutic impact**

**Patient Factors**

*i.e.*

- Tumor burden,
- Immune suppression
- Pretreatment,
- Age,
- Disease setting,
- Other factors
A Multi-Pronged Approach to Cancer Immunotherapy

**Type**
- CD4 immunity (LAMP, Ii inhibition)

**Frequency**
- DC vaccination
- In situ maturation

**Antigen**
- Defined “universal” antigens
- Mixtures (ampl. mRNA)

**Induction of immunity**
- Tregs (ONTAK®)
- ImC (ATRA)
- B7H-1, TGFβRII, DcR3 aptamers

**Enhance survival**
- Costimulation: OX40, 4-1BB CD27, CD40 (mRNA/DC)

**Prevent attenuation**
- CTLA-4, PD-1 aptamers

**Persistence of immunity**
- Stromal antigens
- CD4+ T cell effectors

**Immune suppression**

**Immune evasion**
Activation of T–Cells by APC

From: Abbas et al. Cancer Immunology
Dendritic cell-based vaccines using tumor antigen in the form of mRNA

- Powerful method for stimulating antitumor immunity
- Broadly applicable to all cancer types
- Solution to the problem of treatment-related emergence of resistant variants
**Background:** Phase I Clinical Trial using semi-mature PSA RNA transfected DC

A. Phenotype after RNA Loading

![Graph showing PSA-mRNA Titration](image)

B. Evidence of Immunogenicity

![Bar graph showing spots per 5x10^5 PBMC](image)
Background: Phase I Clinical Trial using semi-mature PSA RNA transfected DC

C. Impact on PSA Velocity

D. Clearance of Circulating Tumor Cells

D. Entire Group

Patient ID  Pre  Post
#2  #3  #5  #7  #9  #13  #16

PSA Log Slope

0 0.05 0.10 0.15 0.20

0 4 8 12

PSA Log Slope

0 1 1.5 2 2.5

0 4 8 12

PSA mRNA Copies/10^7 PBMC

0 1.5 3 4

0 4 8 12

EpCAM mRNA Copies/10^7 PBMC

0 100 200 300 400

0 50
Increase of Tumor-Specific T Cells after Vaccination with semi-mature RCC RNA transfected DC

Patient ID

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G250

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Survival of Subjects immunized with Renal Tumor RNA Loaded DC

Gleave et al. N=188
Mature, but not Immature TERT RNA Loaded DC Elicit A Local Inflammatory Response at the Injection Site
Mature, but not Immature Dendritic Cells are Capable of Migrating to Draining Lymph Nodes
Telomerase (hTERT)  
*A Broadly Expressed Candidate Tumor Antigen*

- hTERT can be processed for **class I presentation** in a broad range of human tumors.
- Telomerase is an attractive candidate for a **broadly expressed tumor rejection antigen**
  - Silent in most somatic tissues
  - Reactivated and over-expressed in the majority of human solid tumors
- Reduced risk of **antigen-escape** tumor cell variants.
Targeting mRNA-encoded antigens into the endosomal/lysosomal compartment

A.  pGEM4Z/hTERT/A64

T7 promoter  hTERT aa1-1132  PolyA

B.  pGEM4Z/hTERT- LAMP/A64

T7 promoter  hTERT aa168-1132  PolyA

gp96  hLAMP
aa1-27  aa 382-416

Leader sequence

TERT mRNA-Transfected DC

Clinical Trial Design

**Dose Schedule A:** 3 cycles of $1 \times 10^7$ cells i.d. per cycle

**Dose Schedule B:** 6 cycles of $1 \times 10^7$ cells i.d. per cycle

- Determine Eligibility
- Metastatic Prostate Cancer
- Informed Consent
- Leukapheresis
- RANDOMIZE
- TERT RNA loaded DC
- LAMP TERT RNA loaded DC
- Leukapheresis
- Follow-up

**Week 0**  
**Week 2**  
**Week 4**  
**Week 6**  
**Week 8**  

Pre-Treatment Phase  
Treatment Phase  
Follow-up
# Patient Characteristics

## Table 1. Characteristics of subjects enrolled

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<tr>
<th>Subject ID No.</th>
<th>Age</th>
<th>Karnofsky Index</th>
<th>Diagnosis of Metastases - Treatment (Months)</th>
<th>Stage (Jewett)</th>
<th>Prior Therapy</th>
<th>Pretreatment PSA (ng/dl)</th>
<th>Metastases (Study Entry)</th>
<th>Assigned Dose Level (Total Dose)</th>
<th>Cell Product (mRNA-Transfected DC)</th>
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Pre-treatment: XRT¹, primary irradiation; XRT², local (palliative) irradiation for painful bony metastases; RP, radical prostatectomy; H, medical hormonal ablative therapy; C, chemotherapy; O, orchietomy. Metastases: LN, lymphadenopathy; BN, bony metastases; ST, soft tissue metastases.
A. DTH Diameter (mm) vs. Vaccine Cycle Number

B. Images of hTERT and LAMP-hTERT

C. Bar graphs of cytokine levels (IL-2, TNF-α, IFN-γ, IL-10, IL-4, IL-5)

D. Graphs showing specific lysis for DC+hTERT and DC+GFP / K562
Stimulation of hTERT-specific T-cell responses After Vaccination with TERT RNA transfected DC
Kinetics of the Antigen-Specific CD8$^+$ T-cell Response

Longitudinal Evolution of CD8$^+$ and CD4$^+$ T cell Responses

**TMS-16-TRT**

**JRL-15-TRT**

**AJG-18-LMP**

**FSH-19-LMP**

Study Week

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<tr>
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<td>600</td>
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<td>1200</td>
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Characterization of Vaccine-induced CD8$^+$ T cells

CD8$^+$ hTERT

- IL-2
- IFN-γ
- TNF-α

CD8$^+$ LAMP hTERT

- IL-2
- IFN-γ
- TNF-α
Impact on PSA Doubling Time and Circulating Tumor Cells

A. Three Weekly Doses (n=7)

- PSA Doubling Time (months)
  - PRE: 4.6
  - POST: 3.8

B. DGE-06-LMP

- PSA mRNA
  - Study Period (weeks): -4, 0, 2, 4, 6, 26

Six Weekly Doses (n=5)

- PSA Doubling Time (months)
  - PRE: 2.9
  - POST: 100.0

B. BRH-05-TRT

- PSA mRNA
  - Study Period (weeks): -4, 0, 2, 4, 6, 26
Conclusions

• Powerful method of stimulating hTERT-specific CD4$^+$ and CD8$^+$ T cell responses in cancer patients.

• Evidence that LAMP-hTERT RNA transfected DC are capable of stimulating higher frequencies of hTERT – specific CD4$^+$ T cells
  — DTH reactions/ELISPOT/cytolytic assays
  — Induction of central T cell memory

• Lack of tolerance with increasing numbers of vaccinations.

• Impact on PSA doubling time and clearance of circulating tumor cells.
Elimination of Regulatory T cells

**Rationale**

- Some studies suggest increased levels of Treg in cancer patients.
- Antibody-mediated elimination of Treg has shown to elicit antitumor immunity in tumor-bearing mice.
- Anti-CD25 mAB therapy was capable of enhancing the therapeutic effects of tumor vaccines.
Elimination of Regulatory T cells

**Approach**

DAB$_{389}$IL-2 is a recombinant fusion protein that contains the catalytic- and membrane translocation domain of diphtheria toxin fused to human IL-2, allowing targeting of CD25$^+$ cells.
Human CD4^+CD25^+ Regulatory T cells

**Definition**

A. CD4^+/CD25^+

B. CD4^+/CD25^{neg}

C. CD4^+/CD25^{int}

D. CD4^+/CD25^{high}
Enhancement of T-cell Immunity after T_{reg} Depletion

A. CD4^{+}/CD25^{high} and PBMC±CD4^{+}/CD25^{high} Viability over Time (hours)

B. Stimulatory Index of PBMC and DC with Treg

C. Specific Lysis (%) for hTERT, fluM1, MART1 RNA and pep with E:T Ratio
Monitoring for Regulatory T cells in a Vaccination Setting

CD4

CD4

CD25

PMA/Ionomycin

allogeneic MLR

97.8 1.3

88.3 9.6

93.0 4.9

0.02 1.0

1.3 1.2

0.2 3.0

7.6 4.2

Isotype

Isotype

GITR

CTLA-4
Depletion of CD4⁺/CD25^{high} Regulatory T cells After DAB₃₈₆ IL-2 Administration

Patient: HMT-04-DAB

CD4

CD25

pre

post (4d)

post (28d)

Isotype

GITR

0.4

0.0

4.6

1.6

3.4

71.9

3.6

0.8

76.5

77.8

2.4

3.4
Depletion of CD4⁺/CD25<sup>high</sup> Regulatory T cells After DAB<sub>386</sub>IL-2 Administration

Patient: JB01-RCC  ONTAK 18µg/kg + RCC RNA DC (2 doses)

Pre 1<sup>st</sup> vaccination =75% depletion efficacy

Pre vaccination

Post 2<sup>nd</sup> vaccination

A. Antigen-specific Proliferation

B. INF-γ ELISPOT (CD4<sup>+</sup> T Cells)

C. INF-γ ELISPOT (CD8<sup>+</sup> T Cells)
# Efficacy of Depleting Regulatory T cells in Metastatic Cancer Patients

## Graph

![Graph showing percent CD4+ T cells](image)

## Table

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<th>Marker</th>
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<th>CD25 Double Positive (% of Single Positive)</th>
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Elimination of $T_{reg}$ is Capable of Enhancing Vaccine-mediated T-cell Responses

A.

B.
Elimination of Regulatory T cells

Conclusions I

• NIH and FDA-approved clinical trial.
• Demonstration of selective Treg depletion following single dose of DAB$_{389}$IL-2.
• Enhancement of T cell responses \textit{in vitro}, predominantly against ‘naturally processed’ self-antigens.
• Safety, no clinical signs of autoimmunity in 10 patients treated thus far.
Elimination of Regulatory T cells

Conclusions II

• No interference with CD4⁺/CD25^{int} memory T cell pool.
• Stimulation of high frequencies of RCC-specific T cells \textit{in vivo} after combined therapy.
• Polarization of RCC-specific CD4⁺ T cells towards Th-1, but not Th-2.
• This strategy could have broad implication for the design of active and passive immune-based protocols.