Adoptive Transfer of Autologous T Cells Targeted to Prostate Specific Membrane Antigen (PSMA) for the Treatment of Castrate Metastatic Prostate Cancer (CMPC)

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Abstract

RATIONALE: Expression of prostate-specific membrane antigen (PSMA) in prostate cancer increases with disease progression and is highest in metastatic disease, especially hormone-refractory disease, and higher grade lesions. We therefore hypothesize that a sensitive targeting strategy using autologous P28z+ T cells that can target to PSMA-expressing tumor cells may offer a unique non-toxic treatment. Chimeric antigen receptors (CARs) that comprise both CD28 and CD3ζ cytoplasmic domains were shown by our group and others to better support T cell activation by cancer cells that present antigen in the absence of activating regulatory ligands.

OBJECTIVES: This is a Phase I dose-escalating trial designed to assess the safety, dose requirement, and targeting efficiency of genetically directed autologous human T cells targeted to PSMA, a molecule widely expressed on prostate cancer cells using CARs. The clinical approach is based on the infusion of PSMA-targeted T cells (utilizing the P28z receptor) in patients with castrate metastatic prostate cancer, following cyclophosphamide administration. The aims of the clinical trial are (1) to assess the safety of PSMA-targeted T cells; (2) to measure biologic and anti-tumor effects using PSA (prostate specific antigen), circulating tumor cells, and clinical imaging modalities; (3) to assess T cell targeting using molecular biology techniques and positron emission tomography (PET) to quantify T cell persistence, accumulation, and track T cell migration; and (4) to measure the immune response to prostate cancer-encoded antigens following PSMA-targeted therapy. This approach has been shown to be feasible in hematologic malignancies where it is currently in clinical trials.

METHODS: We propose to test a total of three T cell doses: 1 x 10^7 CAR$^+$ T cells/kg, 3 x 10^7 CAR$^+$ T cells/kg, and 1 x 10^8 CAR$^+$ T cells/kg, respectively, that have been genetically transduced to target PSMA. To enhance the efficacy of adoptively transferred T cells, all patients will receive pretreatment with cyclophosphamide (Cy) at 300 mg/m^2 intravenously the day prior to receiving autologous targeted T cells. This is primarily a safety and tolerability study with secondary endpoints that will include the study of (1) changes in bone metastases by bone scan, CT (computed tomography) scan, and/or MRI (magnetic resonance imaging; (2) changes in biomarkers of bone metastasis and metabolism; (3) changes in circulating tumor cells (CTCs) pre- and post-treatment; (4) changes in humoral and cell-mediated immunity to PSMA and other known prostate cancer antigens; (5) pattern of change in PSA from pre- to post-adoptive transfer levels; and (6) the persistence and migration of genetically retargeted anti-PSMA autologous T cells using quantitative molecular and/or imaging technologies.

CONCLUSIONS: Patient selection is currently underway in preparation for initiating clinical enrollment and treatment.

Background

Preclinical Proof of Principle

- *in vivo* function of Pz1, a CAR-targeting human PSMA
- Pz1 receptor encompasses the ζ chain of the CD3 complex as its activation domain
- Specifically redirects *in vitro* cytolysis against PSMA-positive tumor cell lines

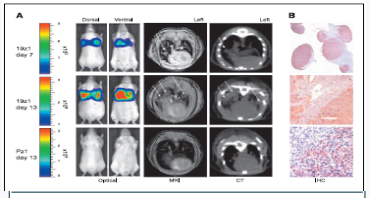
➢ **Hypothesis:** Are expanded Pz1+ T cells active *in vivo*?
- Do they require costimulation after adoptive transfer?
- Three tumor models established three tumor models in SCID-bg/bg mice—orthotopic, s.c., and pulmonary.

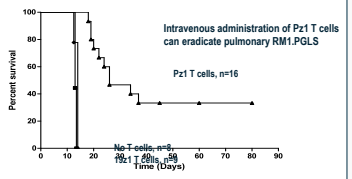
➢ **Conclusion:** Direct administration of Pz1+ T cells in orthotopic and s.c. human prostate tumors eliminated a majority of the tumors.

CHIMERIC ANTIGEN RECEPTORS (CARs)

- Encompass Ig variable regions/receptor ligands as their antigen recognition moiety
- Permits T cells to recognize cell surface tumor antigens in the absence of HLA expression
- Requirements for genetically targeted T cells to function in vivo not clearly defined, hence need to establish *in vivo* conditions
- T-cell activation - mediated by the cytoplasmic domain of the CAR, which is typically derived from the CD3ζ chain or the FcRIγ chain
- Chimeric antigen receptors (CARs) encompass immunoglobulin variable regions or receptor ligands as antigen-recognition elements
- Permit T cells to recognize cell surface tumor antigens in the absence of HLA expression.

Murine Targeting of PSMA-expressing tumors with PSMA -directed CARs





Gade et al., Cancer Res, 2005

Conclusions

➢ Pz1-transduced PBLs - highly antigen specific and cytolytic *in vivo*.

➢ In three tumor models—orthotopic, s.c., lung metastases: Pz1 but not 19z1-transduced T cells induced durable remissions and cures in a substantial fraction of the treated animals.

Rationale for Translation into Humans

PSMA as a Target for Therapeutic Approaches

- PSMA - dimeric type II integral transmembrane protein with glutamate carboxypeptidase activity
- Overexpressed as prostate cancer cells makes transition to castration resistant state
- Abundantly expressed on neovasculature: bladder, pancreas, melanoma, lung, and kidney cancers, but not on normal neovasculature
- Membrane-bound nature of PSMA and its expression signature are attractive features for targeted immunotherapy of prostate cancer.

Genetically redirected adoptively transferred T cells

- Augment T cell expansion
- Generate memory lymphocytes
- CARs that comprise both CD28 and CD3 ζ cytoplasmic domains were shown by our group to better support T cell stimulation by target cells that present antigen in the absence of activating costimulatory ligands.

Aims

Although the primary objective of this study is not the determination of efficacy, the following exploratory assessments will be made:

➢ to assess changes in bone metastases by bone scan, CT scan, and/or MRI

➢ to assess changes in biomarkers of bone metastasis and metabolism

➢ to assess changes in circulating tumor cells (CTCs) pre- and post-treatment

➢ to assess humoral and cell-mediated immunity to PSMA and other known prostate cancer antigens;

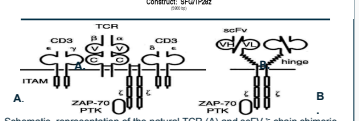
➢ to assess patterns of change in PSA from pre- to post adoptive transfer levels.

➢ to track the persistence, accumulation, and migration of genetically retargeted anti-PSMA autologous T cells using quantitative molecular and/or imaging technologies.

Methods

- P28z receptor - selected for clinical investigation.
- Potential risk of initiating an immune response against normal tissues that express PSMA
- To circumvent possible risks, herpes simplex virus-1 thymidine kinase (hsvtk) gene will be co-transferred with the cDNA encoding the P28z receptor, utilizing the TP28z gamma-retroviral vector.
- Constitutive expression of hsvtk has been extensively investigated and renders T cells sensitive to ganciclovir - providing a means to eliminate diving T cells if required.
- Expression of hsvtk enables PET imaging using radiolabelled FIAU to image the localization of adoptively transferred T cells

SFG-TP28z.3 gammaretroviral vector



Schematic representation of the natural TCR (A) and scFV-ζ chain chimeric molecule (B)

Imaging PSMA-directed T Cells

- ^{124}IodineFIAU as an imaging agent has the advantage of repeatability with a t1/2 of 4 days.

Its usefulness is in:

➢ querying the location of cells at shorter intervals

➢ lower background at 24 hours

➢ greater flexibility of use.

➢ The drug is subfiled under a MSKCC Master Drug File held by the FDA.

Schema

➢ Up to 18 patients will be treated with 3 escalating T cell doses.

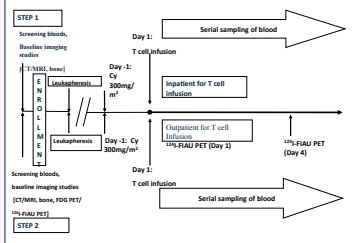
➢ Since there is only limited published data regarding the safety of genetically modified T cells in patients with cancer, the proposed treatment doses in this study are based on safety data in previously published studies using cloned autologous CD4+ or CD8+ T cells in melanoma patients.

➢ The safety of the lympho-depleting cyclophosphamide dose proposed in this study is based on our prior experiences at MSKCC with high dose cyclophosphamide in the treatment of patients with melanoma and CLL.

Rationale for Combining Engineered T Cells With Cyclophosphamide:

➢ Cyclophosphamide (Cy) - active agent in pc
➢ Lympho-depleting chemotherapy may enhance the ability of adoptively transferred tumor specific T cells to proliferate *in vivo* through homeostatic proliferation.
➢ Prior therapy with Cy may transiently ↓ patient CD4$^+$CD25$^+$ regulatory T cells that would otherwise suppress the function of tumor-targeted adoptively transferred T cells.
➢ Administration of Cy prior to adoptive T cell therapy may enhance the expression of stromal cell-derived factor-1 expression in the bone marrow, enhancing the homing of modified T cells to the primary tumor site through binding of SDF-1 with CXCR-4 expressed on the T cell surface.
➢ The infused T cells comprise both CD4+ and CD8+ T cells, both of which will be targeted to PSMA

Treatment Scheme



Pre-treatment, First cohort only:

➢ One day prior to admission for T cell infusion, patients with receive Cy at 300mg/m^2 iv at the Kimmel Center.

➢ The next day, patients will be admitted to the MSKCC GU inpatient service for intravenous hydration, clinical monitoring and blood work for immune monitoring.

➢ If at least 50% of the planned T cell treatment dose is not obtained, patients will be treated with the obtained T cell dose but will be removed from the dose escalation analysis and replaced by the next enrolled subject. Given safety data from a similar trial in CLL [MSKCC IRB#06-138, R. Brentjens, PI], we feel that it is reasonable for only the first cohort to be hospitalized for safety monitoring.

Schema (con't)

Dosing Regimen

Dose Level*	T Cell Dose	Number of patients
1 (Step 1)	1x10^7 CAR + T cells/kg	3-6
2 (Step 2)	3x10^7 CAR + T cells/kg	3-6
3 (Step 2)	1x10^8 CAR + T cells/kg	3-6

*4th Cohort of 3 patients may be added if an anti-PSMA effect is observed either immunologically or radiographically or if there is preferential targeting of the cells at a particular does level. The dose level of the 4th cohort would be from a previously tested.

Cohorts 2, 3

➢ **Pre-treatment:** One day (Day-1) prior to T cell infusion, patients with receive Cy at 300mg/m^2 iv at the Kimmel Center.
➢ The next day, patients will return to the outpatient Center for T cell infusion, intravenous hydration, clinical monitoring, and blood work for immune monitoring.
➢ Tracking of T cells in vivo. An FDG-PET scan will be performed within 24 hours of the [^{124}I]-FIAU PET scans at screening.
➢ FDG-PET scan will be performed at week 12 post treatment.
➢ [^{124}I]-FIAU-labeled cells will be tracked on days 1 and 4 (72 hours) post treatment.

Correlative Studies

Biologic correlates

- CTCs, weeks 4, 12, 24 and every 3 mos.
- Biomarkers for Bone Metastasis and metabolism
- Δs in the number and magnitude of bony lesions as detected by imaging at screening and weeks 12, 24.

Exploratory Immune Monitoring

- PSMA-specific Antibodies:
- Flow Cytometry Assay
- ELISA Assay for PSMA Antibodies
- Cellular Immunity (PSMA-specific T-Cell Assay):
- IFNγ-release ELISPOT
- Persistence of genetically modified T cells: The quantification of persisting, genetically modified T cells in peripheral blood, bone marrow and any other cellular specimens will be performed by quantitative PCR

Results/Conclusions

❖ **Vector sterility studies completed.**

❖ **Certificate of analysis for 293GP-GLVg/TP28z.3 Master Cell Bank (293G P-G LV9/TP28z.3 clone5 MCB completed.**

❖ **First patient enrolled and awaiting T cell reinfusion.**