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14. ABSTRACT CTLA-4 is an inhibitory molecule on T cells that induces T cell downregulation. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a growth and survival factor for dendritic cells. The safety of combining GM-CSF with CTLA-4blockade in prostate cancer patients is being investigated in an ongoing phase I trial. Methods: Sequential cohorts of 3-6 patients receive GM-CSF 250µg/m2/d subcutaneously on days 1-14 of a 28-day cycle withescalating doses of anti-CTLA antibody on day 1 of each cycle x 4. Patients are monitored for clinical autoimmunity with T cellphenotyping performed.Results: Twenty patients have been treated to date. Dose-limiting toxicity (DLT) was not observed in the initial CTLA-4 antibody doselevel. Two DLTs, consisting of a vertebrobasilar TIA possibly related to therapy and a generalized rash requiring steroids wereobserved in the second and third dose levels respectively, resulting in expansion of each to 6 patients. No laboratory evidence ofautoimmunity has been observed in any patient. Expansion of monocytes / dendritic cells and upregulation of PBMC activation markershave been seen, consistent with known GM-CSF effect. A dose response relationship has been seen between anti-CTLA-4 dose andactivation of both CD4+ and CD8+ T cells in the blood. These effects were increased compared to effects seen with anti-CTLA4treatment alone on a separate trial. T cell interferon-gamma production and lytic activity were also enhanced in circulating antigen-specific CD8+ T cells after this combination immunotherapy.Conclusions: CTLA-4 blockade and GM-CSF has demonstrated preliminary safety in advanced prostate cancer. Accrual andimmunologic analyses are ongoing. A phase II trial is being planned of this combination in vaccination-failure prostate cancer patients.						
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Introduction

This research project ultimately aims to develop effective immunotherapy for prostate cancer. Specifically, we are exploring the use of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in combination with blockade of a T cell inhibitory molecule called Cytotoxic T-Lymphocyte-Associated Molecule-4 (CTLA-4). We are initially studying repetitive dosing of an anti-CTLA-4 antibody in combination with subcutaneous GM-CSF to determine the safety of this combination for future clinical testing. We will then move this combination therapy into a phase II trial to look at effects on PSA and other clinical endpoints in patients failing prior vaccination for prostate cancer. Concomitantly, peripheral blood is being collected from patients to evaluate the immune response generated.

Body

Blockade of the T cell inhibitory receptor, CTLA-4, is a potent method of augmenting and potentiating T cell responses. Pre-clinical work has demonstrated enhancement of T cell responses and prostate tumor rejection via antibody-mediated blockade of CTLA-4.(1-3) Addressing a separate and similarly vital component of a coordinated immune response, systemic GM-CSF is a growth factor for dendritic cells (DC) and stimulated DC uptake of antigen to cross-prime T cells. GM-CSF has also produced PSA declines and PSA modulation in multiple prostate cancer disease states.(4, 5) A phase I trial is currently underway combining these approaches based on potential additive or synergistic activity.(6, 7) Men with hormone-refractory, metastatic prostate cancer are being treated with a constant dose and schedule of GM-CSF (250 mcg/m² QD d1 – d14 of a 28-day cycle) plus escalating doses (up to 3 mg/kg) of anti-CTLA-4 antibody (Ipilimumab) given on d1 of each cycle x 4.

With regard to the original proposed Statement of Work, task 1 was to determine if polyclonal T cell activation and clinical autoimmunity occur when CTLA-4 blockade is combined with GM-CSF. Twenty patients have been treated to date on the phase I study. CTLA-4 antibody dose is currently at 3 mg/kg monthly x 4 and no MTD has been reached. Clinical autoimmunity has been limited to one patient with a generalized rash requiring steroids in a previous dose level. No laboratory evidence of autoimmunity has been observed in any patient. There is no evidence of polyclonal T cell activation. Task 2 is being addressed as PSA declines are being tabulated and a manuscript will soon be written regarding the clinical results of this trial. Seven patients have demonstrated a < 50% reduction in their PSA. Task 3 from the original Statement of Work has not yet been addressed, pending outcome from the phase I trial, as described under Task 1. The phase I trial has been slow to completely accrue because of safety limitations (waiting 2 months between cohorts) as required by CTEP.

A dose response relationship has been seen between anti-CTLA-4 dose and activation of both CD4⁺ and CD8⁺ T cells in the blood. These effects were increased compared to effects seen with anti-CTLA4 treatment alone on a separate trial. T cell interferon-gamma production and lytic activity were also enhanced in circulating antigen-specific CD8⁺ T cells after this combination immunotherapy.

In addition, initial immunologic results are available, as seen in the accompanying figures (Figure 1 and Figure 2). Monocytes, and to a lesser degree, dendritic cells, were increased with this combination immunotherapy. This expansion was associated with an up-regulation of activation markers CD25 and CD69. Given that this is a single-arm study, the differential effect of the two agents on these parameters is not able to be surmised.

Further, after 14 days of treatment a slight up-regulation of CD4-positive T cells bearing the activation marker CD69 and CD25 was observed. No observable differences in CD45 RA or CD45 RO were observed in any T cell subset. Last, as a general read-out of the ability of therapy to stimulate immunity, CMV reactive T cells as detected by MHC peptide tetramers were measured. As observed in Figure 3, the percentage of CMV-positive T cells increased with treatment. Of note, these T cells expressed the activation markers CD69 and CD107A, as well as demonstrated production of interferon-gamma. These trends have continued with additional patients treated.

Key Research Accomplishments

1. Anti-CTLA-4 antibody and GM-CSF have demonstrated safety when given in combination to metastatic hormone refractory prostate cancer patients.
2. Collection and immunologic assessment of baseline and serial peripheral blood samples is feasible.
3. This combination immunotherapy in metastatic hormone refractory prostate cancer patients produces expansion of activated monocytes and dendritic cells, as well as activation of an endogenous population of cytotoxic T-lymphocytes *in vivo*.
4. PSA declines have been observed with this combination immunotherapy.

Reportable Outcomes

1. This data was a poster presentation at the ASCO 2004 annual meeting (L. Fong, B. Rini, B. Cavanaugh, E. Small. CTLA-4 Blockade-Based Immunotherapy for Prostate Cancer. Proc Am Soc Clin Oncol 22:14s, 2590, 2004).
2. This data was presented at the National Specialized Program of Research Excellence (SPORE) meeting in Baltimore, MD in July 2004. It was an oral presentation.
3. A serum repository of baseline and treatment samples for all patients is available and stored in the Immunology Core Laboratory of Dr. Larry Fong, who is performing the immunologic assays. This repository of serum will provide valuable companion data to this study, and a potential source of data for future studies.
4. This data will be presented at the 2006 ASCO Prostate Cancer Symposium and the 2006 ASCO Annual Meeting.

Conclusions

CTLA-4 blockade-based immunotherapy in combination with GM-CSF is feasible in metastatic hormone refractory prostate cancer. Without substantial clinical autoimmunity to date, the phase I portion of this study is soon to be completed and will define the maximum tolerated dose of this combination for future clinical testing in prostate cancer. Initial immunologic results suggest an effect of this therapy on both monocytes, dendritic cells, and T cells. Correlation of this response with clinical outcome is forthcoming.

Future Plans (Task 3)

Another immunotherapeutic approach to prostate cancer involves a prime/boost strategy using recombinant vaccinia (rV-PSA) and fowlpox (rF-PSA) viri expressing the PSA antigen (PROSTVAC). A recently completed ECOG trial randomizing biochemically-relapsed prostate cancer patients identified a vaccine approach of rV-PSA prime followed by three rF-PSA boost vaccinations as the most clinically and immunologically potent to move forward to a larger randomized trial.(8) A randomized phase III trial (ECOG 1805 PARADIGM trial) will soon be underway. It is certain that a subset of patients vaccinated on this trial will initially or ultimately fail to respond to this vaccination and experience clinical progression. Further testing of additional immunotherapeutic maneuvers in these patients is a reasonable investigative endeavor. Although taxane-based chemotherapy has a demonstrated modest survival advantage, many patients wish to avoid chemotherapy toxicity and are clinically suitable for investigational therapy prior to the use of chemotherapy.

CTLA-4 blockade-based immunotherapy in the setting of vaccination failure can be applied in this setting. Combination therapy with peptide vaccination and CTLA-4 blockade has produced augmented anti-tumor activity in pre-clinical models.(6, 9) In addition, previous clinical trials in melanoma have demonstrated the potency of combining peptide vaccination with CTLA-4 blockade in regards to both clinical responses and autoimmune toxicity.(10, 11) The high incidence of autoimmunity observed in these trials (43% and 58%) lends caution to a similar approach in prostate cancer patients who are older with more comorbidities compared to melanoma patients. However, a strategy of CTLA-4 blockade upon failure of prior vaccination may be a method to optimize the immunotherapeutic potential of such an approach while minimizing risks. For example, a clinical trial of CTLA-4 antibody in previously immunized metastatic melanoma and ovarian cancer patients demonstrated tumor necrosis/immune cell tumor infiltrate and clinical effects without significant autoimmunity.(12) A phase II trial of this combination in PROSTVAC vaccination failures HRPC patients is planned through the Eastern Cooperative Oncology Group (ECOG).

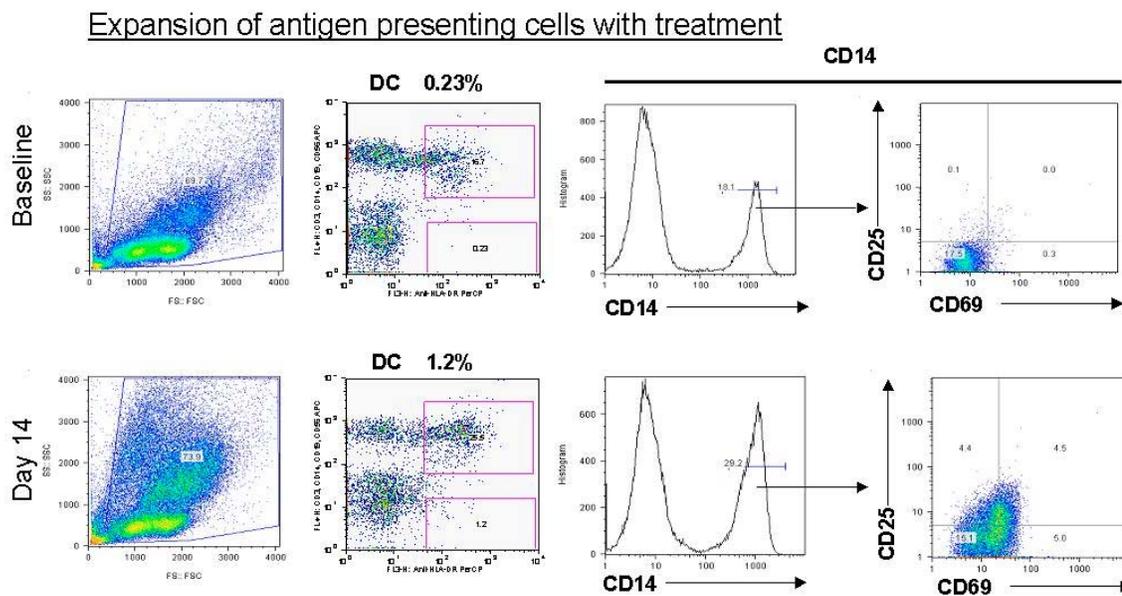
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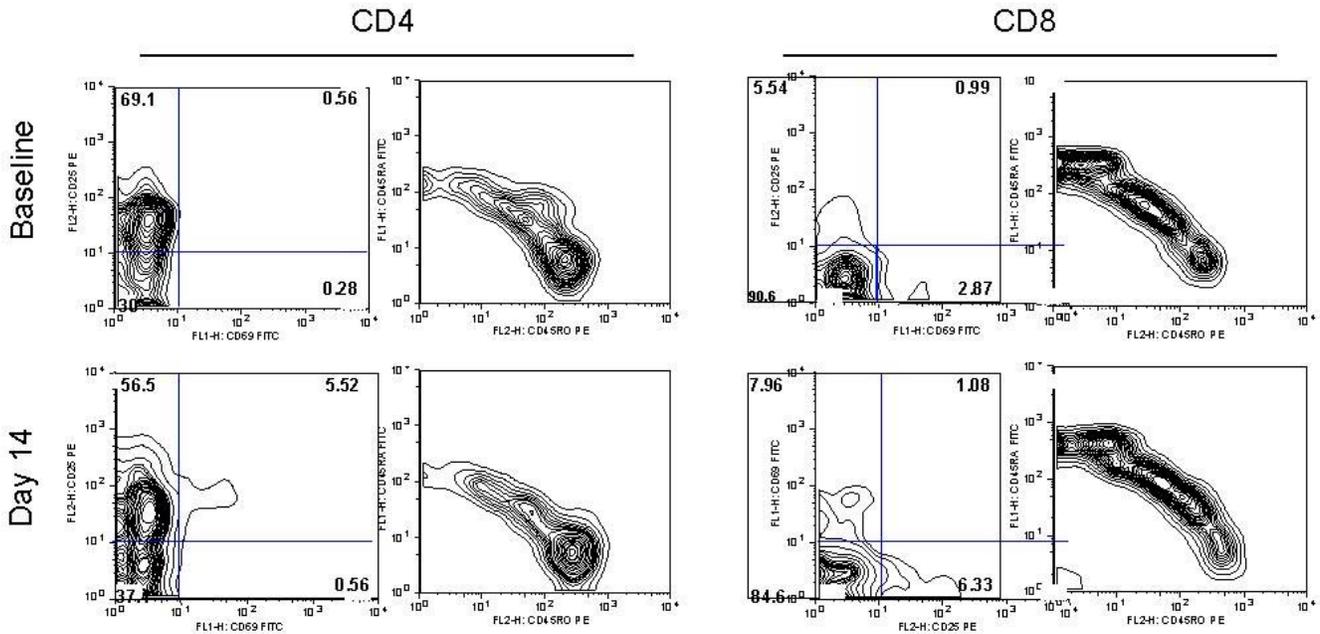
Appendices

Figure 1



- Monocytes and, to a lesser degree, dendritic cells were increased with treatment.
- This expansion was associated with upregulation of activation markers CD25 and CD69.
- This effect is similar to that seen with GM-CSF treatment alone.

Figure 2



After 14 days of CTLA-4/GM-CSF therapy, a slight upregulation of cells double positive for the T cell activation markers CD69 and CD25 was seen in bulk CD4 cells. Whereas in bulk CD8 cells a slight upregulation in CD25 alone was seen. No observable differences could be seen in the coexpression of CD45RA and CD45RO in bulk CD4 or CD8 cells at this time point.

Figure 3

In vivo modulation of antigen-specific CD8⁺ T cells with treatment.

- In patients with CMV reactive T cells detectable by MHC/peptide tetramers, this treatment led to the upregulation of CD69 as well as the functional markers CD107a and INF γ consistent with activation of the T cells *in vivo* by the treatment.

