BIOCHEMICAL AND IMMUNOLOGIC CORRELATES OF CLINICAL RESPONSE IN A COMBINATION TRIAL OF THE GM-CSF-GENE TRANSDUCED ALLOGENEIC PROSTATE CANCER IMMUNOTHERAPY AND IPILIMUMAB IN PATIENTS WITH METASTATIC HORMONE-REFRACTORY PROSTATE CANCER (MHRPC)

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ABSTRACT

Background: A Phase 1 trial is underway to study the GM-CSF-secreting immunotherapy for prostate cancer (GVAX immunotherapy (GVAX IT)) and Ipilimumab (ipi) in mHRPC patients (pts).
Methods: Twelve pts were treated for 24 weeks (wks) with bi-weekly intradermal injections of GVAX IT and monthly Ipi. Pts were enrolled in cohorts of 3; each cohort received an escalating dose of ipi: 0.3, 1, 3 or 5 mg/kg.
Results (as of January 2007): Median follow-up is 15.0 months. All pts had GVAX IT injection site reactions. Five of six pts at the higher ipi doses (3 and 5 mg/kg) developed Grade 2 or 3 immune-related endocrinopathy, consistent with hypophysitis manifested by adrenal insufficiency and/or hypothyroidism, all successfully treated with standard hormone replacement. Two pts were tapered off Synthroid within 6 months (m). There was no induction of the alpha-21-hydroxylase auto-antibody that is seen in 90% of cases of auto-immune adrenal insufficiency. One pt in the 5 mg group developed a Grade 3 dose-limiting alveolitis. PSA responses (declines > 50%) were seen in 5/6 treated at the two higher ipi doses with median response duration of 4.9 m (2 on-going at 7.2 m and 12.8 m). These PSA responses were associated with immune-related endocrinopathy but were not consistently correlated with declines in adrenal androgens. One pt had resolution of measurable disease on abdominal CT scan. Immunomonitoring studies showed T cell and dendritic cell activation, more pronounced at higher doses. Biopsies of injection sites showed T cell infiltration. Multiple tumor-reactive antibodies (abs) induced by tx were identified by serologic analysis (SEREX), including abs to filamin B. Screening against 20 defined prostate cancer antigens demonstrated induction of abs to PSMA and NY-ESO-1.
Conclusions: The GVAX IT and Ipilimumab combination is active in mHRPC. There was an association between PSA response and immune-related adverse events. The PSA responses cannot be accounted for by adrenal insufficiency. The relationship between clinical activity and serologic response to identified antigens is under investigation. Tx of 16 additional pts is planned.

BACKGROUND

GVAX™ Platform

- GVAX (Cell Genesys) refers to a platform based on whole tumor cells that are modified to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF) to stimulate an immune response against cancer.
- GVAX immunotherapy for prostate cancer includes two allogeneic prostate cancer cell lines, LNCaP and PC-3, that contain many common antigens found in metastatic prostate cancer.
- GM-CSF, a potent immune stimulant, induces the dendritic cell growth, maturation and recruitment necessary for initiation of an immune response.
- Activated dendritic cells could then be expected to present prostate cancer antigens to T cells in the lymph node, initiating a polyvalent anti-tumor immune response.

MDX-010 (Ipilimumab)

- Ipilimumab (Medarex and Bristol-Myers Squibb) is a fully human IgG1k anti-CTLA-4 monoclonal antibody.
- In vivo studies in an animal model demonstrated that ipilimumab is specific to the CTLA-4 receptor on T cells and augments immune responses by blocking the inhibitory CTLA-4/B7 interactions.
- Combination therapy with a *B16-GM-CSF* transduced cell line *(*mGVAX) and an anti-CTLA-4 antibody demonstrated improved survival in mice injected with a *B16* melanoma cell line.

METHODS

Study Design: Phase 1/2, open-label, dose-escalation, single-center clinical trial

Patients: Asymptomatic, metastatic hormone-refractory prostate cancer (HRPC) with PSA > 5 ng/mL and no prior chemotherapy or immunotherapy.

Treatment: Patients were assigned sequentially in groups of 3-6 to a dose level

Immunotherapy
- Fixed Dose Level: 5 x 10^8 prime, 3 x 10^8 boost q 2 wks x 12

+

Ipilimumab Dose Groups
- Dose Level 1: 0.3 mg/kg q 4 wks x 6
- Dose Level 2: 1.0 mg/kg q 4 wks x 6
- Dose Level 3: 3.0 mg/kg q 4 wks x 6
- Dose Level 4: 5.0 mg/kg q 4 wks x 6

Endpoints included:

- PSA Response (assessed q 4 wk x 6)
- Objective Tumor Response (assessed q 12 wk x 2)
- Safety: Adverse events were collected

Immunomonitoring: *(Supported in part by the Prostate Cancer Foundation)*

- Monitoring of DC and T cell functions was conducted to verify underlying scientific concepts and to correlate immune responses with clinical efficacy

Patient Disposition

- Dose escalation cohorts: 12 patients were enrolled across the 4 planned dose levels, and all have completed treatment.
- Expansion cohort (up to 16 patients) at Dose Level 3: Enrollment and treatment are on-going.

PSA RESPONSE

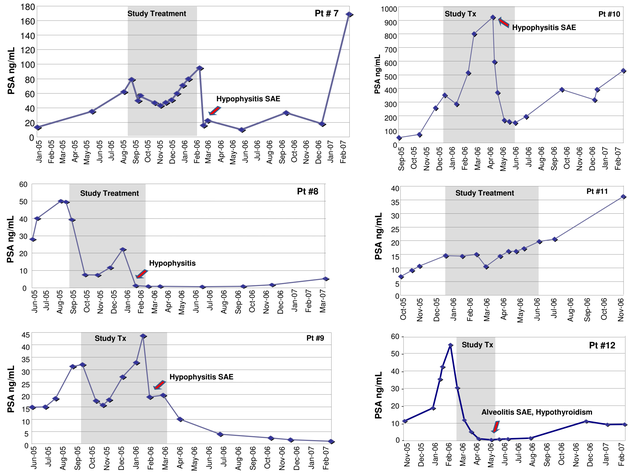
PSA Response

- PSA declines of >50% occurred in 5 of 6 patients at Dose Levels 3-4. Four were durable for at least 6 months (6.6, 8.5, and 16.6 months) with one ongoing at 8.6 months as of April 2007 (median follow-up is 18.1 months).
- Stable disease (<25% change in PSA) occurred in 3 of 6 patients on Dose Levels 1-2.
- Post-treatment declines in PSA on Dose Levels 3-4 are shown in Figure 1.
- An immune-related adverse event (irAE) occurred in 5/6 patients with PSA declines (Table 1), and all irAE dates of onset were 2-7 months after the start of treatment (shown by red arrows in Figure 1).

Table 1. Summary of PSA Responses and irAE's

MDX-010 dose	PSA Response	irAE
0.3 and 1 mg/kg	0/6	0/6
3 and 5 mg/kg	5/6	5/6

Figure 1. PSA Response over time (Dose Levels 3-4). The occurrence of an immune-related adverse event (irAE) is shown by a red arrow ( ).



Adrenal Androgen Levels and PSA Response

- The primary hormone that stimulates the growth of prostate cancer is dihydrotestosterone, which is produced primarily by the testes and also by the adrenal glands. The inhibition of adrenal androgen synthesis may result in disease response even in men who are refractory to androgen ablation therapies. To evaluate whether onset of adrenal insufficiency was correlated with observed PSA responses, the adrenal androgens Androstenedione (Andro) and DHEA were assayed at baseline and at 12 weeks and 24 weeks into treatment in all 12 patients.
- There was an inconsistent relationship between absolute levels of adrenal androgens, declines in adrenal androgens, and PSA response.
  - 7/12 had one or both androgens already below the lower limit of normal at baseline, prior to initiation of treatment.
  - 6/12 had Andro levels decline at some point during treatment; of these 4 had PSA decline, 2 did not.
  - 6/12 had DHEA levels decline at some point during treatment; of these 3 had PSA decline, 3 did not.
  - 5/5 patients with PSA responses had declines of varying magnitude in one or both adrenal androgens, including one patient in whom the PSA decline preceded an androgen decline. There was no consistent temporal relationship between PSA decline and adrenal androgen decline.
- There was no induction of the alpha-21-hydroxylase auto-antibody that is seen in 90% of cases of auto-immune adrenal insufficiency.

TUMOR RESPONSE

Tumor Response

- 11 patients had abnormal bone scans at baseline. Stable disease on bone scan was observed in 6 patients at 3 months, and is ongoing for 3 patients at 9 months and 1 patient at 12 months. This includes 2 patients with resolution of lesions (both in 3 mg/kg cohort; one shown below in Figure 2).
- 3 pts had measurable disease at baseline. Objective tumor response was observed in Patient 12 (5 mg/kg; Figure 3).
- Improvement in bone pain was also reported.

Figure 2. Bone Scan Improvement in Patient 9 (3 mg/kg).

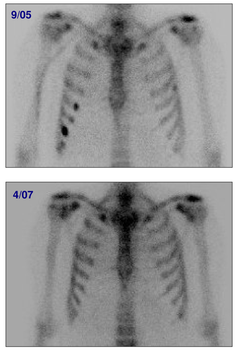
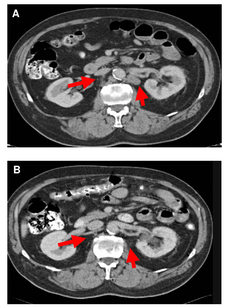


Figure 3. Objective Tumor Response in Patient 12 (5 mg/kg) from 14 Feb 2006 (A) to 16 May 2006 (B).

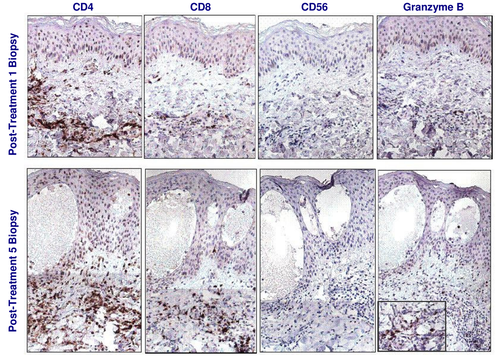


IMMUNOMONITORING STUDIES

Immunomonitoring Studies

- Immunomonitoring studies demonstrated T cell and dendritic cell activation, which was more pronounced at the higher dose levels.
- After one treatment, T cells were found infiltrating the injection site. The number of infiltrating T cells notably increased between the first and fifth treatment. See Figure 4. The majority of infiltrating T cells were CD4+ Th cells, far outnumbering the infiltrating CD8+ T cells. No infiltration by CD56+ NK cells was observed. An increased cytotoxic potential was indicated by an increase in scattered Granzyme B+ lymphocytes after Treatment 5.
- A rise in frequencies of HLA-DR+ activated T cells occurred at the higher dose levels (3-5 mg/kg) but not at the lower dose levels.

Figure 4. Biopsy results (200x) from the injection site taken 48 hours after Treatment 1 (top row) and Treatment 5 (bottom row) in Patient 10 (Dose Level 4). The insert (lower right) shows Granzyme B+ lymphocytes in the deep dermis.



Serologic Analysis of Antibodies

- Multiple tumor-reactive antibodies induced by treatment were identified by serologic analysis (SEREX).
- Antibodies to filamin B were identified in 6/12 patients by ELISA. Antibody responses to filamin B have been reported in two prior studies of GVAX immunotherapy for prostate cancer administered as a single agent. Filamin B is a cytoskeletal protein that is involved in cell division, adhesion, motility, signal transduction, and protein sorting.
- Screening against 20 defined prostate cancer antigens by ELISA demonstrated induction of antibodies to PSMA in 6/12 patients and NY-ESO-1 in 3/12 patients.

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SAFETY

Adverse Events

- Injection site reactions occurred in 100% of patients, similar to those previously reported for GVAX immunotherapy for prostate cancer. (See Figure 5.)
- Flu-like symptoms including fatigue and fever were common. (See Table 3.)

Table 2. Treatment-Related Adverse Events (n=12)

Adverse Event*	Frequency (%)
Fatigue	75
Fever	58
Anorexia	42
Flu-like symptoms	33
Nausea	17
Bone Pain	8
Dry Skin	8

The maximum grade reported for these adverse events was 3 for fatigue and fever, 2 for flu-like symptoms, and 1 for the rest.

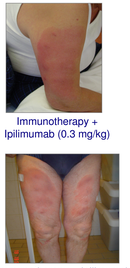
Immune-Related Adverse Events

- No immune-related adverse events (irAE) occurred at the lower dose levels (0.3 and 1.0 mg/kg).
- At the higher dose levels (3 and 5 mg/kg), irAEs occurred in 5 of 6 patients.
  - The irAEs were Grade 2 or 3 endocrinopathies, consistent with hypophysitis manifested by adrenal insufficiency and/or hypothyroidism and similar to those previously reported for ipilimumab. One patient on 5 mg/kg had a Grade 3 dose-limiting alveolitis accompanied by low TSH. See Table 3.
  - The endocrine-related component was successfully treated with standard hormone replacement therapy. Two patients requiring Synthroid were tapered off after recovery of thyroid function, which occurred within 6 months, with one patient subsequently maintaining a PSA response.

Table 3. Summary of Immune-Related Adverse Events

Patient	Primary Event	Onset	Secondary Events
007	Hypophysitis	7 mo	Adrenal Insuff
008	Hypophysitis	5 mo	Adrenal Insuff
009	Hypophysitis	5 mo	Adrenal Insuff, Leukopenia, Hypothyroidism
010	Hypophysitis	4.5 mo	Adrenal Insuff, Hypothyroidism
012	Pulmonary Alveolitis	2 mo	Hypothyroidism

Figure 5. Reactions at sites of intradermal injections of GVAX immunotherapy for prostate cancer



Immunotherapy + Ipilimumab (0.3 mg/kg)

Immunotherapy + Ipilimumab (3 mg/kg) after 1 week

SUMMARY AND CONCLUSIONS

- This study showed a dose response to Ipilimumab (MDX-010) in combination with a fixed dose of GVAX immunotherapy for prostate cancer with regard to immune-related adverse events (irAE) and clinical activity.
- A strong association between tumor response and irAEs was observed: 100% of irAEs were associated with PSA response.
- Durable PSA responses, evidence of bone scan improvement, measurable tumor regression, and improvement in bone pain was observed.
- The PSA responses cannot be fully explained by adrenal androgen suppression.
- This evidence of anti-tumor activity is unprecedented for a combination of immunotherapies in advanced prostate cancer.
- Immunomonitoring studies indicated T cell and dendritic cell activation, which was more pronounced at the higher dose levels. The relationship between clinical activity and serologic response to identified antigens is under investigation.
- Treatment of up to 16 additional pts at Dose Level 3 is underway.

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