# Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial

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## Summary

**Background** **The granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells vaccine (GVAX) has antitumour activity against prostate cancer; preclinical studies have shown potent synergy when combined with ipilimumab, an antibody that blocks cytotoxic T-lymphocyte antigen 4. We aimed to assess the safety of combined treatment with GVAX and ipilimumab in patients with metastatic castration-resistant prostate cancer (mCRPC).**

**Methods** **We did an open-labelled, single-centre, dose-escalation study of ipilimumab concurrent with a fixed dose of GVAX, with a subsequent expansion phase, both at the VU University Medical Centre (Amsterdam, Netherlands). Eligible patients had documented mCRPC and had not been previously treated with chemotherapy. All patients received a $5\times10^8$ cell priming dose of GVAX intradermally on day 1 with subsequent intradermal injections of $3\times10^8$ cells every 2 weeks for 24 weeks. The vaccinations were combined with intravenous ipilimumab every 4 weeks. We enrolled patients in cohorts of three; each cohort received an escalating dose of ipilimumab at 0·3, 1·0, 3·0, or 5·0 mg/kg. Our primary endpoint was safety. This study is registered with ClinicalTrials.gov, number NCT01510288.**

**Findings** **We enrolled 12 patients into our dose-escalation cohort. We did not record any severe immune-related adverse events at the first two dose levels. At the 3·0 mg/kg dose level, one patient had grade 2 and two patients grade 3 hypophysitis; at the 5·0 mg/kg dose level, two patients had grade 3 hypophysitis and one patient developed grade 4 sarcoid alveolitis (a dose-limiting toxic effect). Due to observed clinical activity and toxic events, we decided to expand the 3·0 mg/kg dose level, rather than enrol a further three patients at the 5·0 mg/kg level. 16 patients were enrolled in the expansion cohort, two of whom developed grade 2 hypophysitis, three colitis (one grade 1 and two grade 2), and one grade 3 hepatitis—all immune-related adverse events. The most common adverse events noted in all 28 patients were injection-site reactions (grade 1–2 events seen in all patients), fatigue (grade 1–2 in 20 patients, grade 3 in two), and pyrexia (grade 1–2 in 15 patients, grade 3 in one). 50% or greater declines in prostate-specific antigen from baseline was recorded in seven patients (25%); all had received 3·0 mg/kg or 5·0 mg/kg ipilimumab.**

**Interpretation** **GVAX combined with 3·0 mg/kg ipilimumab is tolerable and safe for patients with mCRPC. Further research on the combined treatment of patients with mCRPC with vaccination and ipilimumab is warranted.**

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## Introduction

The American Cancer Society has estimated that in 2011, 240 890 men were diagnosed with prostate cancer in the USA, accounting for 29% of all malignancies in men. Moreover, an estimated 33 720 men died from prostate cancer in 2011, constituting 11% of all cancer-related deaths in men.[1]

First-line therapy of advanced prostate cancer consists of androgen blockade,[2] but patients will eventually develop castration-resistant prostate cancer.[3] Based on two phase 3 trials,[4,5] chemotherapy with docetaxel is the treatment of choice for patients with metastatic castration-resistant prostate cancer (mCRPC). Investigators have recently reported that both cabazitaxel and abiraterone improved survival as second-line treatment in patients with prostate cancer.[6,7] However, alternatives to hormonal treatment or chemotherapy, such as immunotherapy, are warranted; options include antigen-directed strategies (eg, sipuleucel-T and PSA-Tricom), whole-cell vaccines (eg, granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells vaccine; GVAX), and non-specific immunomodulation (eg, ipilimumab).[8]

A phase 3 trial with sipuleucel-T showed a survival benefit of 4 months relative to placebo;[9] subsequent approval by the US Food and Drug Administration was granted in April, 2010. PSA-Tricom is a prostate cancer vaccine that consists of two different poxviruses that each encode prostate-specific antigen (PSA) plus three immune co-stimulatory molecules. In a randomised phase 2 trial of 125 patients with mCRPC, patients receiving PSA-Tricom had a longer median overall survival.[10] A large phase 3 trial is now planned to confirm these findings.

Another immunotherapeutic approach to prostate cancer is whole-cell vaccination. Irradiated tumour cells expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) generate a long-lasting and specific antitumour immunity in mice.[11] The findings from several phase 1 and 2 trials showed GVAX[12] to be well tolerated and suggested improved survival.[13,14] Cytotoxic T-lymphocyte-associated protein 4 (CTLA4) is a crucial immune checkpoint molecule that downregulates T-cell activation and proliferation. Under homoeostatic conditions it restricts the risk of autoimmunity, but it can also restrict the expansion of tumour-specific effector T cells.[15,16] Ipilimumab is a fully human monoclonal antibody ($IgG_1$) that blocks CTLA4, promotes antitumour immunity, and has been shown in two phase 3 trials to improve overall survival in patients with metastatic melanoma.[17,18] Preclinical studies of the anti-CTLA4 antibody in combination with GM-CSF-secreting tumour-cell vaccines showed potent synergy.[19] We therefore undertook a phase 1 trial in patients with mCRPC to establish whether ipilimumab could be combined safely with GVAX.

## Methods

### Participants

Between Nov 18, 2004, and Dec 19, 2007, we did an open label, single-centre, dose-escalation study of ipilimumab concurrent with a fixed dose of GVAX, with a subsequent expansion phase. Both phases were done at the VU University Medical Centre (Amsterdam, Netherlands). The trial was closed on Nov 18, 2008. We deemed eligible men older than 18 years with histologically confirmed adenocarcinoma of the prostate. All patients had to have castration resistant disease, with evidence of PSA progression, and radiological evidence of metastatic disease (PSA progression and metastatic disease in accordance with the Prostate Cancer Working Group criteria 2; PCWG2).[20] Other eligibility criteria included adequate haematological, renal, and hepatic function; testosterone concentrations less than 1·0 nmol/L; an Eastern Cooperative Oncology Group performance status of 0–2; and a life expectancy of at least 6 months. We excluded patients when they had other histological forms of prostate cancer, evidence of brain metastases, or had received previous gene therapy, chemotherapy, or immunotherapy for prostate cancer. Other exclusion criteria were bone pain that needed treatment with narcotic analgesia, a history of immunodeficiency, or autoimmune disease. We also excluded patients with a history of known hypersensitivity to GM-CSF or to any of the other components of GVAX.

Anti-androgen therapy (ie, bicalutamide) and systemic corticosteroid use had to be withdrawn within at least 6 weeks before the first treatment. For inclusion we required that patients continued treatment with agonists of luteinising hormone releasing hormone or had undergone a bilateral orchiectomy.

All patients provided written informed consent. The protocol was approved by the Central Committee on Research involving Human Subjects in the Netherlands and the institutional review board of the VU University Medical Centre in Amsterdam, Netherlands.

### Procedures

The immunotherapy we used was based on the GVAX platform (Cell Genesys Inc, San Francisco, CA, USA), a cellular vaccine consisting of two prostate tumour-cell lines, PC-3 (CG1940) and LNCaP (CG8711), which were lethally irradiated and genetically modified to secrete GM-CSF.[21] Ipilimumab is a fully human immunoglobulin ($IgG_1K$) purified from a cultured cell line (provided by Medarex/Bristol-Myers Squibb, Plainsboro, NJ, USA). This monoclonal antibody is specific for the CTLA4 antigen expressed on a subset of activated T cells.[22]

We did the dose-escalation phase of our study with the standard three plus three phase 1 trial design. We enrolled patients in cohorts of three for up to four dose levels to establish the maximum tolerated dose of ipilimumab in combination with GVAX. Patients received intradermal injections of GVAX every 2 weeks for a total of 13 injections and were intravenously infused with ipilimumab every 4 weeks for a total of six infusions on the same day as the vaccinations. The first GVAX dose consisted of $5\times10^8$ cells, with subsequent boosts of $3\times10^8$ cells. Our predetermined sequential doses of ipilimumab were 0·3, 1·0, 3·0, and 5·0 mg/kg. When we designed our study, the highest safe dose of ipilimumab in other clinical trials was 5·0 mg/kg. If there were no dose-limiting toxic effects in a cohort, we would enter additional patients in the next dose level. If there was one dose-limiting toxic effect, we would include an additional three patients at this dose. If there were two dose-limiting toxic effects, we would define the previous dose as the maximum tolerated dose. We defined dose-limiting toxic effects as any treatment-related adverse events of grade 4, grade 3 or greater diarrhoea (unresponsive to treatment or irreversible), grade 2 or greater immune-mediated toxic effects (which we defined as an inflammatory process that compromised the function of any organ and was not attributable to another cause, and that was preferably supported by biopsy results) that had the potential to be life threatening with continuation of therapy, immune-mediated toxic effects that did not resolve or improved to grade 2 or less within 14 days of onset, and any clinically significant retinal pigmentation changes or any clinically significant decline in visual

acuity. Exceptions to these criteria were inflammation of grade 4 or less that was attributable to a local antitumour reaction that could potentially be a therapeutic response, and grade 3 skin rashes. We planned to enrol additional patients into an expansion cohort at the maximum tolerated dose, if discovered, to further assess the safety and efficacy of treatment. We would not begin to enrol patients into the expansion cohort until patients at the dose at which dose-limiting toxic effects had been noted had been monitored for other dose-limiting toxic effects for 196 days.

For immunomonitoring, we took blood samples from patients before the start of treatment and every 4 weeks until 4 weeks after the last treatment. We did flow-cytometric analysis either on whole-blood samples or on peripheral-blood mononuclear cells after isolation by density centrifugation (Nycomed AS, Oslo, Norway). We obtained serum before and after prostate GVAX plus ipilimumab immunotherapy and stored it at –80°C. We established peripheral-blood circulating lymphocyte or dendritic cell frequencies and activation status by routine whole-blood (HLA-DR on T cells) or isolated peripheral-blood mononuclear cell (CD40 on dendritic cells) analysis by flow cytometry as described elsewhere.[23]

We obtained full-length filamin B cDNA from Invitrogen (Carlsbad, CA, USA) and prostate-specific membrane antigen (PSMA) cDNA from Origene (Rockville, MD, USA), which we PCR-cloned into a mammalian expression vector with a C-terminal Flag-tag and transfected into 293 cells for protein production. We purified the protein with antibody-affinity purification in accordance with the manufacturer's procedures (Sigma Aldrich, St Louis, MO, USA). For western blot analysis of patients' antibodies to filamin B and PSMA, we separated 150 ng of purified protein by sodium dodecyl sulphate polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. We then blocked and subsequently incubated blots with a 1:500 dilution of patients' sera and a donkey anti-human IgG/IgM HRP-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and visualised by treatment with the enhanced chemiluminescence system (Pierce, Rockford, IL, USA), with subsequent exposure of photographic film (Kodak, Rochester, NY, USA). We scored patients' sera positive for anti-filamin B or PSMA antibodies if we identified an immunoreactive band running at the predicted molecular weight either before or after treatment. We used tetanus toxoid (Calbiochem, San Diego, CA, USA) as a positive control.

We also detected antibodies reactive to PSMA and filamin B with ELISA, as described elsewhere.[24] In brief, we coated 96-well MaxiSorp plates (Nunc, Rochester, NY, USA) with 200 ng per well of protein and then blocked, after which we added patient serum at a 1:100 dilution. Next, we incubated the wells with a donkey anti-human IgG/IgM HRP-conjugated secondary antibody, which we detected with TBM substrate (KPL, Gaithersburg, MD, USA). To establish induction of an antibody response, we divided the optical density value after treatment by the optical density before to establish a fold induction. We judged fold induction levels of 2 or greater to be positive responses. We used tetanus toxoid as a non-vaccine control in ELISA assays.

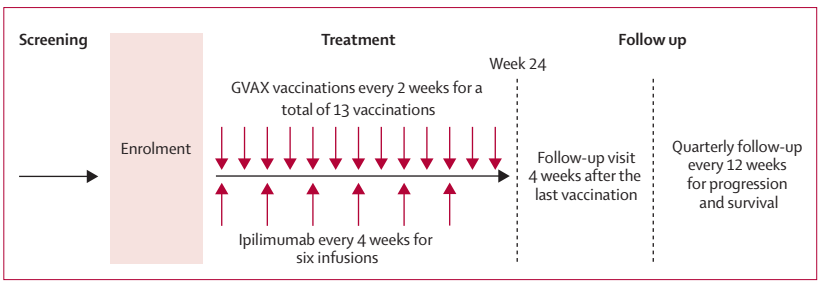


*Figure 1:* **Treatment schedule**

Our primary endpoints were safety, as established by physical and laboratory examinations, autoimmunity, and the incidence and severity of adverse events, and the maximum tolerated dose of ipilimumab in combination with GVAX as established by dose-limiting toxic effects. Our secondary endpoints were time to clinical disease progression as established by radiographic criteria, time to PSA progression, PSA response, immune response as established by analysis of tumour-associated antibodies,[25] and survival. We used the PCWG2 criteria to assess response to treatment.[20]

| | Escalation cohort (n=12) | Expansion cohort (n=16) | p value |
|---|---|---|---|
| Age (years) | 64·5 (44–78) | 67·6 (60–75) | 0·144 |
| ECOG performance score | | | 0·285 |
| 0 | 9 (75%) | 15 (94%) | |
| 1 | 3 (25%) | 1 (6%) | |
| Gleason score | | | 0·529 |
| 2–4 | 0 | 1 (6%) | |
| 5–7 | 8 (67%) | 8 (50%) | |
| 8–10 | 4 (33%) | 7 (44%) | |
| Metastases* | | | |
| Bone | 10 (83%) | 15 (94%) | 0·755 |
| Node | 4 (33%) | 7 (44%) | 0·820 |
| Viscera | 1 (8%) | 4 (25%) | 0·144 |
| Prostate-specific antigen (μg/L) | 48 (15–351) | 117 (25–1206) | 0·077 |
| Lactate dehydrogenase (U/L) | 187 (147–252) | 210 (140–741) | 0·131 |
| Alkaline phosphatase (U/L) | 86 (48–429) | 199 (53–1049) | 0·073 |
| Haemoglobin (g/L) | 137 (118–159) | 126 (111–143) | 0·048 |

Data are median (range) or n (%). ECOG=Eastern Cooperative Oncology Group.
*Patients might have more than one metastasis.

*Table 1:* **Baseline characteristics**

| | Grade 3 | Grade 4 | Total* |
|---|---|---|---|
| Any event | 8 (29%) | 1 (4%) | 28 (100%) |
| Skin | | | |
| Injection-site reactions | .. | .. | 28 (100%) |
| Rash | 1 (4%) | .. | 11 (39%) |
| Pruritus | .. | .. | 7 (25%) |
| Endocrine | | | |
| Hypophysitis | 4 (14%) | .. | 7 (25%) |
| Adrenal insufficiency | 4 (14%) | .. | 5 (18%) |
| Gastrointestinal | | | |
| Nausea | .. | .. | 8 (29%) |
| Diarrhoea | 1 (4%) | .. | 7 (25%) |
| Vomiting | 1 (4%) | .. | 6 (21%) |
| Colitis | .. | .. | 3 (11%) |
| Proctitis | 1 (4%) | .. | 1 (4%) |
| General | | | |
| Fatigue | 2 (7%) | .. | 22 (79%) |
| Pyrexia | 1 (4%) | .. | 16 (57%) |
| Influenza-like illness | .. | .. | 12 (43%) |
| Malaise | .. | .. | 6 (21%) |
| Hepatic | | | |
| Increased AST | 1 (4%) | .. | 2 (7%) |
| Increased ALT | 1 (4%) | .. | 1 (4%) |
| Hepatitis | 1 (4%) | .. | 1 (4%) |
| Neurological | | | |
| Headache | .. | .. | 7 (25%) |
| Dysgeusia | .. | .. | 3 (11%) |
| Other | | | |
| Anorexia | .. | .. | 6 (21%) |
| Decreased weight | .. | .. | 3 (11%) |
| Alveolitis | .. | 1 (4%) | 1 (4%) |
| Hyponatraemia | 1 (4%) | .. | 1 (4%) |
| Injection-site pain | 1 (4%) | .. | 1 (4%) |
| Leucopenia | 1 (4%) | .. | 1 (4%) |

Some patients experienced more than one adverse event. We also list adverse events with at least grade 3. ALT=alanine aminotransferase. AST=aspartate aminotransferase. *Events for all grades.

***Table 2:*** **Adverse events related to either study drug**

### Statistical analyses

We compiled baseline characteristics for the two cohorts (ie, escalation and expansion) and assessed these for differences with Fisher's exact test and the independent two-sample *t* test. We used Pearson's analysis to establish correlations between variables and the Kaplan-Meier method to estimate the median time of survival. We assessed differences between immunomonitoring variables before treatment (week 0, visit 1) and during or after treatment (week 4, visit 3; week 8, visit 5; week 12, visit 7; week 16, visit 9; week 20, visit 11; week 24, visit 13, and follow up) with the repeated measures ANOVA with a post-hoc Dunnett's multiple comparisons test. For data collection we used Microsoft Excel (version 2007) and for statistical analysis we used SPSS version 17.0. This study is registered with Central Committee on Research involving Human Subjects in the Netherlands, number P03.1786C, and ClinicalTrials.gov, number NCT01510288.

### Role of the funding source

The sponsors of the study had no role in data collection, analysis, data interpretation, or writing of the report. IL, HMP, KH, NS, and WRG were responsible for the design of the trial. WRG, AJMvdE, JV, SJAMS, and TDdG had full access to all raw data and share responsibility for integrity of the data and the accuracy and completeness of the data analyses. WRG had final responsibility for the decision to submit for publication.

## Results

Figure 1 shows the treatment schedule. We included 12 patients in our dose-escalation cohort (0·3–5·0 mg/kg ipilimumab), and a subsequent 16 patients in our expansion cohort at a dose of 3·0 mg/kg ipilimumab. Table 1 lists the baseline characteristics of the patients.

We did not identify any severe adverse events (grade 3–4) at doses of 0·3 and 1·0 mg/kg ipilimumab. By contrast, we detected clinical signs (ie, adrenal insufficiency and hypothyroidism) of autoimmune hypophysitis in all three patients at the third dose level (3·0 mg/kg ipilimumab;

| | 0·3 mg/kg escalation cohort (n=3) | 1·0 mg/kg escalation cohort (n=3) | 3·0 mg/kg escalation cohort (n=3) | 5·0 mg/kg escalation cohort (n=3) | Expansion cohort (3·0 mg/kg; n=16) | All doses (n=28) |
|---|---|---|---|---|---|---|
| Alveolitis | .. | .. | .. | 1 (33%) | .. | 1 (4%) |
| Grade 4 | .. | .. | .. | .. | .. | 1 |
| Colitis | .. | .. | .. | .. | 3 (19%) | 3 (11%) |
| Grade 1 | .. | .. | .. | .. | 1 | 1 |
| Grade 2 | .. | .. | .. | .. | 2 | 2 |
| Hepatitis | .. | .. | .. | .. | 1 (6%) | 1 (4%) |
| Grade 3 | .. | .. | .. | .. | 1 | 1 |
| Hypophysitis | .. | .. | 3 (100%) | 2 (67%) | 2 (13%) | 7 (25%) |
| Grade 2 | .. | .. | 1 | .. | 2 | 3 |
| Grade 3 | .. | .. | 2 | 2 | .. | 4 |

Some patients experienced more than one adverse event.

***Table 3:*** **Immune-related adverse events to either study drug**

one grade 2, two grade 3); these were manageable but potentially life threatening. When we suspected hypophysitis we did blood tests to confirm diagnosis and started hormone replacement therapy (hydrocortisone, levothyroxine, or both) within 24 h, resulting in rapid relief of the patients' symptoms. After consultation with the safety monitoring board, we decided not to categorise hypophysitis as a dose-limiting toxic effect, since it was easily manageable. On relief of symptoms, we continued to give GVAX and ipilimumab to these patients. At the fourth dose level, we again diagnosed two patients with hypophysitis (both grade 3). One of these patients also developed a severe pulmonary sarcoid alveolitis after three doses of ipilimumab and six doses of GVAX and underwent a thoracoscopy with lung and lymph-node biopsy, which showed the presence of many small non-compact granulomas with associated lymphocytic infiltrates. We treated this patient with high-dose prednisone and they slowly recovered. After 2 months of high-dose prednisone, the patient started tapering off prednisone and was successfully weaned off after another month. We judged the alveolitis a dose-limiting toxic effect, and the patient received no further ipilimumab or GVAX.

In accordance with our protocol another three patients should have been treated at a dose of 5·0 mg/kg. However, because of the recorded toxic and antitumour effects in all three patients at the third dose level, we decided instead to amend our protocol and to expand the third dose cohort (3·0 mg/kg ipilimumab), as advised by the independent safety monitoring board.

Nine (75%) of the 12 patients in the escalation cohort and 13 (81%) of 16 patients in the subsequent expansion cohort completed all planned treatments. One patient did not complete treatment because of a dose-limiting toxic effect in the escalation phase, and the remaining five patients experienced disease progression and were taken off protocol to receive alternative treatment.

Table 2 summarises the adverse events related to treatment for all 28 patients. The most common adverse events were grade 1 or 2 injection-site reactions, fatigue, pyrexia, influenza-like illness, and rash. Adverse events deemed to be immune related are shown in table 3; two more cases of hypophysitis (both grade 2) were noted in the expansion cohort, together with three cases of colitis (one grade 1, two grade 2), and one case of grade 3 hepatitis. All cases of hypophysitis, both in the escalation and expansion phases, manifested as adrenal insufficiency, hypothyroidism, or both. The proportion of patients affected by immune-related adverse events was much lower in the expansion cohort than in the third and fourth dose levels (table 3). The timing of onset for these immune-related adverse events varied, ranging from 2 to 7 months after the start of treatment. All immune-related adverse events were at our third and fourth dose levels, all had previously been reported after giving ipilimumab,[26–28] and all were successfully treated with appropriate medical treatment, study drug discontinuation, or both.

In figure 2 we summarise the maximum relative PSA changes during the immunotherapy of our two cohorts. Seven (25%) of the 28 patients had a more than 50% decline of PSA from baseline. We did not record any PSA responses (≥50% decline) in the first two cohorts (0·3 and 1·0 mg/kg ipilimumab). Five (23%) of the 22 patients, treated with either 3·0 or 5·0 mg/kg ipilimumab, had

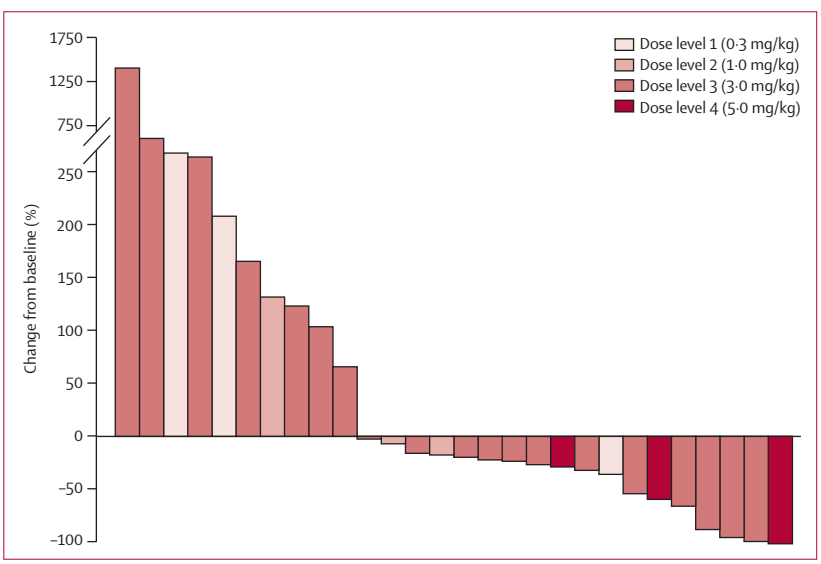


***Figure 2:*** **Relative changes in PSA responses from baseline**
Maximum relative changes (rise or fall) in PSA levels of patients with mCRPC from baseline, either during treatment, or until progression after treatment. Each bar represents one patient. PSA=prostate-specific antigen.

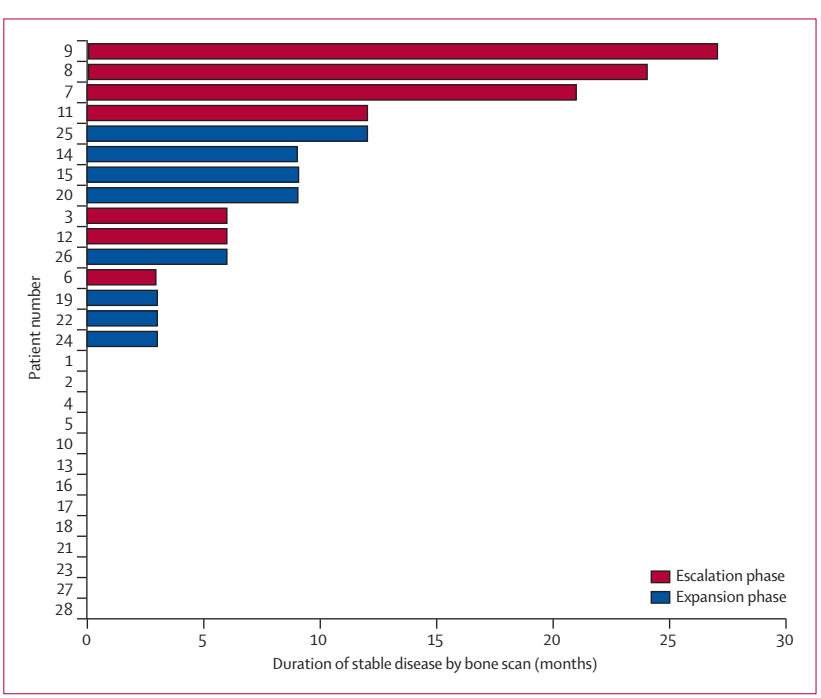


***Figure 3:*** **Duration of stable disease according to PCWG2 criteria from start of treatment**
Each bar represents one patient. We did bone scans every 3 months until progressive disease. PCWG2=Prostate Cancer Working Group criteria 2.

confirmed partial PSA responses (>50% decline from baseline). The median duration of response was 12 months (range 2–21). PSA kinetic changes included delayed declines after initial progression (data not shown). Moreover, we noted a strong association between PSA response and immune-related adverse events in the patients in the escalation phase, with all patients with an immune-related adverse event having a PSA response. In the expansion cohort we did not identify an association, although four patients had immune-related adverse events. Seven patients enrolled in the escalation phase and eight patients in the expansion cohort had at least stable disease on bone scans; in two patients in the escalation phase there was clear regression of metastases. Duration of stabilisation ranged from 3 to 27 months (figure 3). We noted complete regression of abdominal lymphadenopathy in one of four patients with measurable disease at baseline.

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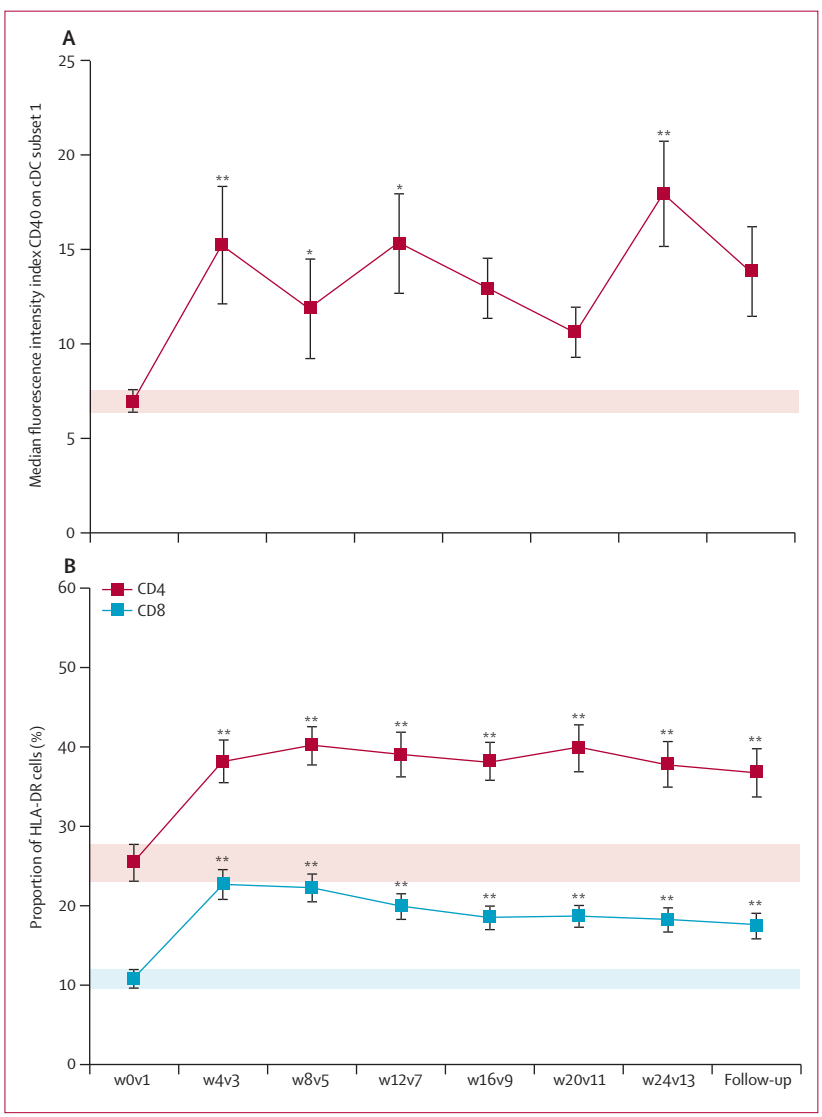


***Figure 4:*** **Response of peripheral blood BDCA-1/CD1c cDC and T cells**
We established cDC and T-cell activation before (week 0, visit 1; w0v1) and after (w4v3, w8v5, w12v7, w16v9, w20v11, w24v13, and follow up) treatment by use of flow cytometry. (A) Median fluorescence intensity index of CD40 on CD11c$^{high}$/CD14$^{-}$/BDCA1$^{+}$ cDC. (B) Proportion of HLA-DR cells within CD3/CD4 (red squares) and CD3/CD8 T cells (blue squares). Data is mean ± standard error of the mean for 28 patients. The shaded bar shows the pretreatment value. cDC=conventional dendritic cells. *p<0·05 compared with baseline. **p<0·01 compared with baseline.

Testosterone concentrations were less than 0·1 nmol/L in six (22%) of 27 patients (one patient not measurable) 4 weeks after completing vaccinations, with no correlation with survival. In the other 21 patients (78%) concentrations ranged from 0·13 to 0·49 nmol/L (castration level <1·0 nmol/L).

As a measure of immune activation induced by GVAX plus ipilimumab, we monitored expression levels of the activation markers CD40 and HLA-DR over the course of treatment on BDCA-1/CD1c conventional dendritic cells and T cells in the peripheral blood, respectively. Both CD40 on conventional dendritic cells and HLA-DR on CD4 and CD8 T cells were upregulated on treatment with GVAX plus ipilimumab (figure 4). For HLA-DR on T cells, this upregulation was dependent on the ipilimumab dose, with significant upregulation only at high dose levels (3·0 and 5·0 mg/kg). T-cell activation by HLA-DR upregulation was greater in patients with a partial response or stable disease than in patients with progressive disease (appendix). Whereas HLA-DR upregulation on T cells was not associated with overall survival (data not shown), CD40 upregulation on CD1c conventional dendritic cells was associated with improved overall survival (>70% increase, with n=22, median overall survival 38·5 months [95% CI 30·5–47·6] *vs* <70% increase, with n=6, median overall survival 15·5 months [11·0–19·3]).

As an indication of tumour-specific immune reactivity, we tested antibody responses to filamin B and PSMA, both of which are antigens expressed by GVAX. For filamin B, we noted therapy-induced increases in seroreactivity in 13 (46%) of 28 patients, but this did not correlate with improved survival (median overall survival 33·8 months [95% CI 16·6–51·0] for filamin B negative patients *vs* 20·9 months [0·0–51·3] for those who were filamin B positive; p=0·703; figure 5). We noted PSMA seroconversions in 12 (43%) of 28 patients and in four (80%) of five patients with a partial response. Patients who developed a PSMA-specific antibody response had a median overall survival of 46·5 months (95% CI 30·2–62·8), whereas patients without a PSMA-specific antibody response had a significantly (p=0·028) shorter median overall survival of 20·6 months (19·0–22·2; figure 5).

As of November, 2011, the observed median overall survival of all patients was 29·2 months (95% CI 9·6–48·8).

## Discussion

Our findings show that treatment with GVAX plus ipilimumab is feasible, safe, and clinically active. Furthermore, we have shown that this combined

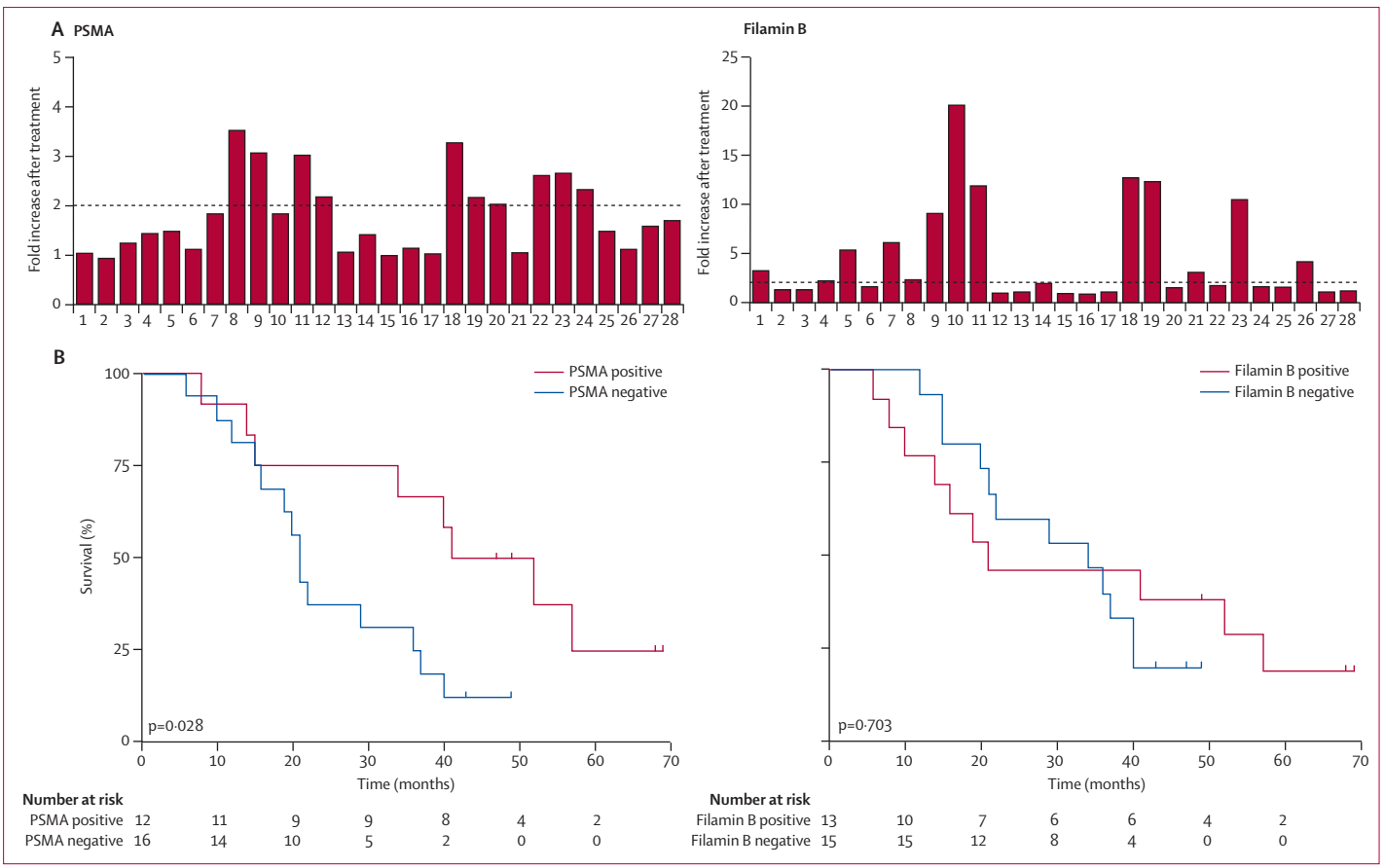


***Figure 5:*** **Treatment-induced autoantibodies against PSMA and filamin B**
(A) Antibody response against PSMA and filamin B measured by ELISA and expressed as fold increase over pretreatment levels. We measured anti-PSMA and anti-filamin B antibodies before and after (week 24, visit 13 or follow up) treatment. (B) Kaplan-Meier curves of overall survival for seroreactivity against PSMA and filamin B after treatment. We judged as a positive response positive seroconversion on immunoblot, two-fold or greater induction of antibody response over pretreatment, or both. We assessed statistical significance of the survival distribution between autoantibody positive (red lines) and autoantibody negative (blue lines) by log-rank testing. PSMA=prostate-specific membrane antigen.

immunotherapy results in the systemic activation of conventional dendritic and T cells and induces a specific antibody response against PSMA. Hypophysitis, secondary hypothyroidism, and adrenal insufficiencies were more common in our trial than previously reported for ipilimumab monotherapy,[26–29] suggesting that the recorded toxic effects after our combined treatment are greater than either agent alone. We do not yet know the cause of these endocrine insufficiencies, but they might be immune mediated. The cause could be further assessed by testing post-therapy seroreactivity against a pituitary cDNA expression library. Our findings show that toxic effects of the combination of GVAX plus ipilimumab is manageable and safe at 3·0 mg/kg ipilimumab; we did not establish a maximum tolerated dose because we expanded at 3·0 mg/kg. Higher doses are conceivable, because several phase 2 and 3 trials have now shown manageable toxic effects at 10·0 mg/kg ipilimumab.[30–32]

Our findings of durable PSA responses, improvement in bone scan, and tumour regression on CT scan suggest that this form of immunotherapy has clinical activity in mCRPC. Nevertheless, evidence is accumulating that these endpoints could be of limited validity in immunotherapy trials.[33] The response patterns evident with immunotherapeutic agents extend beyond those of cytotoxic agents and can present after a period of stable disease in which there is no tumour shrinkage or even initial evident increase in tumour burden. We noted such a PSA response pattern in five patients, with initially rising PSA levels at the start of treatment, subsequent rapid decreases, and thereafter rising PSA levels with a subsequent second rapid and profound decline that lasted 2–9 months.

We noted that the patients treated with GVAX and ipilimumab had a median overall survival of 29·2 months (95% CI 9·6–48·8). To place our findings in perspective (panel), the median overall survival of patients in the placebo group (with similar patient characteristics) was 21·7 months in the phase 3 trial of sipuleucel-T.[9] In two phase 2 trials assessing GVAX alone in a similar group of patients with mCRPC, investigators noted a median overall survival of 26·2 months and 35 months.[13,14]

### *Panel:* Research in context

**Systematic review**

Before we designed our trial we reviewed all preclinical and clinical data available for the GVAX immunotherapy and ipilimumab. Phase 2 data for prostate GVAX alone were encouraging.[13,14] Similarly, ipilimumab was successfully applied for the treatment of patients with melanoma and some responses were noted in patients with prostate cancer (Small E, University of California, San Francisco, personal communication). The rationale for the combination therapy was based on preclinical data[19] and on clinical responses recorded in patients with advanced melanoma or ovarian cancer who received ipilimumab after previous vaccination with irradiated, autologous tumour cells engineered to secrete GM-CSF.[22]

**Interpretation**

Our findings suggest that the combination of GVAX and ipilimumab has a manageable toxic effect profile and is clinically and immunologically active. Our findings warrant further clinical exploration of combined treatment with tumour vaccines and ipilimumab.

Median overall survival for patients treated in a randomised study of PSA-Tricom was 25·1 months and for sipuleucel-T was 25·8 months.[9,10] In these two trials, tumour responses were relatively scarce, whereas overall survival was improved. It is conceivable that subsequent conventional therapies might work synergistically with the induced immune response. In view of these findings, a general consensus is growing that we need to reconsider how to measure the efficacy of immunotherapy and not use short-term endpoints like response or progression-free survival, but rather use long-term survival endpoints, preferably in randomised studies.[34,35]

Vaccination-induced tumour-specific immunoglobulin responses might have direct effector functions but can also be regarded as surrogate markers for specific T-cell activation. In one study, GVAX reactive antibody responses seemed to be associated with improved survival.[14] In another study, induced seroreactivity against filamin B, a cytoskeletal protein contained in the GVAX vaccine and previously linked to cancer, was a non-significant predictor of improved survival.[13] By contrast, we did not identify improved survival in patients who developed an antibody response against filamin B, but rather identified a survival advantage for patients who underwent de-novo PSMA-specific seroconversion. PSMA is abundantly expressed on prostate cancer epithelial cells and its expression correlates with tumour progression.[36] Our findings suggest a possible involvement of anti-PSMA immunity in the recorded efficacy of the GVAX plus ipilimumab combination therapy, but also suggest PSMA seroreactivity as a possible biomarker for clinical benefit.

On the basis of our study, GVAX given concurrently with 3·0 mg/kg ipilimumab is tolerable and safe for patients with mCRPC. The recorded clinical and immunological activity warrant further research pertaining to the combined treatment of patients with mCRPC with vaccination and immune-stimulatory antibodies.

#### Contributors

AJMvdE, IL, HMP, KH, NS, and WRG contributed to the study design. AJMvdE, JV, HPvdB, RJAvM, HEG, WRG were involved in collecting clinical data and analysing data. AJMvdE, JV, SJAMS, RJAvM, TMvdS, TCH, KJ, RJS, AGMS, BMEvB, TDdG, and WRG were involved in collection of immunomonitoring data and testosterone levels, analysing the data, and statistical analysis of data. AJMvdE, JV, TDdG, and WRG drafted the first version. All authors contributed to revisions of the report. All authors approved the final draft of this report.

#### Conflicts of interest

TCH, KJ, KH, and NS worked at Cell Genesys Inc during the design of the study. They all left the company before contributing to this manuscript. IL worked at Medarex/Bristol-Myers Squibb, but left the company before contributing to this report. AJMvdE, HMP, and WRG have served as consultants and received honoraria from Bristol-Myers Squibb. TDdG and WRG received an educational grant from Cell Genesys Inc. The other authors declare that they have no conflicts of interest.

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