Tumour cell lysate-loaded dendritic cell vaccine induces biochemical and memory immune response in castration-resistant prostate cancer patients

D Reyes1,2,3, L Salazar1,3, E Espinoza3, C Pereda1,3, E Castellón1, R Valdevenito2, C Huidobro2, M Inés Becker4, A Lladser5, M N López1,3,6 and F Salazar-Onfray*,1,3

1Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago 8380453, Chile; 2Service of Urology, University of Chile Clinical Hospital, Santiago 8380453, Chile; 3Millennium Institute on Immunology and Immunotherapy, University of Chile, Santiago 8380453 Chile; 4Fundación Ciencia y Tecnología para el Desarrollo, Santiago 7750269, Chile; 5Cell Therapy Laboratory, Blood Bank Service, University of Chile Clinical Hospital, Santiago 8380453, Chile and 6Cell Therapy Laboratory, Blood Bank Service, University of Chile Clinical Hospital, Santiago 8380453, Chile

Background: Recently, we produced a tumour antigen-presenting cells (TAPCells) vaccine using a melanoma cell lysate, called TRIMEL, as an antigen source and an activation factor. Tumour antigen-presenting cells induced immunological responses and increased melanoma patient survival. Herein, we investigated the effect of TAPCells loaded with prostate cancer cell lysates (PCCL) as an antigen source, and TRIMEL as a dendritic cell (DC) activation factor; which were co-injected with the Concholepas concholepas haemocyanin (CCH) as an adjuvant on castration-resistant prostate cancer (CRPC) patients.

Methods: The lysate mix capacity, for inducing T-cell activation, was analysed by flow cytometry and Elispot. Delayed-type hypersensitivity (DTH) reaction against PCCL, frequency of CD8+ memory T cells (Tm) in blood and prostate-specific antigen (PSA) levels in serum were measured in treated patients.

Results: The lysate mix induced functional mature DCs that were capable of activating PCCL-specific T cells. No relevant adverse reactions were observed. Six out of 14 patients showed a significant decrease in levels of PSA. DTH+ patients showed a prolonged PSA doubling-time after treatment. Expansion of functional central and effector CD8+ Tm were detected.

Conclusion: Treatment of CRPC patients with lysate-loaded TAPCells and CCH as an adjuvant is safe: generating biochemical and memory immune responses. However, the limited number of cases requires confirmation in a phase II clinical trial.

Prostate cancer (PC) is the second leading cause of male tumour-related deaths in developed countries (Jemal et al, 2009). As of now there is no curative treatment for local-advanced or disseminated PC, and the only available handling is androgen deprivation therapy (ADT; Huggins and Hodges, 2002). Androgen deprivation therapy has showed important short-term effects in diminishing tumour growth, however, a significant percentage of treated patients have developed hormone-independent neoplastic cells; becoming resistant to the treatment and displaying a fast tumour progression (Pienta and Bradley, 2006). Docetaxel-based chemotherapy has showed limited clinical benefits in patients with castration-resistant PC (CRPC); improving in 2 months the patients' overall survival, nevertheless, it shows to have imperative toxic side effects (Petrylak et al, 2004; Tannock et al, 2004). The search for more effective therapies has increased the interest in exploring the use of cellular immunotherapy as treatment against PC.

*Correspondence: Dr F Salazar-Onfray; E-mail: fsalazar@med.uchile.cl
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Dendritic cells (DCs) are professional antigen-presenting cells because of their ability to efficiently activate T cells and to generate an adaptive immune response. Dendritic cells have been used in novel antitumour therapeutic strategies for several types of cancers (Hart, 1997; Banchereau et al., 2003). In PC, different approaches using prostate antigen-loaded DCs (peptides, proteins and mRNA) have been developed and tested in clinical trials (Tjoa et al., 1999; Mu et al., 2005; Fuessel et al., 2006). These strategies have proved to elicit immunological responses in patients, although only marginal clinical benefits have been achieved (Tjoa et al., 1999; Mu et al., 2005; Fuessel et al., 2006). A phase III clinical trial with Sipuleucel-T, an antigen-presenting cell-based immunotherapy against PC using prostatic acid phosphatase (PAP) as target tumour-associated antigen (TAA), has shown to improve overall survival of CRPC patients (Kantoff et al., 2010) and has been newly approved by the FDA for the treatment of advanced PC patients.

In our laboratory, we have performed two clinical trials using monocyte-derived DC vaccines for the treatment of advanced malignant melanoma (Escobar et al., 2005; López et al., 2009; Aguilera et al., 2011). Our data show that using these monocyte-derived DCs loaded with allogeneic melanoma lysates constitutes a safe procedure; able to increase the overall survival of stage IV melanoma patients and to inhibit tumour recurrence of stage III patients (López et al., 2009; Aguilera et al., 2011). In stage IV patients, delayed-type hypersensitivity (DTH) response against the melanoma lysate, which represents specific memory immune responses, was correlated with enhanced overall survival; where DTH+ patients showed a median overall survival of 33 months vs 11 months for DTH− patients (López et al., 2009; Aguilera et al., 2011). These results validate the use of allogeneic tumour lysates as an antigen source for inducing antitumour immunological memory, and DTH reaction as a useful immune-monitoring method in cancer immunotherapy. In addition, during those clinical trials, we demonstrated that TRIMEL, a heat-shocked melanoma lysate, has the ability to promote the maturation and activation of monocyte-derived DCs; and thus enabling them to activate antigen-specific T cells (Aguilera et al., 2011), which further suggests the lysate’s role not only as antigenic source but also as a maturation stimulus. The lysate’s effect seems to be mediated by the induction of danger signals, such as the release of HMGB1 and membrane mobilised calreticulin, which is differently induced in distinct cell lysates (Aguilera et al., 2011).

One major obstacle to a successful immunotherapy is breaking the self-tolerance to tumour antigens. Induction of antitumour T-cell responses by DC-based immunotherapies relies on the capability of vaccine formulations to strongly activate and to induce mature DCs (Joffre et al., 2009). To this end, clinically tested adjuvants, such as the hapten carrier protein keyhole limpet haemocyanin (KLH), have been used to enhance the efficacy of anticancer vaccines (Chiang et al., 2011; Del Campo et al., 2011). On the other hand, one of the significant drawbacks to the use of KLH as a TAA carrier in antitumour vaccines is their poor solubility and propensity to aggregate and precipitate (Gathuru et al., 2009). This flaw has increased the interest in finding other haemocyanins with similar or better adjuvant/immunomodulatory properties, and enhanced structural stability. Recently, the haemocyanin derived from the gastropod Concholepas concholepas (Concholepas concholepas haemocyanin (CCH)) has been tested in vivo; showing its ability to activate innate immunity, inducing a Th1 cytokine profile and eliciting strong antitumour effects in a murine bladder cancer model, and therefore demonstrating its potential as an immunotherapeutic agent (Moltedo et al., 2006). In addition, CCH is a protein with a high solubility and stability (Idakieva et al., 2008).

At this point, we investigated whether the treatment of CRPC patients with DCs loaded with a PC cell lysate (PCCL), used, as antigen source and TRIMEL as DC activation factor, in combination with CCH as adjuvant, may constitute a safe therapy that is able to induce clinically relevant cellular responses. Consequently, the association between the level of prostate-specific antigen (PSA) in serum and specific immunological memory responses against PCCL, detected by DTH and CD8+ memory T cell (Tm) populations present in peripheral blood, was analysed.

**Materials and Methods**

**Patients.** Twenty CRPC patients were recruited from January 2007 to December 2009 for a prospective phase I study. Inclusion criteria included histologically confirmed PC; progressive disease during combined ADT and androgen blockade (AB) according to PSA working group 2 criteria, an Eastern Cooperation Oncology Group performance status of 0 and 1, an estimated life expectancy of >6 months, withdrawal of other oncological treatments (as radiotherapy or chemotherapy) at least for 4 weeks, haematocrit levels over 30%, normal white blood cell count, normal liver function and serum creatinine under 1.5 mg dl⁻¹. Exclusion criteria included steroidal therapy and second oncological disease. The present trial’s primary end points were the immune response induction and the overall biosafety. Secondary end point was the vaccine effect on the PSA levels. The Bioethical Committee for Human Research of the University of Chile approved this study. Patients and healthy donors needed to understand and sign an informed consent statement. Non-orchietomised patients were maintained under ADT with LHRH agonist or oestrogen throughout the study.

**Haemocyanin Source.** Haemocyanin from Concholepas concholepas in PBS (0.1 M sodium phosphate, 0.15 M NaCl; pH 7.2) purified under sterile and pyrogen-free conditions and tested as LPS-free (Inmunocyanin) was provided by Biosonda Corporation (Santiago, Chile). All solutions were prepared using water for human irrigation (Baxter Healthcare Corp., Deerfield, IL, USA) and filtered through a 0.02 μm membrane filter (Millipore, Billerica, MA, USA).

**Cell Lines and Cell Lysates.** DU145, LNCaP and PC3 are PC cell lines (ATCC, Manassas, VA, USA). Prostate cancer cell lines were cultured in serum-free AIM-V medium (Invitrogen, Carlsbad, CA, USA), mixed in equal numbers, heat shocked at 42 °C for 1 h, incubated for 2 h at 37 °C and then lysed through repeatedly freeze-thaw cycles in liquid nitrogen. Next, the cell lysate was sonicated and irradiated (80 Gy). Lysate protein concentration was determined by Bradford’s method using a Biophotometer (Eppendorf, Hamburg, Germany). TRIMEL is a standardised melanoma lysate generated at the Institute of Biomedical Sciences, Faculty of Medicine of the University of Chile (Aguilera et al., 2011). Microbiological tests were performed to rule out mycoplasma, retrovirus and bacterial presence in the cell lines and the lysates. The absence of trace amounts of LPS in the lysates was also confirmed.

**Generation of Tumour Antigen-Presenting Cells (TAPCells).** Adherent monocytes from peripheral blood mononuclear cells (PBMCs) of healthy donors (n = 3) were cultured for 22 h in serum-free AIM-V medium (Invitrogen), supplemented with recombinant human interleukin 4 (rHIL-4; 500 U ml⁻¹; US-Biological, Marblehead, MA, USA), and recombinant human granulocyte-macrophage colony-stimulating factor (800 U ml⁻¹; Schering Plough, Kenilworth, NJ, USA). Then, monocytes were stimulated for 24 h with rhTNF-α (20 U ml⁻¹; US-Biological), TRIMEL (100 μg ml⁻¹) plus rhTNF-α (20 U ml⁻¹) (Trime); PCCL (100 μg ml⁻¹) plus rhTNF-α (20 U ml⁻¹) (PCCL100); PCCL (90 μg ml⁻¹) plus TRIMEL (10 μg ml⁻¹) plus rhTNF-α (20 U ml⁻¹) (PCCL90 + Trime10), PCCL (80 μg ml⁻¹) plus TRIMEL (20 μg ml⁻¹)
plus rhTNF-α (20 U ml⁻¹) (PCCL80 + Trimel20) or with medium only (activated monocytes (AMs)).

For vaccine production, PBMCs from PC patients (n = 20) were obtained by leukapheresis at the Blood Bank Service of the University of Chile Clinical Hospital as previously described (López et al, 2009). Adherent monocytes from PBMCs, cultured as described above for 24 h, were then cultured for an additional 24 h with a mix of the PCCL (80 µg ml⁻¹) and TRIMEL (20 µg ml⁻¹) plus rhTNF-α (20 U ml⁻¹; US Biological). Afterwards, the cells were harvested and cryopreserved in liquid nitrogen until their use.

**Phenotypic characterisation of TAPCells.** Tumour antigen-presenting cells were phenotypically characterised by flow cytometry using the following monoclonal antibodies (mAbs): HLA-ABC, HLA-DRDQDP, CD80 and CD83 conjugated with fluorescein isothiocyanate (FITC; eBioscience, San Diego, CA, USA), CD86-FITC (BD PharMingen, San Jose, CA, USA) and anti-CD11c conjugated with phycoerythrin (PE; eBioscience). Briefly, 1 × 10⁶ cells were recovered from the culture plate and incubated with conjugated mAbs for 30 min at 4°C. Cells were fixed with paraformaldehyde (PFA) and analysed in a flow cytometer FACSort (Becton Dickinson, Franklin Lakes, NJ, USA). Data analysis was performed using the WinMDI 2.8 software (Purdue University, West Lafayette, IN, USA).

**ELISPOT assay for IFN-γ secretion.** Peripheral blood mononuclear cells from healthy donors (n = 3) were cultured for 2 h in serum-free AIM-V medium (Intriviron). Non-adherent peripheral blood lymphocytes (PBLs) were collected and cultured in RPMI medium supplemented with rhIL-2 (20 U ml⁻¹) along with autologous prostate-loaded TAPCells (Pro80 + Trimel20) in a 10:1 ratio. On day 4 of culture, rhIL-2 (20 U ml⁻¹) was added to the medium. At day 8, autologous TAPCells were again added to the culture. Finally, at day 14, PBL were rescued from the culture. Pre-stimulated and non-stimulated PBL were co-cultured overnight with 2 × 10⁶ autologous TAPCells at different effectors/target ratios (E:T 20:1, 10:1 vs 5:1). IFN-γ secretion was tested by ELISPOT assay, as described (Escobar et al, 2005).

**Clinical protocol and DTH assay.** At days 1, 10, 30 and 60, patients were immunised subcutaneously with a mix of 2 × 10⁵ autologous TAPCells in 400 µl of frozen medium (90% autologous AB serum 10% DMSO) plus 150 µg CCH in 200 µl of saline solution as an adjuvant. Before each immunisation, 50 ml of peripheral blood was collected in heparinised tubes, separated by centrifugation with a mix of the PCCL (80 µg ml⁻¹) and TRIMEL (20 µg ml⁻¹) plus rhTNF-α (20 U ml⁻¹; US Biological). Later, the cells were harvested and cryopreserved in liquid nitrogen until their use.

One month after the end of the therapy, patients were evaluated for in vivo DTH reaction against PCCL and controls. Skin tests were performed by intradermal injections of 100 µl of PCCL (4 µg ml⁻¹), CCH 100 µl (1 µg ml⁻¹), KLH 100 µl (1 µg ml⁻¹) or 100 µl of saline solution (as negative control) at three different sites. A positive DTH reaction was defined as skin induration and erythema equal or larger than 5 mm at 48 h after injection.

**Toxicity and quality of life.** Toxicity was evaluated during every immunisation and DTH test, using the NCI Common Terminology Criteria for Adverse Events Cancer Therapy Evaluation Program (CTCAE, Bethesda, MD, USA) v.3.0. In addition, patients had a 24-h emergency phone number to report adverse events.

Quality of life was evaluated at immunisation 1, 3 and 4, and also at DTH test. We used the European Organization for Research and Treatment of Cancer Quality of Life Core Questionnaire (EORTC QLQ-C30), in Spanish language and validated in Chile. Scores for every scale were calculated according to EORTC instructions.

**Clinical response.** Serum PSA levels were measured monthly from the onset of the therapy. The PSA baseline is the level showed the week before treatment. The PSA Nadir is the absolute lowest level that the PSA drops after treatment. Prostate-specific antigen doubling-time (PSADT) was calculated using all available serum PSA measuring over the last 12 months before the treatment. We utilised the Memorial Sloan–Kettering Cancer Centre PSADT calculator, based on the formula PSADT = log2 × t/lnBG – logA), available on the website (http://nomograms.mskcc.org/Prostate/PsADoublingTime.aspx).

In addition, we evaluated metastatic disease with bone scan and abdomino-pelvic CT scan 1 week before and 1 week after the treatment.

**Determination of Tm populations.** T-cell populations from treated patients PBMCs were collected before the first immunisation and at the DTH test time. Peripheral blood mononuclear cells were stained using the following mAbs: CD8–FITC, CD45RO–Pe-Cy5 and CD62L–PE; CCRP7–PE or CD28–PE (e-Bioscience). Stained samples were acquired on a FACScan (Becton Dickinson) and analysed using the WinMDI 2.8 software.

**Determination of intracellular cytokines (IFN-γ, perforin and granzyme B).** Peripheral blood mononuclear cells obtained before the first immunisation and at the DTH test time were cultured overnight on RPMI 10% FBS with autologous DCs loaded with PCCL. Next, the cells were incubated with Brefeldin A (10 µg ml⁻¹; e-Bioscience) for 4 h, collected and stained with anti-CD8–FITC and anti-CD45RO–PECy5 mAbs. After that, cells were fixed with PFA 1%, permeabilised with saponin 0.1% and incubated with anti-IFN-γ–PE, anti-granzyme B–PE (e-Bioscience) or anti-perforin–PE (Becton Dickinson) mAbs. Stained samples were acquired on a FACScan (BD) and analysed using the WinMDI 2.8 software.

**Statistical analysis.** The normal distribution of the data was evaluated using Skewness/Kurtosis, Shapiro–Wilk and Shapiro–-Francia tests. For the analyses of paired samples, t-test and Wilcoxon rank-sum test were applied. For non-paired samples, Mann–Whitney tests were used. For grouped samples, ANOVA tests were utilised. Statistical significance was considered at a P-value < 0.05. Results are shown as means (95% confidence interval, CI). Statistical analyses were performed using Graph Pad Prism 4 software (GraphPad Software, Inc. La Jolla, CA, USA).

**RESULTS**

**Combination of PCCL with TRIMEL induces APCs maturation and activation.** We have previously demonstrated that TRIMEL, a conditioned melanoma lysate, has the ability to induce a fast, full mature and activated DC phenotype on APCs that we named TAPCells (Aguilera et al, 2011). Similarly, we have shown that other tumour cell lysates (derived from renal, breast and colon cancer) increased the expression of maturation markers on DCs (Aguilera et al, 2011). Considering this ability of conditioned tumour lysates, we decided to generate fast-differentiating TAPCells using PCCL as antigenic source and as maturation stimulus. We observed that heat-shocked PCCL (PCCL100) slightly increased the expression of MHC I and II molecules and co-stimulatory molecules CD80 and CD86, although at lower levels compared with Trimel alone (Figure 1A). For that reason, we combined PCCL with TRIMEL as maturation stimulus at different proportions. We observed that the combination of PCCL (80 µg ml⁻¹) with TRIMEL (20 µg ml⁻¹) (PCCL80 + Trimel20) increased the expression of MHC class I and II molecules and maturation markers CD83, CD80 and CD86 similarly to Trimel alone (100 µg ml⁻¹; Figure 1A).

Next, we evaluated the ability of PCCL-loaded TAPCells to activate T lymphocytes. We stimulated PBLs from healthy donors with...
PCCL-loaded autologous TAPCells for 2 weeks. After two rounds of stimulation, APC-stimulated and non-stimulated PBLs were evaluated for IFN-γ secretion in an ELISPOT assay. PC cell lysate-loaded autologous TAPCells were used as target cells. We observed that stimulated PBLs increased two- to eight-fold the IFN-γ secretion compared with non-stimulated PBLs (Figure 1B). Altogether, these results showed that the combination of PCCL with TRIMEL induces the differentiation of mature, functionally activated APCs (TAPCells); and therefore this lysate combination was clinically tested.

**Patient demographic description and treatment adherence.** Twenty CRPC patients were recruited for this study. Patient’s demographic characteristics are described in Table 1. Eleven patients received ADT as primary treatment and nine were initially treated as organ-confined PC (eight patients underwent retropubic radical prostatectomy and one patient received external beam radiotherapy). During ADT, all patients were submitted to AB using flutamide or bicalutamide as anti-androgens. In addition, 15 patients were treated with oestrogens as second-line hormonal therapy, and one patient received a third-line hormonal treatment with ketoconazol plus steroids. Only one recruited patient had been previously treated with docetaxel-based chemotherapy.

In all, 14 out of 20 patients presented metastatic disease, all of them compromising pelvic nodes and bone tissues, but none of these patients showed visceral metastases. For CRPC diagnosis, seven patients showed progressive metastatic disease under ADT plus AB, but all patients showed biochemical progression.

In total, 14 out of 20 recruited patients completed the treatment protocol. Two patients did not perform the DTH test because they presented a fast rise of serum PSA and decided to abandon the study. Two patients did not complete the treatment because of the lack of adherence to the protocol. One patient needed to use oral steroids after the second immunisation and he was also excluded from the study. Another patient presented a lower extremity.
paraparesis, 10 days after the second immunisation; this patient had a history of lumbar spine metastases and an MRI confirmed spinal cord compression, secondary to lumbar metastases. The patient started oral steroids and was excluded from the treatment protocol.

**Treatment toxicity and its effect on patient quality of life.** Observed adverse events are described in Table 2. A total of 10 adverse events were registered, being 8 cases of pain and erythema in the inoculation site after the third or fourth immunisation. All skin inflammatory events were self-limited, with a maximum duration of 48 h. None of the patients required any treatment for those adverse events. The last adverse event was self-limited asymptomatic hypertensive urgency, presented 12 h after the third immunisation and lasting for 6 h. That patient had the clinical antecedent of arterial hypertension. No grade 4 or greater adverse events were observed in this study.

In spite of this, the EORTC QLQ-C30 symptom section showed an increase in nausea and vomiting symptoms during the treatment protocol (Supplementary Figure 1A). The patients did not mention these emetic symptoms. The score of nausea and vomiting item was significantly greater after the third and fourth immunisation (Supplementary Figure 1B). We did not observe any change in the other eight symptoms evaluated by the EORTC QLQ-C30 (Supplementary Figure 1C).

*DC vaccine induces immunological memory responses.* To determine whether the treatment had the ability to induce specific cellular immune responses, we performed IFN-γ secretion ELISPOT assays before the onset of the therapy and after the fourth immunisation, using PBL as effector cells and tumour cell lysate-loaded autologous DCs as stimulators. We observed that 7 out of 14 patients showed an effector cell number dependence increase in IFN-γ secretion after the therapy as showed for 2 representative patients PT129 and PT134 (Figure 2A). The rest of the patients showed no difference between before and after vaccination as exemplified for PT78 and PT92 (Figure 2A). None of the patients showed decreased IFN-γ secretion at the end of the therapy.

Moreover, we evaluated the presence of in vitro specific cellular immune response against the PCCL and the adjuvant CCH in vivo using the DTH test as a clinically relevant parameter, since in our previous studies in malignant melanoma, the DTH test and not the in vitro immune assays, were correlated with overall survival. In this report, 9 out of 14 patients showed a positive DTH reaction against the PCCL, whereas all 14 patients developed a positive DTH test against the CCH adjuvant (Figure 2B). In some patients, DTH reactions against KLH were also detected indicating a cross-reaction with CCH after immunisation (Figure 2B). No patients showed a DTH reaction when saline solution was injected as a control. These results suggest that TAPCell-based immunotherapy is able to induce specific immune responses in a fraction of the patients, and the 100% reactivity against the adjuvant indicates that the treatment was able to induce specific cellular immune responses, increased after treatment from a mean of 9.6% to 19.8% of CD8+ T cells in all-treated patients (Figure 3B). In contrast, we observed no changes in neither the expression of the co-stimulatory molecule CD80 nor the expression of the PD-L1 molecule in the treated T cells (data not shown).

**PCCL/TRIMEL-loaded TAPCells vaccine induces an increase of functional CD8+ IFN-γ+ memory T-cell population.** To further gain insight into the DC vaccine induction of specific immune responses detected by ELISPOT and DTH test, we decided to evaluate the induction of a CD8+ memory T-cell population (Tm), which was previously correlated with the antitumour efficacy (Klebanoff et al, 2006). We observed that the total CD8+ T-cell population remained stable during the whole treatment period (from 10.1% to 13.56% of CD8+ T cells, P = 0.239; Figure 3A). Similarly, the total CD8+ T-cell population remained also stable until the end of the therapy (data not shown). Interestingly, IFN-γ+ Tm cells, a population related to the DTH reaction and antitumour responses, increased after treatment from a mean of 9.6% to 19.8% of CD8+ T-cell population in all-treated patients (Figure 3B). In contrast, we observed no changes in neither the expression of the cytotoxic molecules granzyme B nor the perforin in Tm cells, nor changes in the central or peripheral Tm subsets (Supplementary Figure 3). No statistical differences were observed in the CD4+ population (data not shown).

Immunotherapy reduces serum PSA levels and improves PSADT in immune-responder patients. We evaluated the

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**Table 1. Patient’s characteristics at baseline (n = 20)**

| Event                                      | All grades, N | Grades 3–5 |
|--------------------------------------------|---------------|------------|
| Age (years)–mean (range)                   | 69 (54–84)    |            |
| Initial treatment, n (%)                   |               |            |
| Curative intention                         | 9 (45)        |            |
| Primary ADT                                | 11 (55)       |            |
| ADT method, n (%)                          |               |            |
| Bilateral orchietomy                       | 9 (45)        |            |
| LHRH agonists                              | 11 (55)       |            |
| Second-line treatment, n (%)               |               |            |
| Oestrogens                                 | 15 (75)       |            |
| Ketoconazol plus prednisone                | 1 (5)         |            |
| Chemotherapy                               | 1 (5)         |            |
| Duration of ADT, months (range)            | 24.5 (6–81)   |            |
| Metastases, n (%)                          |               |            |
| Bone                                       | 14 (70)       |            |
| Lymph node                                 | 14 (70)       |            |
| Visceral                                   | 0 (0)         |            |
| None                                       | 6 (30)        |            |
| Progression, n (%)                         |               |            |
| Biochemical                                | 13 (65)       |            |
| Metastases                                 | 7 (35)        |            |
| Patients that completed treatment protocol, n (%) | 14 (70)     |            |

**Table 2. Adverse events**

| Event                                      | All grades, N | Grades 3–5 |
|--------------------------------------------|---------------|------------|
| Any event                                  | 10            | 1          |
| Pain and erythema in punctue site          | 8             | 0          |
| Lower extremities paraparesis              | 1             | 1          |
| Hypertensive crisis                        | 1             | 0          |

Abbreviations: ADT = androgen deprivation therapy; LHRH = luteinizing hormone-releasing hormone.
capacity of TAPCell-based immunotherapy to generate clinical responses, measured as serum PSA levels and changes in bone scan 1 month after the end of treatment protocol (DTH time). We observed decreased levels of PSA serum in 10 patients, including a 30% greater decrease than the PSA baseline in 4 patients and 50% greater in another 2 patients (Figure 4). All Nadir PSA levels were observed between third immunisation and DTH test evaluation. It is worthy to note that 9 out of 10 patients who presented decreased serum PSA levels were DTH-positive. There was no correlation between biochemical responses and in vitro T-cell responses measured by ELISPOT.

Correspondingly, we evaluated whether the treatment could improve the calculated PSADT, which was previously described as a prognostic factor in CRPC (Semeniuk et al., 2006). We observed that calculated PSADT in treated patients increased from a median of 5.1 months before treatment to 6.4 months, 1 month after treatment (DTH time), and up to 12.1 months, 3 months after the end of the therapy (Figure 5B; P < 0.05, ANOVA). In contrast, non-responder patients showed a calculated PSADT of 3 months, without any change during the treatment. Despite the effect of the PSADT, we observed no changes in metastatic lesions at the end of the treatment protocol.

**DISCUSSION**

The use of DC-based vaccines as an immunotherapeutic strategy against cancer pursues to induce and enhance T-cell-mediated antitumour immune responses. This strategy resembles physiologic mechanisms, where DCs loaded with tumour antigens are able to
Figure 3. PCCL-loaded TAPCells immunotherapy increases IFN-γ-producing memory T-cell population. PBMCs obtained before the first immunisation (Pre) and at DTH test time (Post) were co-cultured overnight with autologous PCCL-loaded TAPCells, and analysed by FACS. (A) Proportion of CD45RO⁺ T cells over CD8⁺ population. (B) Proportion of IFN-γ⁺ T cells over CD8⁺ CD45RO⁺ population (P<0.05 t-test).

Figure 4. PCCL-loaded TAPCells immunotherapy reduces serum PSA levels. Serum PSA was evaluated monthly from the treatment onset until DTH test. Graphic shows change of PSA from baseline. The PSA Nadir is the absolute lowest level that the PSA drops after treatment. Every bar corresponds to a different patient.

Figure 5. Treatment improves PSADT in immunologic-responder patients. PSADT was calculated using all available previous PSA. (A) Mean of PSADT in all-treated group. (B) Mean of PSADT by immunologic response, determined by DTH test (P<0.05, ANOVA).
prime tumour antigen-specific T cells. Effective T-cell priming requires adequate DC maturation and activation. Resting DCs or DCs exposed to an anti-inflammatory environment displayed an immature phenotype and generated T-cell tolerance (Joffre et al., 2009) that could explain reports showing that DC-based immunotherapies can stimulate the proliferation of regulatory T cells (López et al., 2006). Our protocol to generate TAPCells has demonstrated to produce mature DCs with the capacity to induce specific T-cell activation and clinical responses in malignant melanoma patients (López et al., 2009; Aguilera et al., 2011). The cancer cell lysate used in these studies as the antigenic source contains ‘danger’ signals, such as HMGB1 and Calreticulin, which are capable to trigger TLR4 and rage on the APC-promoting DC immunogenicity (Tittarelli et al., 2012). Other tumour lysates also contain danger signals that can induce DC maturation and activation, but our melanoma cell lysate (TRIMEL) displayed a comparatively stronger activity (Aguilera et al., 2011). For this reason, we used a low proportion of TRIMEL together with PCLL in this study. The presence of TRIMEL seems to contribute to the increase in the activation and maturation of DCs (Figure 1), and further adding to a better immune response of vaccinated patients.

DTH test is one of the main methods used for monitoring immune responses in clinical trials (de Vries et al., 2005). A positive DTH response is mediated by the activation of antigen-specific CD4⁺ memory T cells against an antigen, which induces the production of Th1 cytokines like IFN-γ and the recruitment of CD8⁺ T cells and other inflammatory cells (Kobayashi et al., 2001). Although some reports have suggested that the DTH test is not an appropriate method for immune monitoring because of the difficulty to standardise it and the generation of a DTH response against non-antigenic compounds; other studies have showed that DTH test is a reproducible and cost-effective method for predicting clinical benefits (de Vries et al., 2005). Delayed-type hypersensitivity reaction has showed to be an in vivo good prognosis predictor in some cancer immunotherapy trials (de Vries et al., 2005; López et al., 2009).

In our previous works, 60% of treated melanoma patients showed a positive DTH reaction against melanoma lysate, and such DTH response correlated with improved median survival (López et al., 2009; Aguilera et al., 2011). In this report, we observed a similar proportion of DTH-positive responder patients against the PCCL (immune-responder). We can assume that the induction of DTH against the PCCL reflects the generation and activation of memory CD8⁺ T cells and other inflammatory cells (Kobayashi et al., 2001). Although some reports have suggested that the DTH test is not an appropriate method for immune monitoring because of the difficulty to standardise it and the generation of a DTH response against non-antigenic compounds; other studies have showed that DTH test is a reproducible and cost-effective method for predicting clinical benefits (de Vries et al., 2005). Delayed-type hypersensitivity reaction has showed to be an in vivo good prognosis predictor in some cancer immunotherapy trials (de Vries et al., 2005; López et al., 2009). In our previous works, 60% of treated melanoma patients showed a positive DTH reaction against melanoma lysate, and such DTH response correlated with improved median survival (López et al., 2009; Aguilera et al., 2011). In this report, we observed a similar proportion of DTH-positive responder patients against the PCCL (immune-responder). We can assume that the induction of DTH against the PCCL reflects the generation and activation of memory CD8⁺ T cells and other inflammatory cells (Kobayashi et al., 2001). Although some reports have suggested that the DTH test is not an appropriate method for immune monitoring because of the difficulty to standardise it and the generation of a DTH response against non-antigenic compounds; other studies have showed that DTH test is a reproducible and cost-effective method for predicting clinical benefits (de Vries et al., 2005). Delayed-type hypersensitivity reaction has showed to be an in vivo good prognosis predictor in some cancer immunotherapy trials (de Vries et al., 2005; López et al., 2009). In our previous works, 60% of treated melanoma patients showed a positive DTH reaction against melanoma lysate, and such DTH response correlated with improved median survival (López et al., 2009; Aguilera et al., 2011). In this report, we observed a similar proportion of DTH-positive responder patients against the PCCL (immune-responder). We can assume that the induction of DTH against the PCCL reflects the generation and activation of memory CD8⁺ T cells and other inflammatory cells (Kobayashi et al., 2001). Although some reports have suggested that the DTH test is not an appropriate method for immune monitoring because of the difficulty to standardise it and the generation of a DTH response against non-antigenic compounds; other studies have showed that DTH test is a reproducible and cost-effective method for predicting clinical benefits (de Vries et al., 2005). Delayed-type hypersensitivity reaction has showed to be an in vivo good prognosis predictor in some cancer immunotherapy trials (de Vries et al., 2005; López et al., 2009). In our previous works, 60% of treated melanoma patients showed a positive DTH reaction against melanoma lysate, and such DTH response correlated with improved median survival (López et al., 2009; Aguilera et al., 2011). In this report, we observed a similar proportion of DTH-positive responder patients against the PCCL (immune-responder). We can assume that the induction of DTH against the PCCL reflects the generation and activation of memory CD8⁺ T cells and other inflammatory cells (Kobayashi et al., 2001). Although some reports have suggested that the DTH test is not an appropriate method for immune monitoring because of the difficulty to standardise it and the generation of a DTH response against non-antigenic compounds; other studies have showed that DTH test is a reproducible and cost-effective method for predicting clinical benefits (de Vries et al., 2005). Delayed-type hypersensitivity reaction has showed to be an in vivo good prognosis predictor in some cancer immunotherapy trials (de Vries et al., 2005; López et al., 2009).
analysed patients strongly limits the conclusions raised by this study, it becomes necessary to design and develop a phase II trial to establish the real clinical impact of our treatment on PSA-DT and overall survival. At this respect, a more accurate selection of patients, excluding non-responding advanced bone metastatic patients, need to be performed to increase the effect of our therapy in a future phase II clinical trial.

Immunotherapy may constitute an interesting alternative for some patients, particularly if used in combination with other systemic therapies. In fact, emerging evidence indicates that the antitumour activities of chemotherapy rely on several off-target effects, particularly directed to the host immune system, that cooperate for successful tumour eradication (Bracci et al, 2013; Ma et al, 2013). Among the indirect ways of immune cell stimulation, some cytotoxic drugs have been shown to enhance an immunogenic type of cell death in tumour cells, resulting in the emission of specific signals that trigger phagocytosis of cell debris and promote the maturation of DCs, ultimately resulting in the induction of potent antitumour responses. At this respect, some experiences, combining immunotherapy with chemotherapy have been explored in melanoma and retinoblastoma (Liu et al, 2013; Weber et al, 2013).

In conclusion, our study suggests that TAPCell-based immunotherapy against PC is a safe approach that is capable to induce memory T-cell responses as determined in vivo (DTH) and in vitro (cytokine release) that could be associated with clinical responses including, decreased serum PSA levels and enhanced PSADT, and encouraging to continue further studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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