Phase I study with an autologous tumor cell vaccine for locally advanced or metastatic prostate cancer

Milton Berger [1], Fernando T. Kreutz [2,3], Jorge L. Horst [1], Aline C. Baldi [3], Walter J. Koff [1]

[1] Department of Urology, Hospital de Clínicas de Porto Alegre, and [2] Department of Biophysics, Federal University of Rio Grande do Sul, and [3] FK Biotecnologia SA, Porto Alegre – RS, Brazil

ABSTRACT - Purpose: To attempt the isolation and primary culture of prostate tumor cells, to use the cultured cells for active immunotherapy, and to evaluate the safety and efficacy in a Phase I clinical trial. **Methods:** Tumor fragments were collected from 50 patients with prostate-specific antigen (PSA) ≥ 10 ng/mL, $\leq$cT2 PCa who underwent radical retropubic prostatectomy (RRP) and 6 patients with metastatic PCa who underwent transurethral resection of the prostate (TURP). Cultured tumor cells were incubated with IFN-α, irradiated, and cryopreserved. Seven vaccine inoculations were performed into $\geq$pT3 and/or N+ patients, and M+ patients, with the first two doses admixed with Bacille Calmette-Guérin (BCG). Follow-up was performed with measurement of delayed-type hypersensitivity (DTH) reactions, PSA and hemato-chemical tests, and bone scans. **Results:** No cell culture was obtained in the TURP group. Cell culture and vaccine production were obtained in 37 cases (74%) in the RRP group. Eleven $\geq$pT3 and/or N+ patients were vaccinated. Toxicity was generally limited to the inoculation sites. DTH reactions ≥ 10 mm were observed in 2 patients and ≥ 5 mm in 6 patients. Two patients had a decrease in PSA levels after vaccine administration. **Conclusions:** The autologous cell vaccine is safe and seems to induce a positive immune cellular response. Primary cell culture and vaccine production can be obtained for most RRP patients, but not for TURP patients using our method. There seems to be some influence of the vaccine in PSA evolution after RRP.

INTRODUCTION

Prostate cancer (PCa) is a major public health problem. In Brazil and USA, PCa is the second cause of mortality by cancer and the most prevalent non-skin cancer among men (1,2). Despite the good results achieved with surgery or radiotherapy for localized disease (3). patients with locally advanced disease represent a high-risk population for recurrence and should be considered for adjuvant therapy(4). Moreover, novel therapeutic interventions are needed for patients with metastatic PCa, especially those with hormone-refractory disease, whose median survival is less than 1 year (5).

The evidence that an immune response could be mounted against cancer has driven the investigation of immunotherapy for the treatment of PCa (6). Active specific immunotherapy involves the presentation of tumor antigens to the immune system in order to induce an immune response, cellular or humoral. In theory, the tumor itself is the best antigenic source to induce an immune response, since it presents a unique range of antigens peculiar to the affected individual (7). In this report we describe a phase I study with an autologous tumor cell vaccine, immunomodulated with interferon (IFN) and Bacille Calmette-Guérin (BCG), in patients with locally advanced or metastatic PCa.

MATERIALS AND METHODS

Study design and patient selection
In the first phase of the study (material collection), patients with clinically localized PCa scheduled to undergo radical retropubic prostatectomy (RRP) and patients with metastatic PCa scheduled to undergo transurethral resection of the prostate (TURP) agreed with collection of material from the

Corresponding author: Milton Berger MD, PhD Rua Ramiro Barcelos, 1172 / 213 Porto Alegre – RS 90035-002, Brazil Phone/fax: + 55 51 33118945 + 55 51 33124516 mberger.uro@uol.com.br

surgical specimens (tumor samples) for establishment of primary tumor cell culture. RRP patients should have at least one pre-operative prostate-specific antigen (PSA) determination ≥10 ng/mL and TURP patients should have documented metastases and infravesical obstruction, this being the indication of surgery.

In the second phase of the study (vaccine inoculation), RRP patients whose pathological examination revealed extracapsular extension, seminal vesicle invasion (≥pT3) and/or lymph node metastases (N+), and TURP M+ patients received the autologous cell vaccine.

Both groups (RRP and TURP) should meet the additional inclusion criteria to start the second phase: leukocyte count ≥4000/μL; platelet count ≥100.000/μ; normal renal and hepatic functions; ECOG (Eastern Cooperative Oncology Group) performance 0-2; successful establishment of primary tumor cell culture and enough cell expansion to produce the proposed number of vaccine doses; and for TURP patients, life expectancy of at least 3 months; and at least a 4-week interval between another treatment (such as chemotherapy or radiotherapy) and the beginning of the protocol. Exclusion criteria were use of immunosuppressive doses of corticosteroids; presence of autoimmune disease; presence of active infection; and presence of metastatic disease in the central nervous system.

The study was approved by the institution's Scientific Committee for Research and Ethics in Health. Patients signed an informed consent form for each of the study phases (material collection and vaccine inoculation).

The TNM 2002 classification was used for pre-operative and post-operative staging.

Material collection and vaccine preparation
For RRP patients, tumor fragments were collected just after the excision of the surgical specimen, under aseptic conditions in the operating room. The prostate was painted with sterilized China ink and cut in 7-10 mm-thick slices. The tumor areas were identified visually and by palpation, based on the information of pre-operative digital rectal examination and transrectal ultrasound-guided biopsy. For TURP patients, after resection of the

periurethral portions of the gland, chips of prostate tissue closer to the periphery were collected. Approximately 1 cm^3 of tumor tissue was removed and placed in culture medium (RPMI-1640 with 20% fetal bovine serum and antibiotics) for transportation. A fragment of the material to be placed in culture medium was separated for pathological examination.

The material was processed in the first 24 hours. Samples were mechanically reduced to 1-3 mm^3-fragments and incubated with RPMI-1640 medium, supplemented with 10% fetal bovine serum and antibiotics. Culture status was checked daily and the culture medium changed on a weekly basis, depending on cell growth.

When cell growth was sufficient to prepare at least seven vaccine doses (7 x 10^7 cells), IFN-α2b (Blauferon B, Blaufiegel) was added to the culture for 72 hours. After this period, cells were removed from culture medium, irradiated (200 Gy), and cryopreserved. (Aroldo Braga Filho, M.D., Serviηo de Radioterapia do Hospital Sγo Lucas (PUC-RS), Porto Alegre).

Vaccination schedule and follow-up
The patient only started the protocol once seven doses, each containing 10^7 autologous tumor cells, were available. Four initial doses were administered weekly. BCG (Fundação Ataulpho de Paiva, Brazil) was added as adjuvant on the 1st and 2nd doses (10^7 organisms) on all patients. The 5th and 6th doses were administered monthly and the 7th dose was administered three months after the 6th dose. Therefore, the vaccine was administered over a period of six months. Each dose was diluted with saline and had a total volume of 0.150 mL.

Inoculations were performed intradermally in the upper limbs. After 72 hours of each inoculation, the delayed-type hypersensitivity (DTH) reaction was measured (largest diameter in millimeters of induration). DTH was not measured after the first two doses. At each visit, medical history and physical examination were performed, and adverse reactions were recorded according to Common Toxicity Criteria. Hematological tests, renal and hepatic function tests, and PSA measurements were performed before the 1st, 5th, 6th and 7th inoculation, and at the 8th and 12th follow-up months.

Table 1. Baseline characteristics of TURP and RRP groups

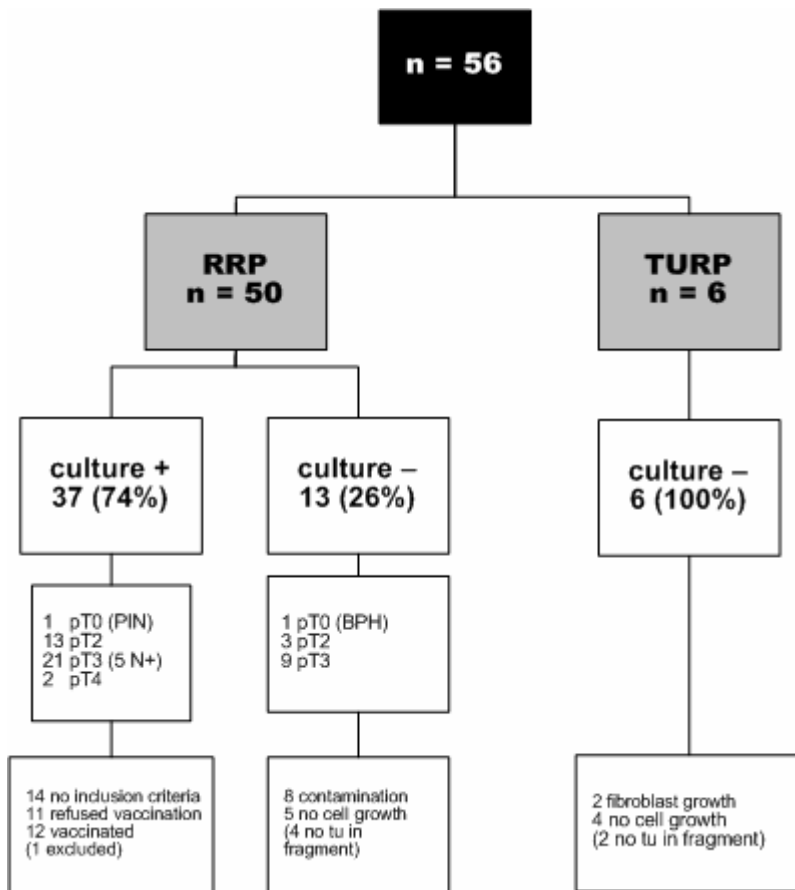|		mean	median	range
TURP n = 6	*age*	73	73	67 - 78
	pre-op PSA	269	195	109 – 500
	Gleason score	9	9	8 – 10
	TNM	T2-4 Nx M1		
RRP n = 50	*age*	66	67	43 – 79
	pre-op PSA	18.1	15.6	8.05 – 52.5
	Gleason score	7	7	6 – 9
	cTNM	T1c (19), T2 (31)		
	pTNM	T0 (2), T2 (16), T3 (30), T4 (2) N+ (5)		

Figure 1. Primary cell culture results according to collection method

Table 2. Relevant characteristics of the vaccinated patients

initials	age	pre-op PSA	pTNM	Gleason score	Other Treatments
PPL	68	15.8	T3a N0	7 (3+4)	
MRS	70	27.9	T3a N0	9 (5+4)	Hormonal blockade *
JaB	68	22.0	T3b N1	9 (5+4)	Hormonal blockade **
OAC	63	19.5	T3b N1	9 (5+4)	Hormonal blockade **
AAS	68	18.4	T3b N1	9 (5+4)	Hormonal blockade **
AI	65	23.4	T3b N0	6 (3+3)	
JoB	69	52.5	T3a N0	7 (3+4)	
APS	68	13.0	T3b N1	7 (4+3)	Hormonal blockade **
ASO	68	20.0	T3b N0	6 (3+3)	
RC	47	24.5	T4a N0	7 (3+4)	
RG	66	27.1	T3b N0	7 (4+3)	

- Treatment was initiated during vaccination; ** Treatments were initiated before vaccination;

Bone scans were performed at the 6th, 9th, and 14th follow-up months. Any test or investigation could be repeated any time at the physician's discretion. Patients were not denied any conventional treatment considered appropriate, such as hormonal therapy or radiotherapy, either before, during or after the protocol. The software programs Epi Info version 3.2.2 and SPSS version 8.0 were used for descriptive statistics and logistic regression analyses, respectively.

Table 3. Adverse effects on the eleven vaccinated patients

Adverse Effects	No. Pts
erythema	11
induration	11
pruritus	11
ulceration	1
regional adenopathy	2
fever	2

RESULTS

From December/2001 to July/2004, a total of 56 patients were enrolled in the first part of the study. Six patients underwent TURP (TURP group) and 50 patients underwent RRP (RRP group). The baseline characteristics of both groups are presented in Table 1. All patients in the TURP group had cT2-4 Nx M1 hormone-refractory disease. All patients in the RRP group had clinically localized disease (T1c and T2). Thirty-two of the 50 RRP patients (64%) were upstaged to ≥T3 disease after surgery. Two RRP patients had pT0 disease; one had diffuse

high-grade prostatic intraepithelial neoplasia (PIN) and the other one had benign prostatic hyperplasia (BPH). Five pT3 patients had lymph node metastases (N+).

Cell culture and vaccine production
In the RRP group, primary cell culture was established in 37 cases (77%), including the pT0 patient with PIN, and cell expansion in culture sufficient for vaccine production was achieved for all cases. Primary cell culture could not be established in 13 cases (23%), due to contamination in 8 and no cell growth in 5 cases. In 4 cases, the material sent to pathology did not show tumor cells (one of them was the pT0 patient with BPH) (Figure 1). Using a multivariate logistic regression model, no association was verified between pre-operative PSA [OR=0.94,IC95%(0.88;1.02)] and definitive Gleason score [OR=0.81,IC95% (0.18;3.57)] with the establishment of primary cell culture. Primary cell culture could not be established in any of the TURP cases, due to fibroblast growth in 2 and no cell growth in 4 cases. In 2 cases, the material sent to pathology did not contain tumor cells (Figure 1).

Vaccination
Fourteen patients did not meet the inclusion criteria to receive the vaccine (1 pT0 and 13 pT2 patients). From the 23 ≥pT3 remaining patients, 11 refused and 12 agreed to receive the vaccine (Figure 1). One patient was excluded from the study due to an unrelated laryngeal cancer.

The relevant characteristics of the vaccinated patients are shown in Table 2. The four N+ patients

(JaB, OAC, AAS and APS) started hormonal blockade before the study. One patient with a Gleason 9 tumor and post-op PSA elevation (MRS) started hormonal blockade during the study. The other 6 patients received only the vaccine after surgery.

Vaccine toxicity is shown in Table 3. All patients presented some degree of erythema, induration and pruritus after the inoculation of the first two BCG-containing vaccine doses. One patient presented ulceration that resolved with local treatment. Two patients presented regional adenopathy and short duration low-grade fever, also after the administration of the vaccine with BCG. No other adverse effect was seen during the study.

Two patients had DTH reactions ≥10 mm (AAS, APS), 6 patients had reactions ≥5 mm (PPL, MRS, JaB, OAC, ASO, AI), 2 patients had reactions <5 mm (JoB, RC), and 1 patient did not have any DTH reaction (RG).

The evolution of PSA records after surgery and vaccination of the 6 patients who received only the vaccine is shown in Figure 2.

DISCUSSION

Since the demonstration of the antitumor properties of interleukin-2 in patients with metastatic melanoma and renal cancer, cancer immunotherapy has evolved with progress in knowledge of general and tumor immunology (8). The objective of cancer immunotherapy is to induce a T cell response against tumor antigens, since specific T lymphocytes could recognize and destroy the cells that display the tumor antigens (9). Both CD3+CD4+ helper and CD3+CD8+ cytotoxic T lymphocytes (ThL and CTL) are important for the generation of an effective immune response to tumors, although CTLs are the final effectors. In order to induce such a response, adequate exposure of tumor antigens to antigen-presenting cells (APCs) is necessary, since APCs, whose prototype is the dendritic cell (DC), are responsible for processing and presenting tumor antigens to T cells in the lymphatic tissue (10). Tumor antigens can be presented to the immune system either in the form of autologous or allogeneic whole tumor cells, or in the form of a defined, specific tumor antigen (total protein, peptide, DNA, RNA) (11). Being

intracellular proteins, the exposure of tumor antigens on the cell surface is realized by major histocompatibility complex (MHC) class I molecules. Often, MHC class I molecules are downregulated in tumors, including PCa (12). IFN-α stimulates the expression of MHC class I molecules and could potentiate the exposure of tumor antigens (13). This is the reason for the utilization of this cytokine in the preparation of the vaccine. In addition, antigens are best exposed to the immune system in a stimulatory context, i.e. of greater antigenicity. BCG is a non-specific immunostimulant and has been used as an adjuvant for tumor vaccines. Its properties seem to be related to activation of DCs and mobilization of CD4+ and CD8+ T cells in the lymph nodes draining the inoculation site (14), what justified its use as a vaccine adjuvant in this trial. BCG has become the standard of care for patients with superficial bladder cancer (15). Although not precisely understood, its mechanism of action may be related to a possible direct antiproliferative effect and, more importantly, to activation of local vesical DCs, induction of a Th1 immune response, activation of CTLs and especially Natural Killer (NK) cells (16). Toll-like receptors (TLRs), expressed by immune cells, recognize several bacterial structures such as bacterial wall components or bacterial DNA. Immuno-stimulatory CpG motifs in bacterial DNA recognized by specific TLRs in DCs lead to a Th1 immune response (17). Activation of innate immunity may be an important step in the induction of acquired immunity, and TLRs may constitute the link between these two components of the immune system (18).

The intradermal route of administration of the autologous vaccine was favored by the abundant presence of Langerhans cells, the cutaneous DCs (19). The number of tumor cells per dose, number of BCG organisms per dose, and dose of radiation were based on studies of autologous vaccines for colon, kidney and prostate cancers (20,21).

One difficulty related to autologous cell vaccines is the occasional failure to establish primary cell culture and cell expansion sufficient to produce the planned vaccine doses (21). In our study, we were able to obtain primary cell culture in 74% of the RRP cases (37 out of 50) and enough cell expansion was achieved in all of them. Cell culture could also be obtained in a pT0 patient with diffuse high-grade
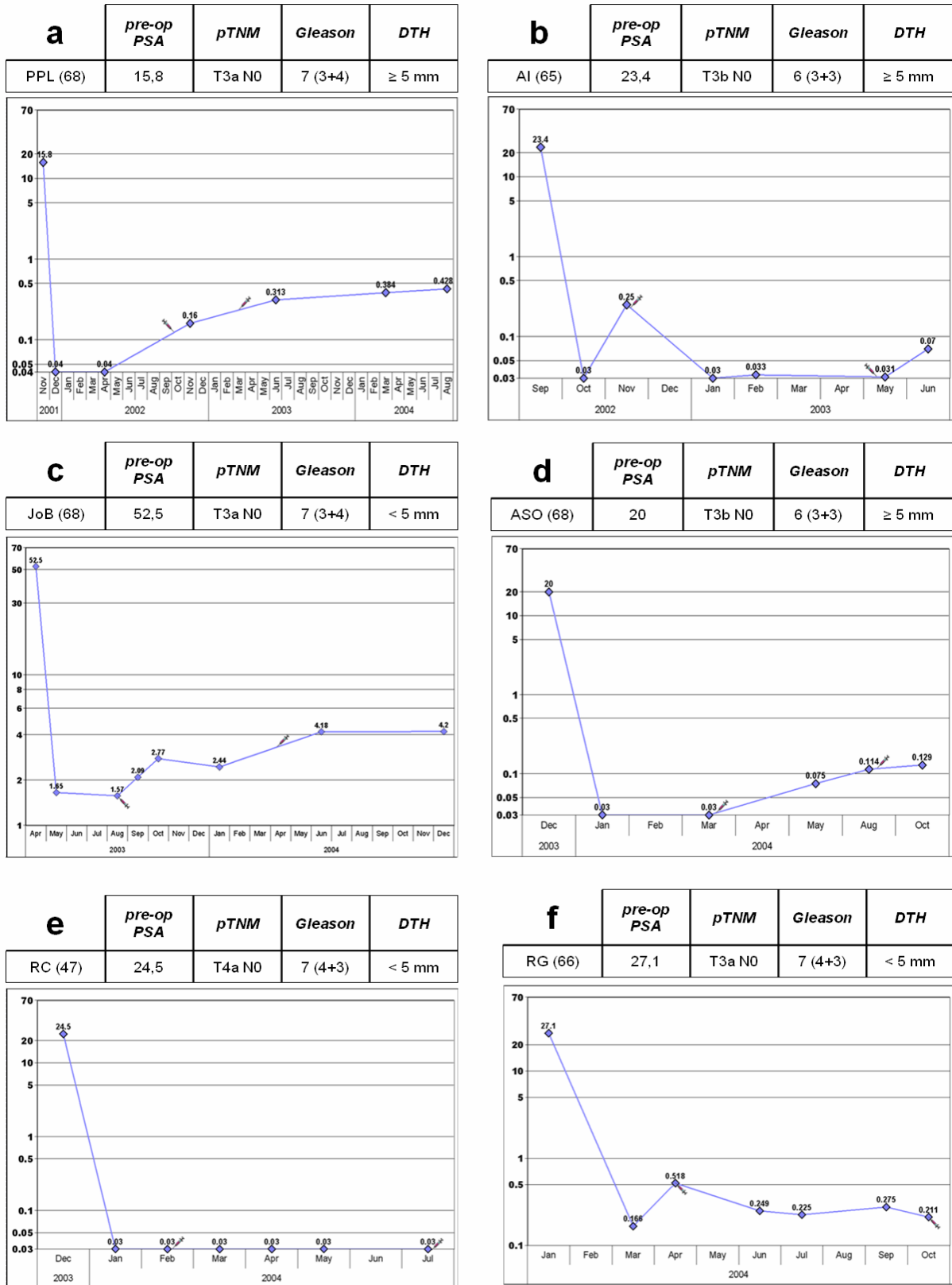
Figure 2 – Evolution of PSA curves of the six patients who received only the vaccine after surgery (syringe indicates start and completion of vaccination; PSA values plotted in logarithmic scale).

PIN. We have precisely identified tumor areas in the surgical specimens of 45 (90%) of the 50 RRP patients (Figure 1). We believe that patient selection (PSA ≥ 10 ng/mL) has favored this result. Primary cell culture could not be established in all 6 TURP cases. Cell damage caused by electrical current could explain this finding. Therefore, we could not assess the vaccine in patients with metastatic disease.

DTH reactions are an in vivo functional test that suggests T cell response (22), and can be considered a predictor of peripheral immunity by T lymphocytes (23). Reactions greater than 5 mm are usually referred to attest cellular immune response. We have observed DTH reactions ≥ 5 mm in 6 patients and ≥ 10 mm in 2 patients and we believe that such findings are suggestive of immunological mobilization. These responses can be considered specific against the modified tumor cells. DTH was not measured after the first two doses due to the fact that BCG by itself produces an intense DTH response unrelated to the tumor cells.

This study corroborates the low toxicity of whole-cell vaccines in PCa immunotherapy (21,24). We have found mild skin reactions on inoculation sites in all vaccinated patients. More intense reactions (ulceration, low-grade fever, and adenopathy) were found in 3 patients after the first two BCG-containing doses (Table 3).

It is difficult to evaluate the effect of the vaccine on PSA evolution in the 5 patients who received hormonal blockade. The evolution of PSA records for the 6 patients who received only the vaccine after surgery is shown in Figure 2.

Patients AI and RG, both with pre-op PSA > 20 ng/mL and seminal vesicle invasion, had increases in early post-op PSA to significant levels (0.25 and 0.518 ng/mL, respectively) that decreased after the start of vaccination (Figure 2, b and f). Patient JoB, with pre-op PSA > 50 ng/mL and a post-op nadir of 1.65 ng/mL, seemed to have a stabilization of PSA levels around 2.0 ng/mL after vaccination for about one year (Figure 2c). Patient RC, with pre-op PSA > 20 ng/mL and microscopic invasion of external sphincter and positive surgical margins at this level (pT4a), had undetectable PSA levels after surgery and vaccination with no other adjuvant treatment (Figure 2e). Patients PPL and ASO, with

extracapsular extension and seminal vesicle invasion, respectively, have presented a progressive slow increase in PSA levels after surgery and vaccination (Figure 2, a and d). It is likely that surgery alone has been responsible for these outcomes and it is premature to state that vaccination had an impact over PSA evolution after treatment; nevertheless, we could hypothesize some association. Considering that these patients belong to a high-risk group for disease progression after surgery and given the occurrence of DTH reactions in most of them, it would be necessary to evaluate the vaccine in a larger number of patients to further confirm its clinical benefit.

The advantages of an autologous whole cell cancer vaccine are: multiple tumor-associated antigens can be targeted; the need for prior identification of the antigen is circumvented; the fact that autologous cells are used increases the chance of a MHC dependent stimulatory mechanism being in effect. Although the individualized preparation of the vaccine is labor intensive, we were able to prepare vaccines in 80% of the samples containing tumors. The material lost due to contamination was mainly due to lack of training in collecting of tumor cells for culture. Once the proper training was given the efficacy on preparing the culture material was increased.

The possible mechanisms of immunoactivation are still under investigation. The modified culture cells could be engulfed by DCs for the subsequent processing and presentation of tumor antigens to T cells. It is also possible that T cells would recognize tumor antigens presented by the irradiated tumor cells themselves. In this particular study we did not evaluate the increase on MHC class I expression on the modified tumor cells. On previous studies (unpublished data) we demonstrated an increased on MHC class I expression on different tumor cells after IFN- α2b treatment.

For future studies, vaccine efficacy in addition to quantification of PSA levels in a larger number of patients would be performed. These would also include measurement of cellular immunity such as the induction of T cell Th1 cytokine production upon specific re-stimulation with vaccine ex vivo and the assessment of the frequency of T cells specific to prostate cancer specific epitopes. A more selective adjuvant such as CpG could also be used

to investigate the role of a more focused Th1 response.

CONCLUSIONS

Immunotherapy is a novel approach to PCa treatment and may eventually become part of the anticancer armamentarium, along with surgery, radiotherapy and chemotherapy, especially in the setting of minimal residual disease.[9] This study demonstrates that the autologous tumor cell vaccine immunomodulated with IFN-α2b and BCG is safe, with adverse effects restricted to the inoculation sites. Additionally, it has been shown that primary tumor cell culture can be established with tissue samples derived from RRP specimens and cannot be established with samples derived from TURP specimens, using our method. The vaccine seems to induce cellular immune responses, as measured by DTH reactions. A study with a larger number of patients will be performed to assess the impact on disease progression.

REFERENCES

[1]   INCA (Instituto Nacional do Câncer). Available at http://inca.gov.br/ (accessed March 2005).

[2]   Jemal, A., Murray, T., Ward, E., Samuels, A., Tiwari, R.C., Ghafoor, A., Feuer, E.J., Thun, M.J. Cancer Statistics, 2005. CA Cancer J. Clin., 55: 10, 2005.

[3]   Kupelian, P.A., Potters, L., Khuntia, D., Ciezki, J.P., Reddy, C.A., Reuther, A.M., Carlson, T.P., Klein, E.A. Radical prostatectomy, external beam radiotherapy < 72 Gy, external beam radiotherapy > or = 72 Gy, permanent seed implantation, or combined seeds/external beam radiotherapy for T1-T2 prostate cancer. Int. J. Radiat. Oncol. Biol. Phys. 58: 25, 2004.

[4]   Khan, M.A., Partin, A.W. Management of high-risk populations with locally advanced prostate cancer. Oncologist, 8: 259, 2003.

[5]   Cho, D., Di Blasio, C.J., Rhee, A.C., Kattan, M.W. Prognostic factors for survival in patients with hormone-refractory prostate cancer (HRPC) after initial androgen deprivation therapy (ADT). Urol. Oncol., 21: 282, 2003.

[6]   Pantuck, A.J., Zisman, A., Henderson, D., Wilson, D., Schreiber, A., Belldegrun, A. New biologicals for prostate cancer prevention: genes, vaccines, and immune-based interventions. Urology, 57 Suppl: 95, 2001.

[7]   Ward, S., Casey, D., Labarthe, M.C., Whelan, M., Dalgleish, A., Pandha, H., Todryk, S. Immunotherapeutic potential of whole tumour cells. Cancer Immunol. Immunother., 51: 351, 2002.

[8]   Rosenberg, S.A. Development of effective immunotherapy for the treatment of patients with cancer. J. Am. Coll. Surg., 198: 685, 2004.

[9]   Berzofsky, J.A., Terabe, M., Oh, S., Belyakov, I.M., Ahlers, J.D., Janik, J.E., Morris, J.C. Progress on new vaccine strategies for the immunotherapy and prevention of cancer. J. Clin. Invest., 113: 1515, 2004.

[10] Smyth, M.J., Godfrey, D.I., Trapani, J.A. A fresh look at tumor immunosurveillance and immune-therapy. Nat. Immunol., 2: 293, 2001.

[11] Mocellin, S., Mandruzzato, S., Bronte, V., Lise, M., Nitti, D. Part I: vaccines for solid tumors. Lancet Oncol., 5: 681, 2004.

[12] Blades, R.A., Keating, P.J., McWilliam, L.J., George, N.J., Stern, P.L. Loss of HLA class I expression in prostate cancer: implications for immunotherapy. Urology, 46: 681, 1995.

[13] Belardelli, F., Ferrantini, M., Proietti, E., Kirkwood, J.M. Interpheron-alpha in tumor immunity and immunology. Cytokine Growth Factor Rev., 13: 119, 2002.

[14] Li, Q., Normolle, D.P., Sayre, D.M., Zeng, X., Sun, R., Jiang, G., Redman B.D., Chang, A.E. Immunological effects of BCG as an adjuvant in autologous tumor vaccines. Clin. Immunol., 94: 64, 2000.

[15] Morales, A., Eidinger, D., Bruce, A.W. Intracavitary bacillus Calmette-Guerin in the treatment of superficial bladder tumours. J. Urol., 116: 180, 1976.

[16] Bohle, A., Brandau S. Immune mechanisms in bacillus Calmette-Guerin immunotherapy for superficial bladder cancer. J. Urol., 170: 964, 2003.

[17] von Meyenn, F., Schaefer, M., Weighardt, H., Bauer, S., Kirschning, C.J., Wagner, H., Sparwasser, T. Toll-like receptor 9 contributes to recognition of Mycobacterium bovis Bacillus Calmette-Guerin by Flt3-ligand generated dendritic cells. Immunobiology, 211: 557, 2006.

[18] Akira, S., Takeda, K. Toll-like receptor signalling. Nat. Rev. Immunol., 7: 499, 2004.

[19] Simons, J.W., Mikhak, B., Chang, J.F., DeMarzo, A.M., Carducci, M.A., Lim, M. Weber, C.E., Baccala, A.A., Goemann, M.A., Clift, S.M., Ando, D.G., Levitsky, H.I., Cohen, L.K., Sanda, M.G., Mulligan, R.C., Partin, A.W., Carter, H.B., Piantadosi, S., Marshall, F.F., Nelson, W.G. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer. Cancer Res., 59: 5160, 1999.

[20] Vermorken, J.B., Claessen, A.M., van Tinteren, H., Gall, H.E., Ezinga, R., Meijer, S., Scheper, R.J., Meijer, C.J., Bloemena, E., Ransom, J.H., Hanna, M.G. Jr., Pinedo, H.M. Active specific immunotherapy for stage II and III human colon cancer: a randomised trial. Lancet, 353: 345, 1999.

[21] Nelson, W.G., Simons, J.W., Mikhak, B., Chang, J.F., DeMarzo, A.M., Carducci, M.A., Kim, M., Weber, C.E., Baccala, A.A., Goeman, M.A., Clift, S.M., Ando, D.G., Levitsky, H.I., Cohen, L.K., Sanda, M.G., Mulligan, R.C., Partin, A.W., Carter, H.B., Piantadosi, S., Marshall, F.F. Cancer cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer as vaccines for the treatment of genitourinary malignancies. Cancer. Chemother. Pharmacol., 46 Suppl: S67, 2000.

[22] Clay, T.M., Hobeika, A.C., Mosca, P.J., Lyerly, H.K., Morse, M.A. Assays for monitoring cellular immune responses to active immunotherapy of cancer. Clin. Cancer Res, 7: 1127, 2001.

[23] Disis, M.L., Schiffman, K., Gooley, T.A., McNeel, D.G., Rinn, K., Knutson, K.L. Delayed-type hypersensitivity response is a predictor of peripheral blood T-cell immunity after HER-2/neu peptide immunization. Clin. Cancer Res., 6: 1347, 2000.

[24] Eaton, J.D., Perry, M.J., Nicholson, S., Guckian, M., Russell, N., Whelan, M., Kirby, R.S. Allogeneic whole-cell vaccine: a phase I/II study in men with hormone-refractory prostate cancer. BJU Int, 89: 19, 2002.