Use of Human Glandular Kallikrein 2 for the Detection of Prostate Cancer: Preliminary Analysis

Partin AW, Catalona WJ, Finlay JA, et al.
Urology. 1999;54:839-845.

Intrigued by the differences in expression and biologic activity of PSA and hK2, Partin and associates characterized the role of hK2 in the detection of prostate cancer using a prototype assay that uses 2 antibodies against total hK2 (thK2). They analyzed 937 archival serum samples from 922 men with PSA levels greater than 2 ng/mL, all with prostate biopsies revealing either cancer (184 men) or benign prostatic tissue (738 men). Of the men studied, 86% had a benign digital rectal examination (DRE). Median age, PSA level, and thK2 levels were similar, while median %fPSA and thK2/fPSA values differed significantly between men with and without prostate cancer.

Multivariate logistic regression analysis identified %fPSA as the best predictor of the presence of prostate cancer, followed by thK2/fPSA. The investigators constructed cut points of %fPSA and thK2/PSA that stratified patients into prostate cancer risk groups. In men with benign DRE results and tPSA levels of 2 to 4 ng/mL, the use of both %fPSA and thK2/fPSA identified 40% of all cancers while requiring biopsy in only 16.5% of the men. Combining %fPSA and thK2/fPSA was also useful in men with benign DRE results and PSA levels between 4 and 10 ng/mL with an initial negative biopsy. In this population, a thK2/fPSA ratio greater than 0.18 predicted a subset of men at greater risk for harboring cancer, supporting a strategy of repeated biopsy in these men.

In summary, Partin and associates demonstrated the reliability of a direct immunoassay for thK2. Multivariate logistic regression was used to compare the utility of %fPSA and thK2/fPSA in prostate cancer detection. While %fPSA outperformed thK2/fPSA, the information provided by these markers was complementary, allowing the greatest specificity for prostate cancer detection. The use of thK2/fPSA in men with PSA levels of 2 to 4 ng/mL identified 40% of all detectable cancers while sparing biopsy in 83% of men without detectable cancer. The thK2/fPSA ratio also appeared to offer promise in helping counsel men with a prior negative biopsy on the need for subsequent biopsy.

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Vaccine-Based Immunotherapy for Prostate Cancer

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After a long period of smoldering activity in cancer immunotherapy, interest in cancer vaccines has caught fire. This interest is reflected in the logarithmic increase in the number of open clinical trials for the treatment of prostate and other cancers. The renewed enthusiasm has been fanned largely by a more sophisticated understanding of the intricacies of immune system regulation and by the availability of better reagents (recombinant cytokines, dendritic cells, etc) to manipulate the immune response.

The concept of active immunotherapy of cancer is based on the theory that tumor cells escape detection and destruction by the host's immune system through various strategies: deficient tumor antigen processing and presentation, lack of immune costimulation, production of inhibitory factors by tumor cells, and insufficient helper activity from CD4 cells. One method of inducing the immune system to recognize and destroy cancer cells is cancer vaccination. Cancer vaccines attempt to facilitate the presentation of tumor antigens to the immune system; typically, in conjunction with factors that augment immune system activation, generating cellular and humoral antitumor activity.

Several distinct approaches to prostate cancer vaccination are currently being investigated. One important factor is the choice of material used as the tumor antigen. Some investigators have used irradiated, intact autologous prostate cancer cells derived from patients' own tumors or allogeneic prostate cancer cells from previously established prostate cancer cell lines, such as LNCaP and PC-3. Others have used prostate cancer cellular homogenates, which contain a multitude of poorly defined tumor- and non–tumor-specific antigens, or have used more well-defined single antigens; often, prostate-specific proteins, such as PSA, prostate-specific membrane antigen (PSMA), or the cancer-associated mucin MUC-1.[1] Another important factor is the method chosen to augment the normally muted immune response to these antigens. Approaches currently being evaluated include the use of dendritic cells, which are highly efficacious in presenting antigens to T cells, and the use of various cytokines, such as tumor necrosis factor, interleukin (IL)-2, IL-4, IL-10, IL-12, and granulocyte-macrophage colony-stimulating factor (GM-CSF), to improve the immune system's response to the desired antigens.

GM-CSF is a potent inducer of hematopoietic differentiation, stimulating the production of macrophages and dendritic cells from monocyte precursors. In vivo administration of exogenous GM-CSF induces differentiation of bone marrow-derived antigen presenting cell-like macrophages, which are critical for antigen presentation to T cells. In addition, GM-CSF also stimulates the activation of quiescent dendritic cells. In 1997, Simons and associates[2] at Johns Hopkins University in Baltimore reported on the feasibility, safety, and bioactivity of a GM-CSF–gene transduced autologous tumor vaccine for patients with metastatic renal cell carcinoma. Although initial clinical responses were limited, this vaccine was shown to induce a specific immune response to tumor cells. Recently, Simons and colleagues reported the results of a small phase I study investigating the feasibility and safety of prostate cancer vaccines using autologous prostate cancer cells similarly engineered to express GM-CSF in patients with metastatic prostate cancer (first review).

Prostate cells express tissue-specific proteins, such as PSA and PSMA, which represent a unique potential antigenic target for antitumor immune response targeted specifically to prostate cells. Sanda and coworkers at the University of Michigan have explored the use of a vaccinia viral vector carrying the PSA complementary DNA (cDNA) as a potential vaccination strategy for prostate cancer patients (second review). Since PSA is already present in patients as a "self" protein, it should be nonimmunogenic. It is possible, however, that once PSA is processed by antigen-presenting cells in the context of the vaccinia virus, it may enter different processing and packaging pathways, unmasking PSA peptide epitopes that can generate a humoral and cellular immune response. Alternatively, vaccinia virus proteins may act as an adjuvant to augment an otherwise weak anti-PSA response.

Finally, the characterization of dendritic cells, thought to be the most powerful antigen-presenting cells of the body, has stimulated intense interest as a potential cancer vaccine reagent. Dendritic cells are bone marrow–derived "professional" antigen-presenting cells. They have distinct pathways of differentiation that can be subdivided into myeloid- and lymphoid-derived lineages. Dendritic cells are vital for the stimulation of cytotoxic and helper T-cells because of their ability to recognize, phagocytose, and process antigens in the peripheral tissues, which they transport to and present in secondary lymphoid organs. In the clinical setting, a convenient source of myeloid-derived dendritic cells is peripheral blood monocytes, which can be induced to differentiate into dendritic cells using GM-CSF and IL-4. Dendritic cell–based tumor vaccines have produced encouraging early results in various cancers, such as melanoma and renal cell carcinoma. Murphy and colleagues[3-6] from Washington University have done extensive preclinical and clinical research on dendritic cell–based immunotherapy of prostate cancer. Moreover, they have studied a prostate cancer vaccine strategy using autologous dendritic cells as a vehicle to present prostate antigens, such as PSMA-derived peptides to T cells (third review).

Numerous centers around the country are investigating use of new tumor vaccines to treat patients with prostate cancer and other tumors. Preclinical and early clinical data suggest that tumor vaccine therapies are feasible and safe. Most trials report only mild systemic side effects, such as hypotension, fever, chills, and fatigue; reactions at the vaccination site, such as erythema, swelling, and pruritus; and discomfort during injection. These approaches have shown that a systemic immune response to human prostate cancer can be generated, although clinical importance of the current generation of tumor vaccines remains to be established in phase II and III studies.

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

Simons JW, Mikhak B, Chang JF, et al.
Cancer Res. 1999;59(20):5160-5168.

In a small-dose escalation study, Simons and associates evaluated the safety of vaccinating patients who had metastatic prostate cancer with irradiated, autologous prostate cancer cells, modified ex vivo to produce high levels of GM-CSF. Tumor cells were obtained from each patient at surgery and were established in culture. The cells were then genetically modified through transduction with a replication-defective retrovirus containing a cDNA encoding the GM-CSF gene. After expansion and irradiation (to prevent further proliferation), the vaccine cells were reinjected subcutaneously into the patient.

Eleven immunocompetent patients with metastatic prostate cancer found incidentally at radical prostatectomy were enrolled. The vaccine was administered subcutaneously into the limbs. Two dose levels of vaccine (1×10^7 and 5×10^7 cells) secreting human GM-CSF at 150 to 1500 ng/million cells/24 h were assessed. Vaccination was repeated every 2 weeks until exhaustion of the vaccine supply, yielding between 3 and 6 vaccination cycles.

Primary cultures of prostate tumor cells failed to establish for 3 patients, leaving 8 of the 11 patients for evaluation. Further difficulties with in vitro expansion of primary cultures shifted 4 patients from the planned high-dose group (5×10^7) to the low-dose group (1×10^7). Thus, the high-dose group comprised 3 patients and the low-dose group, 5.

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In contrast to these difficulties, ex vivo retroviral GM-CSF gene transfer was successful in all 8 patients.

No dose-limiting toxicity was observed. Toxicity was limited to injection pain, erythema, swelling, and pruritus at the vaccination site as well as mild, low-grade fever; malaise; and chills. No significant alterations in serum electrolyte, chemistry, and hematologic counts were observed. Vaccine site biopsies revealed the presence of prostate tumor–derived vaccine cells and inflammatory infiltrates composed of antigen presenting cells, such as Langerhans cells and macrophages as well as neutrophils, T cells, and eosinophils. The authors observed a dose response with higher intensity of cellular infiltrates in the high-dose group. T-cell antitumor response was evaluated with serial cutaneous delayed-type hypersensitivity (DTH) reactivity tests to irradiated, untransduced, autologous prostate cell targets. Two patients displayed DTH reactivity before vaccination and 7 patients, after vaccination. Both vaccine and DTH site biopsies displayed massive eosinophil recruitment. Modest numbers of B-cells were also observed in the biopsies. Comparison of prevaccination with postvaccination serum analyses showed an increase in antibody titers to prostate tumor antigens in 3 of the 8 patients. While new antibodies to 3 undefined prostate antigens were identified, no anti-PSA antibodies and no PSA-specific T-cell recognition were detected. Although efficacy is not an end point of phase I studies, it is noteworthy that all 8 patients exhibited disease progression.

As noted by the authors, establishing and expanding human prostate cancer cells is technically difficult, limiting dose and number of cycles of autologous tumor vaccines. Use of allogeneic tumor cells, which, in contrast to autologous tumor cells, are not from the patient's own tumor but are histologically similar human tumor cell lines, may be a practical alternative. In support of this argument, all 3 of the newly identified prostate antigens were also found in LNCaP cells. The Johns Hopkins team, therefore, switched to GM-CSF–gene transduced LNCaP and PC-3 for the phase II trial.

Recombinant Vaccinia-PSA (Prostvac) Can Induce a Prostate-Specific Immune Response in Androgen-Modulated Human Prostate Cancer

Sanda MG, Smith DC, Charles LG, et al.
Urology. 1999;53:260-266.

Sanda and colleagues administered vaccinia-PSA to 6 patients with biochemical recurrence after radical prostatectomy. The vaccinia virus has an attractive safety profile, having been used for more than 200 years for smallpox immunization. These investigators used the vaccinia virus as a vector for introducing recombinant PSA as a tumor-associated antigen with the goal of enhancing the cellular recognition of prostate cancer cells. All patients were androgen responsive and received antiandrogen therapy for a median duration of 7 months (range, 2 to 14 months) before inclusion in the study. Antiandrogen therapy was interrupted at the beginning of the study period to allow tumor cells to re-express PSA, the target for the vaccine therapy. Seven days after each of the patients received the last administration of goserelin depot 3.6 mg, they were given a single intradermal injection of vaccinia-PSA at a dose of 2.65×10^7 or 2.65×10^8 plaque-forming units (3 patients per dose level). In all 6 patients, the vaccinia-PSA virus proved safe, with minimal toxicity limited to constitutional symptoms, such as vaccine site erythema and vesicle formation, fever, chills, fatigue, and diarrhea. Induction of humoral immunity to PSA by the vaccine was evaluated with Western blot analysis of anti-PSA antibody in patient sera. Vaccine-related anti-PSA IgG was induced in only 1 patient and anti-PSA IgM, in none. Two patients had detectable anti-PSA IgG and 5 patients had detectable anti-PSA IgM before vaccination. Five of the 6 patients exhibited a PSA level rise in a median time of 1 month (range, 0 to 4 months) after restoration of serum testosterone levels. The interval from androgen restoration to PSA rise was used as a surrogate end point of antitumor activity. The fact that vaccination occurred concurrently with cessation of hormone therapy makes it difficult to determine the effect of either on clinical response. The observation that 2 patients had anti-PSA antibodies before vaccination and that 1 patient seroconverted despite a rapidly rising PSA level questions the clinical effects of a humoral response to prostate cancer.

Evaluation of Phase I/II Clinical Trials in Prostate Cancer With Dendritic Cells and PSMA Peptides

Tjoa BA, Simmons SJ, Bowes VA, et al.
Prostate. 1998;36:39-44.

Infusion of Dendritic Cells Pulsed With HLA-A2-Specific Prostate-Specific Membrane Antigen Peptides: A Phase II Prostate Cancer Vaccine Trial Involving Patients With Hormone-Refractory Metastatic Disease

Murphy GP, Tjoa BA, Simmons SJ, et al.
Prostate. 1999;38:73-78.

Phase II Prostate Cancer Vaccine Trial: Report of a Study Involving 37 Patients With Disease Recurrence Following Primary Treatment

Murphy GP, Tjoa BA, Simmons SJ, et al.
Prostate. 1999;39:54-59.

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Follow-up Evaluation of a Phase II Prostate Cancer Vaccine Trial

Tjoa BA, Simmons SJ, Elgamal A, et al.
Prostate. 1999;40:125–129.

Murphy and associates have explored a prostate cancer tumor vaccine approach that employs dendritic cells to present prostate antigens to autologous T cells. This approach requires cultivating peripheral blood mononuclear cells harvested from enrolled patients. The cells are then cultured in the presence of cytokines to induce differentiation of monocytes into antigen-presenting cells. These cells are pulsed with the desired antigen (PSMA) and then given back to the patient intravenously, as a vaccine. The authors have chosen two 9–amino-acid fragments from each end of the PSMA molecule (PSM-P1 and PSM-P2) with a high affinity for the class I human leukocyte antigen (HLA-A0201) molecule.

In their initial phase I clinical trial comprising 51 patients with metastatic hormone-refractory prostate cancer, Murphy and coworkers[6] demonstrated that the administration of autologous dendritic cells pulsed with HLA-A0201–derived peptides from PSM-P1 and -P2 was safe. Toxicity was limited to transient hypotension. Seven partial responders were observed.

On the basis of phase I data, this group expanded its study assessing the efficacy of autologous dendritic cells pulsed with PSMA peptides. One hundred seven patients were enrolled in an open-labeled, phase II clinical trial comprising 3 main groups of patients. The first group consisted of 33 men with metastatic hormone-refractory prostate cancer who had participated in the previous phase I study. Another group consisted of 33 patients with hormone-refractory prostate cancer with no previous immunotherapy. The third group was made up of 41 patients with evidence of local recurrence after failure of a primary treatment, including surgery, radiotherapy, brachytherapy, or hormone therapy. All study participants received a total of 6 infusions of dendritic cells pulsed with PSMA-derived peptides at 6-week intervals. In addition, approximately half of the study patients also received a 7-day course of systemic adjuvant therapy in a nonrandomized fashion in the form of subcutaneous injections of 75 $\mu g/m^2/d$ of GM-CSF starting on the day of infusion. Clinical response as defined by the National Prostate Cancer Project Criteria—a 50% decrease in PSA level or a significant resolution of lesions on scintigraphy—was evaluated. A panel of immunogenic recall antigens for the evaluation of T-cell immune function as assessed by DTH skin test was used. Patients who were receiving hormonal therapy before enrollment in the study continued the same therapy throughout the trial.

Of the 33 patients with advanced prostate cancer who participated in the primary study, 9 (27.3%) displayed partial response, 11 (33.3%) exhibited no significant change in disease, and 13 had disease progression. Seven patients died during the study. Four of the 9 partial responders also responded in the phase I study. Four of the remaining 5 partial responders did not receive autologous dendritic cells pulsed with PSM-P1 or -P2 in the phase I study. Immune response as assessed with DTH tests did not add any conclusive information.

Of the group of 33 patients with hormone-refractory prostate cancer who had no previous immunotherapy, only 25 were evaluable, 2 (8%) of whom were complete responders and 6 (24%) of whom were partial responders. One patient (4%) showed no significant disease change, and 16 (64%) exhibited disease progression. Of these, 7 died and 2 withdrew from the study. Once again, the level of DTH response was equivalent in the responders to that in the nonresponders. In fact, 6 of the 8 responders exhibited no change and 2 exhibited a decrease in immune reactivity.

The third group consisted of patients with locally advanced prostate cancer, all of whom did not respond to primary treatment and 35% of whom also did not respond to secondary treatment. Of 41 subjects who were initially enrolled in the study, 37 subjects were evaluable. One complete (4%) and 10 partial (27%) responders were observed. Eight patients (22%) showed no significant change, and 18 (49%) exhibited disease progression. As in the previous studies, DTH tests did not reveal any significant information.

Overall, the response rate of the phase II study groups was 30% (ranging from 27% to 32%). Although a majority of the responders expressed HLA-A2, a high number did not, which should have abrogated correct antigen presentation in this group of patients. The authors hypothesize that the clinical improvement in these patients is caused by the ability of the dendritic cells to capture and present other antigens after being infused into the patients, as well as by the affinity of PSM-P1 and -P2 to other HLA molecules. A follow-up evaluation, with an average of 291 days for the hormone-refractory group with no previous immunotherapy and of 557 days for the recurrent group (follow-up days include a treatment period of 221 days plus a follow-up period), revealed that only 58% of the initial responders were still responsive. Disease progression of the initial responders occurred between 20 and 131 days after the end of the trial. The average response duration was 149 days for the hormone-refractory group and 187 days for the group with recurrence. Responders were found to have a statistically significant decrease in post-study versus prestudy serum PSA when compared with nonresponders, who exhibited an increase in PSA levels.

While this approach seems intriguing and promising, these studies are not yet sufficient to allow a reliable determination of the efficacy and appropriate clinical scenario for the use of autologous dendritic cells (pulsed with PSMA

peptides) as a prostate cancer vaccine. Further, well-controlled clinical trials are needed.

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Erectile Dysfunction

Diagnostic Index and Dysfunction Treatment

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Two areas with potential clinical impact are discussed: the advantages of a patient-friendly questionnaire for diagnosis of erectile dysfunction, and the diagnosis of and treatment for andropause.

Development and Evaluation of an Abridged, 5-Item Version of the International Index of Erectile Function (IIEF-5) as a Diagnostic Tool for Erectile Dysfunction

Rosen RC, Cappelleri JC, Smith MD, et al.
Int J Imp Res. 1999;11:319-326.

Nothing irks an epidemiologist more than using a test that is not reliable and reproducible. Therefore, the group of epidemiologists from the Robert Wood Johnson Medical School set out to identify and validate a questionnaire that could be used by any physician to diagnose erectile dysfunction. They came up with a questionnaire—the International Index of Erectile Function (IIEF)—that consists of 15 questions covering 5 domains of erectile function and is reliable and reproducible.[1] It has a high degree of sensitivity and specificity for detecting patients with erectile dysfunction. Although the original IIEF test covers 15 questions and is ideal for use in clinical trials of drugs for treating patients with erectile dysfunction, it is not patient-friendly, particularly if it were used as a diagnostic tool by physicians in clinical practice. This suggested the need for a more abbreviated version of the IIEF that would be easy to administer in the office setting. The authors took it upon themselves to devise an abbreviated 5-item version of the original 15-item test that focused primarily on erectile function and intercourse satisfaction (Table 2). Their data indicated that this abbreviated version was excellent in detecting the presence and severity of erectile dysfunction, thereby supporting its use as a valid diagnostic instrument in the clinical setting. The sensitivity of the test was 0.98, and the specificity was 0.88.

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Testosterone Supplementation in the Aging Male

Kim Y-C.
Int J Imp Res. 1999;11:343-352.

Treatment of Endocrinologic Male Sexual Dysfunction

Nehra A.
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Is there such a condition as andropause?[1] There is heated debate in the endocrinologic world whether such a condition occurs and whether treatment of this condition should be offered to elderly patients. The major clinical manifestations of andropause—if there is such a condition—are decreased cognition, decrease in muscle mass and strength, osteopenia, and loss of libido. It is accepted that since testosterone levels decline with aging, such effects may be reversed with exogenous testosterone, potentially improving the quality of life of older men. The deleterious side effects of exogenous testosterone, however, must be considered in these elderly men. Although prostate cancer and/or benign prostatic hyperplasia are not induced by exogenous testosterone, the hormone may induce already present cancer cells to begin growing. Yet when prostate-specific antigen is measured, most studies show very little, if any, change during androgen therapy.