1 SCIENTIFIC ABSTRACT

a. Immunotherapy has been an attractive possibility for therapy of prostate carcinoma. Prostate cancer [1, 2] has been considered an ideal candidate for immunotherapy development as its long natural history, starting with non-metastatic biochemical relapse (elevating prostate specific antigen in serum following complete therapy of primary tumor) allows time for the generation of potentially effective anti-tumor immune responses. Thus, numerous attempts to treat prostate cancer by immunotherapy have started to yield encouraging results. For example, a prostate cancer vaccine consisting of autologous antigen-presenting cells combined with a single prostate-associated antigen, has been tested in successful phase 3 clinical trials (1, 2). That trial demonstrated that tolerance to a tumor-associated antigen can be broken leading to enhanced T cell response that resulted in one complete and durable remission and examples of improved survival (3, 4). Single–antigen targeting of prostate cancer is attractive as prostate cancer cells, even after relapse following hormonal treatment, usually continue to express the characteristic prostate–specific antigen (PSA), prostatic acid phosphatase (PAP) and prostate–specific membrane antigen (PSMA). However, targeting single antigens carries a higher risk of tumor escape from immune recognition than targeting numerous antigens. Mechanisms of escape include the loss of expression of tumor–associated antigens and/or MHC class I molecules, defects in proteosomal antigen processing and/or peptide epitope presentation (5) and adverse events of immune modulation (6). While targeting of a single tumor antigen can result in effective tumor cell killing through epitope spreading, recent results in immunotherapy of glioblastoma suggest that multiple antigenic targets are important for clinically relevant immune responses (7). The notion that a broader spectrum of tumor-associated antigens may increase the chance of clinical success has led, inter alia, to the use of allogeneic prostate cancer cell lines in the presence of adjuvant (8) or modified to express cytokines such as GM–CSF (reviewed in (9)).

b. A whole-cell vaccine is safe and effective in men suffering from asymptomatic androgen–independent prostate cancer. Despite the initial success of therapy, many men develop recurrent and subsequently androgen independent disease. Immunotherapy for control of disease progression is particularly attractive at this stage in view of its documented anti-tumor activity, low toxicity and the potential to alter the natural history of the disease (1, 2).

   Recently a study has been completed testing the efficacy of an allogeneic whole-cell prostate carcinoma (APCC) vaccine in a phase 2 clinical trial in pain–free patients with minimal or no symptoms and no bone metastases (8). The vaccine contained prostate cell lines designated OnyCap23, LnCaP and P4E6. (For the rationale in selecting these cell lines, see the investigator brochure, Appendix IV.) Each dose contained eight million irradiated cells from each of the three cell lines and was injected intradermally. The first two doses (Weeks 0 and 2) were co-administered with Bacille Calmette Guerin and the subsequent 12 monthly doses (starting on Week 4) without it. This treatment was safe, non-toxic and effective in reducing PSA velocity in 42 percent of patients. The treatment was also suggestive of clinical benefit as median time to progression (TTP) of 58 weeks compared favorably to historical controls (TTP = 26 weeks) (10).

c. Irradiated tumor cells are an effective source of tumor–associated antigens. Recently our colleagues at Mayo Clinic showed that irradiated tumor cells combined with dendritic cells (DCs) can raise protective immunity against tumors much more effectively than combination of dendritic cells with tumor cell lysates or boiled tumor cells (11). This observation is in line with evidence that radiation-induced necrosis elevates the levels of heat shock proteins that render tumor cells highly immunogenic

(12). In addition, human monocyte–derived DCs incubated with killed allogeneic tumor cells effectively cross prime naïve $CD8^+$ T cells (13) lending support to the hypothesis that mature monocytes can substantially enhance immunogenicity of irradiated APCC lines.

d. Dendritic cells could enhance clinical efficacy of allogeneic tumor cell vaccines. So far, allogeneic tumor cells have been either co-injected with inflammatory adjuvants (e.g., Bacille Calmette Guerin; (14)) or transfected with cytokines (e.g., GM–CSF (9)). The relative theoretical disadvantage of this approach (with or without adjuvants) is the lack of ability to control antigen presentation. Pivotal for controlled initiation and maintenance of immune response to infections and tumors are dendritic cells (DCs) (15). Activated autologous DCs have been administered to patients to stimulate the immune system and empower it to select and expand tumor–specific effector T cells. The advantages of ex vivo DC culture are the ability to 1) control vaccine quality and quantity 2) circumvent tumor mediated immune suppression 3) ensure potency of individualized vaccines and 4) control antigen delivery.

Injection of tumor–cell vaccines can result in their processing and consequent epitope presentation by macrophages (16), immature DCs, mature DCs, etc; each of these cells can affect immunity differently, even with opposing effects (e.g., the tolerizing and suppressive effects of immature DCs v. immunizing effects of mature DCs; (17)). Perhaps the most relevant observation is that prostate cancer cells actively inhibit DC maturation, the critical step in immune activation, and kill mature DCs (18). The use of ex vivo processed DCs has been considered an effective means to offset these immunosuppressive effects of prostate cancer and stimulate therapeutic immunity against the disease.

The aim of this clinical study is to test the hypothesis that combination of a proven effective APCC vaccine co-administered with ex vivo generated DCs (DC–APCC) will be more clinically effective than the APCC alone. This protocol includes a two arm (30 patients each) randomized phase II study of APCC compared with DC–APCC. To our knowledge, this is the first controlled clinical study of the adjuvant effects of DCs in combination with cultured allogeneic tumor cells. Patients will be randomized either to the APCC arm or DC–APCC arm. They will be monitored for tumor response, changes in PSA velocity and markers of immune response.