Optimizing Prostate Cancer Treatment by Combining Local Radiation Therapy with Systemic Vaccination

Commentary on Gulley et al., Combining a Recombinant Cancer Vaccine with Standard Definitive Radiotherapy in Patients with Localized Prostate Cancer.

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Prostate cancer is the most common malignancy in men and recurs in up to 30% to 40% of patients within 10 years of diagnosis despite initial treatment by prostatectomy or radiation therapy (1). This suggests that primary treatment may be limited by failing to address occult metastatic disease at the time of diagnosis. In fact, recurrence is often heralded by an increase in serum prostate-specific antigen (PSA) before occult metastases become evident. In this situation, androgen ablation is generally used, but there is controversy over the timing of salvage treatment and this is often temporarily effective due to the appearance of hormone resistance (2). Thus, new therapeutic strategies for patients at the time of diagnosis to prevent recurrence are high priorities. The increased expression of PSA and relatively limited tissue distribution in adult men suggested that PSA might serve as an effective target for vaccine development. This has been confirmed in several preclinical models in which various vaccine vectors have shown T cell–dependent therapeutic responses against PSA-expressing tumors (3–5). The safety of PSA vaccines have been shown in early-phase clinical trials and data from these preliminary studies documented the ability to generate PSA-specific T-cell responses following vaccination in selected men with advanced prostate cancer (6, 7). The last few years have also identified a number of strategies for increasing the immunogenicity, and potentially the clinical effectiveness, of tumor vaccines. In the May 1 issue of Clinical Cancer Research, Gulley et al. (6) utilized many of these new strategies to combine systemic vaccination with localized radiation therapy at the time of initial prostate cancer treatment. They reported minimal toxicity and 13 of 17 evaluable patients had at least a 3-fold increase in PSA-specific T cells, whereas no such T cells were isolated from patients receiving standard radiotherapy alone. These results show the safety profile and feasibility of combining systemic vaccination against PSA with local radiotherapy in the early management of prostate cancer. The general rationale for radiation therapy and vaccination will be reviewed and the potential logic for this combination as a more general paradigm in the approach to cancer patients with localized disease will be discussed.

Role of the Immune System in Prostate Cancer Treatment

The potential of the immune system to recognize and eradicate cancer has been recognized for over a century. Yet, only in the past several years have the molecular and cellular basis of immune recognition and host defense against tumors been elucidated. In murine studies, using knockout technology, protection against chemically induced sarcomas and spontaneous epithelial tumors was shown to depend on the presence of T lymphocytes and IFN-γ (8). These insights strongly supported the widely held notion that T cells are central for immune-mediated rejection of tumors and increased the focus on strategies designed to activate and promote T-cell responses as a general approach to cancer immunotherapy.

The activation of T cells depends on recognition of cognate antigen by the T-cell receptor. The T-cell receptor recognizes processed peptide epitopes presented on MHC molecules on the surface of target cells, or in the case of initial priming, on professional antigen-presenting cells. The most potent antigen-presenting cell is the dendritic cell; these cells promote T-cell activation by engulfing antigen and processing exogenous protein into peptide fragments that can be presented by class I MHC molecules to CD8+ T cells and class II MHC molecules to CD4+ T cells following dendritic cell maturation. In addition, mature dendritic cells provide additional costimulatory signals to responding T cells, which lower the threshold for T-cell activation and promote T-cell survival following recognition of cognate antigen (9).

Thus, effective immunity against cancer requires presentation of tumor-associated antigens (TAA) by professional dendritic cells that prime and activate T cells in the context of appropriate costimulatory signals. Once activated, antigen-specific T cells traffic to sites of tumor growth and mediate tumor rejection upon encountering antigenic epitopes expressed on the tumor cell surface by class I MHC molecules. This general process, although highly simplified, has led to an intense focus on strategies for promoting dendritic cell antigen presentation and T-cell activation (Fig. 1). A critical component of this process is the necessity of isolating antigens capable of mediating T-cell engagement and serving as targets on tumor cells to guide T-cell–mediated rejection. The ideal antigen would be highly expressed by tumor cells, would display unique immunogenic
epitopes, and would not be expressed by normal tissue. Few antigens have been able to fulfill all of these objectives, although prostate cancer provides a unique opportunity for immunotherapy because many prostate antigens are exclusively expressed by prostate epithelial cells or are expressed at only very low levels in other body tissues (10). Because patients with prostate cancer often have the primary gland removed by surgery or damaged by radiation, the risk of serious autoimmune or related toxicity is minimized. PSA represents one such antigen and has been widely evaluated as a potential TAA.

The Biology Behind

The general mechanism by which tumor cells are recognized and rejected by the immune system is shown. Tumor cells secrete soluble antigens or are engulfed in apoptotic or necrotic form by professional antigen-presenting cells, such as dendritic cells (DC). The dendritic cell process antigen and present class I – restricted peptides to CD8+ T cells and class II – restricted peptides to CD4+ T cells in the presence of costimulatory signals expressed on mature antigen-bearing dendritic cells. The CD4+ T cells provide help for CD8+ T-cell priming through local cytokine release and dendritic cell conditioning. The CD8+ T cells circulate to sites of tumor growth and mediate cytotoxicity of tumor cells through the release of perforins and granzyme B following recognition of class I – restricted peptide fragments or through fas/FasL interactions.

Recombinant Poxviruses as Expression Vectors

A critical issue in vaccine design is the selection of a vector for expression of targeted TAA and a variety of approaches have been utilized, as mentioned above. The use of viral vaccines offers several advantages, including the inherent immunogenicity of the virus, high levels of gene expression, and a more “natural” way to initiate immune responses. The poxviruses represent a family of related double-stranded DNA viruses distinguished by their host specificity and DNA restriction maps; they have been extensively studied as vaccines in preclinical models. The pioneering work of Panicali, Moss, Paolotti, and others showed that poxviruses could be used for recombinant gene expression and first suggested the potential for expressing prokaryotic and eukaryotic genes as a method for vaccination (11). Vaccinia virus is the prototypical poxvirus and was used for smallpox prevention with great success. Similar to other poxviruses, vaccinia virus replicates within the cytoplasm of infected cells and induces cell lysis, releasing new virion capable of infecting surrounding cells. The host immune response to vaccinia virus, including foreign transgenes expressed by recombinant vectors, includes strong neutralizing antibody titers and a significant cell-mediated T-cell response. The ability to express large eukaryotic genes, induce potent immunity, and lack of nuclear integration suggested that recombinant poxviruses could be useful for vaccines targeting highly specific antigens. In 1988, a human melanoma antigen, p97, was expressed by vaccinia virus and induced cellular immune responses in both mice and primates (12). This was followed by Schlom’s group, reporting that a vaccinia virus expressing human carcinoembryonic antigen (CEA) had therapeutic activity against a model CEA colon carcinoma and induced CEA-specific antibody and T-cell immunity (13). Early-phase clinical trials in patients with advanced cancer showed that recombinant vaccinia vectors were well tolerated, induced CEA immune responses in some patients, but had little clinical response. The rapid appearance of strong neutralizing antibodies against the vaccinia vector itself appeared to inhibit the ability to boost immunity against weak foreign transgenes expressed by recombinant vectors.
The avipox viruses are a family of poxviruses that infect birds and are unable to replicate in mammalian cells. Because infection with avipoxviruses does not produce new virions, the degree of neutralizing antibodies generated following mammalian infection is quite low. This allows viral particles to persist for a longer period of time and express foreign transgenes resulting in significantly enhanced T-cell immunity. The feasibility of using avipoxvirus vectors was established in several clinical trials using recombinant fowlpox virus and the canarypoxvirus, ALVAC (14). Further studies in animal models suggested that heterologous prime-boost vaccination schedules using two different poxvirus vectors induced stronger immune responses against foreign antigens compared with single-agent immunization protocols (15). This observation has been extended to clinical trials although the mechanism for enhanced immunity and therapeutic activity remains uncertain. Nevertheless, the optimal application of the prime-boost approach is being actively explored in both the preclinical and clinical settings.

Because rhesus monkeys share 94% sequence homology with human PSA, a recombinant vaccinia virus expressing human PSA was tested in these primates (3). All vaccinated monkeys developed short-lived anti-PSA IgM antibody titers and PSA-specific T-cell responses that could be detected up to 270 days following vaccination. This group also showed that T cell lines generated in vitro could recognize cells infected with recombinant vaccinia virus expressing PSA in a human leukocyte antigen–restricted manner (16). These results prompted a phase I clinical trial of vaccinia-PSA in men with increasing PSA levels after prostatectomy or radiation therapy (6). This trial enrolled 33 men and the vaccine was administered with or without granulocyte macrophage colony-stimulating factor. There were only mild cutaneous and constitutional symptoms reported and PSA levels were stable for at least 6 months in 14 of the 33 men. Nine of these patients remained stable for 11 to 25 months and six were progression-free at the time of the report. A dose escalation phase I trial was also completed in 42 men with metastatic prostate cancer (7). This trial documented that the vaccine was well tolerated, although no antitumor responses were observed. In 1997, the availability of a recombinant fowlpox virus expressing PSA led to a cooperative group phase II clinical trial evaluating the prime-boost approach using vaccinia-PSA and fowlpox-PSA in a three-arm randomized protocol design (17). In this trial, men with increasing PSA levels after primary treatment were eligible for a series of three vaccinations with fowlpox alone, vaccinia prime and fowlpox boosters, or fowlpox prime and vaccinia booster. Whereas no PSA responses were observed, 45.3% of men were free from PSA progression at 19.1 months and 78.1% had no evidence of clinical disease progression. There was a trend favoring those men receiving vaccinia-PSA as a prime and fowlpox-PSA as boosters.

**Optimizing Poxviruses for Priming Tumor-Specific T-Cell Responses**

In addition to prime-boost strategies, there are now several other methods for enhancing the immunogenicity of poxvirus vaccines based on an improved understanding of how the immune system is activated and how tumors escape immune detection. Whether these strategies will lead to improved therapeutic or clinical responses remains to be seen, but there is evidence that this will be the case. As mentioned above, the central role of dendritic cells in priming antitumor T-cell responses has been recognized as a critical mediator of effective tumor recognition by the immune system. This process is incompletely understood but likely proceeds in a stepwise fashion in vivo and is sensitive to regulation by cytokines, which can be used to promote dendritic cell maturation and T-cell priming (18). The addition of granulocyte macrophage colony-stimulating factor as a vaccine adjuvant has been evaluated and seems to increase immune responses by attracting dendritic cells to the vaccine site and promoting maturation of antigen-bearing dendritic cells (19). Other cytokines, including members of the IFN, interleukin-2, and interleukin-12 families, have also shown promise in augmenting vaccine-induced tumor immunity in animal models. Thus, including various cytokines in the treatment regimen may be a powerful way to help increase immune activation in the clinic.

In addition to cytokines, the role of costimulatory molecules in mediating antitumor immunity has become better understood over the past decade. Costimulatory molecules are generally expressed on the cell surface of antigen-presenting cells and bind to lymphocyte surface ligands through which they mediate signal transduction and activation or inhibition of effector immune cells (9). These molecules have been described as a type of “rheostat” that modulates the level of immune cell activation. Costimulation seems to be especially important when dealing with self-antigens, such as TAA, that ordinarily elicit weak T-cell receptor engagement. The low levels of costimulation provided when encountering self-antigens probably prevent host autoimmunity under normal physiologic conditions. This response may be inappropriate in cases where tumors have become established, and the lack of costimulation by tumor cells and during TAA presentation may be one mechanism of tumor escape. This system can be manipulated...
by coexpressing TAA with specific costimulatory molecules as evaluated in the poxvirus system by several groups demonstrating improved therapeutic responses when TAA were coexpressed with the B7.1 (CD80) costimulatory molecule in murine models (Fig. 2; refs. 20, 21). The Schlom group reported further improvement in T-cell priming and antitumor activity when a triad of costimulatory molecules, including B7.1, intercellular adhesion molecule-1, and LFA-3 were used together in a recombinant poxvirus system (Fig. 2; refs. 22, 23). Preliminary clinical data documented that vaccination with poxviruses expressing TAA and costimulatory molecules is well tolerated and may induce more consistent T-cell responses. In a phase I trial using recombinant vaccinia and fowlpox viruses expressing CEA, B7.1, intercellular adhesion molecule-1, and LFA-3 administered with granulocyte macrophage colony-stimulating factor, 23 of 58 patients (40%) with metastatic CEA-expressing tumors had stable disease for at least 4 months with 14 patients having stable disease for over 6 months and one patient achieved a complete pathologic response (24). This study also showed CEA-specific T-cell responses in the majority of vaccinated patients.

Whereas optimizing vaccines through molecular manipulation represents an important goal for tumor immunotherapists, the identification of patient populations most likely to benefit from vaccination and the potential for synergistic activity when vaccines are coupled with other treatment modalities are areas that have received less attention. Whereas it is generally agreed that early vaccination at a time when tumor volumes are low is better than trying to vaccinate patients with metastatic disease, this concept has not been validated. Prostate cancer is an ideal tumor to examine because recurrence rates are significant. Given the magnitude of antitumor responses currently possible and the fact that most solid tumors require multimodality therapy, it is logical to consider combining vaccines with other therapeutic options. Cytotoxic chemotherapy, hormonal manipulation, and radiation therapy are all standard treatment options for a variety of malignant tumors. Whereas somewhat counterintuitive that activating immune responses in the setting of cytotoxic chemotherapy or radiation treatment, there is little direct evidence of how specific interactions will affect tumor responses. Prostate cancer provides another opportunity to evaluate such interactions, with radiation in particular, because radiotherapy is frequently used as standard treatment for primary localized prostate cancers.

The report by Gulley et al. combines many of the above-mentioned strategies in an effort to optimize the treatment of prostate cancer. They selected a group of men who had completed radiation therapy for localized prostate cancer and evaluated the addition of a prime boost vaccination regimen using vaccinia and fowlpox viruses coexpressing PSA and three costimulatory molecules. In addition, vaccinated patients also received local granulocyte macrophage colony-stimulating factor and low dose interleukin-2. Whereas the sample size was too small to detect clinical end points, they reported that 13 of 17 patients who completed the intended vaccinations exhibited at least a 3-fold increase in PSA-reactive T cells by ELISPOT assay. An important conclusion from this trial is that local radiotherapy did not seem to adversely impact on the generation of vaccine-induced immune responses, paving the way for further studies evaluating the clinical efficacy of vaccine and radiation therapy combinations.

**Mechanism of Radiation Therapy in Prostate Cancer**

The observation that radiation therapy does not prevent vaccine-mediated immune responses provides an opportunity to consider putative mechanisms whereby radiation may exert an additive or synergistic therapeutic benefit. In addition, it is possible that once these mechanisms are understood, radiation may become a useful tool for promoting vaccine-induced antitumor immunity. Radiation therapy is used in almost half of all cancer patients. In virtually all instances, the radiation seeks to provide loco-regional control. Whereas radiation is effective in achieving local control, it does so by delivering the maximum possible radiation to surrounding normal tissue; this precludes repeat administration to the same area in virtually all instances. Systemic delivery of radiation is limited to administration of radiopharmaceuticals, the most widely used being iodine-131 for thyroid cancer; current radiopharmaceuticals deliver low-dose rate radiation, which is usually inadequate for adequate tumor control.

Targeted radiation induces apoptotic cell death, not only of the cancer cell but of surrounding vasculature as well (25). Radiation seems to act on the plasma membrane, activating sphingomyelinase that generates ceramide; radiation-induced DNA damage may also result in ceramide production de novo in mitochondria by ceramide synthase. Ceramide acts as a second messenger in apoptosis initiation via the mitochondrial system or by activation of BAX (26). Previous studies in BAX-deficient mice showed reduced baseline microvascular endothelial apoptosis, suggesting that endothelial apoptosis is also an important factor regulating tumor growth and that radiation may result in tumor control by microvascular damage (27).

Radiation sensitizes tumor cells to antigen-specific CTL by up-regulation of Fas (28); combinations of tumor-targeted sublethal radiation and CTL may promote more effective antitumor responses in vivo (29). Radiation, by functionally affecting dendritic cell antigen-presentation pathways, has been shown to modulate MHC-class I antitumor immunity (30). Other postulated mechanisms include cytotoxic synergy of innate immune cells (TLR) and radiation (31). Furthermore, blockade of “protective” T cells (e.g., by anti–CTLA-4 antibody) has also been shown to enhance radiation cytotoxicity (32). Thus, preliminary evidence suggests that local radiation may result in an enhanced systemic antitumor immunity (Fig. 3).

The study by Gulley et al. presents convincing evidence, in a clinical trial, that local external radiation induces a specific systemic T-cell response to vaccine given concurrently. These preliminary results hold promise for the generation of an immune response by radiation, including perhaps radiolabeled antibody therapy, which would combine the potential of low-dose-rate radiation (29) and antibody-mediated cytotoxicity. Outcome data with this promising multimodality therapy are awaited, especially in other diseases where the possible increase in immune response as a consequence of androgen deprivation would not confound results.

**Concluding Remarks**

Significant advances in our understanding of the immune system and how it interacts with tumor cells has led to improved designs in vaccine approaches to cancer. The
poxvirus platform represents a good example of how new insights in tumor immunology can be incorporated into better treatment strategies. Although originally envisioned as a system to display single TAAs to the immune system, the poxvirus system can be exploited to express other immunomodulatory molecules, including multiple costimulatory molecules. The addition of cytokines and a prime/boost schedule also enhance the ability of these vectors to elicit potent immune responses against specific TAAs. The recognition that sublethal radiation sensitizes tumor cells to immune detection suggested the potential for synergy between radiation therapy—delivered either externally or potentially with radiolabeled antibodies with immune effector function—and vaccination. Prostate cancer represents a logical model to evaluate this hypothesis because tumor cells are sensitive to radiation and PSA-targeted vaccines have been developed for the clinic. The report by Gulley et al. in the May 1 issue showed the feasibility of this approach and the potential for instituting such treatment early in the course of the disease before the appearance of high-volume metastatic disease. Future investigation is needed to better define the clinical response rate and benefit of such novel combinations through expanded clinical trial using appropriate controls. The timing of radiation with respect to vaccination will also need more careful analysis through further work on relevant animal models and translation of important findings into the design of clinical trials. The application of new combinations, such as systemic vaccination with localized prostate irradiation, will also benefit from integrated multidisciplinary teams dedicated to working together at the interface between laboratory and clinic.

References


