Gene and Immune-based Therapies for Genitourinary Malignancies: Current Status and Future Prospects

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In recent years we have witnessed improvements in many fields of cancer research and therapy, including enhancement of preventive medicine and early detection programs, improved surgical techniques, and major achievements in radio- and chemotherapy. However, the ability to cure the majority of cancer patients remains elusive. At the same time, it has become increasingly apparent that cells of the immune system play a key role in the recognition and elimination of neoplastic cells. Recently, therefore, new cancer therapies have been directed at modulating and exploiting components of the immune system in order to augment the immunogenicity of, and thus eradicate cancer cells. The science of vectorology has occupied itself with the task of designing and constructing new methods of delivering genes into tumor tissue with high efficiency. These methods, which are tumor or organ specific, are also capable of preventing systemic toxic effects.

The above mentioned achievements in basic science have not bypassed the clinical realm of genitourinary oncology. The use of intravesical immunotherapy for superficial bladder tumors was the first immune-based therapy for bladder cancer and has become a gold standard. Developments in immunotherapy have resulted in an improved outlook for patients presenting with advanced renal cell carcinoma as well as for those who develop both distant and local recurrences after curative treatment. These advances represent only the beginning of new directions to come. Clearly, the future prospects of cancer therapy will be built upon the foundation of current investigative efforts in gene and immune therapy.

This article reviews the current role of immunotherapy and gene therapy in the treatment of metastatic and recurrent genitourinary neoplasms. First, we will discuss the fundamentals of immunotherapy and gene therapy. Next, we will review the applications of each of these therapeutic modalities individually with respect to prostate, bladder and renal cell carcinoma. We will then address future directions in gene and immunotherapy; and, finally, we will list the current clinical trials, focusing on gene and immune therapies. We contend that all the data on current cancer clinical trials should be available for the public to choose from [Tables 1 and 2].

One of the fundamental dilemmas in gene therapy today involves the selection of genes to be used in clinical trials. Unlike many other genetic diseases, tumorigenesis is a multi-step pathway involving initiation, proliferation, loss of contact inhibition, invasion and ultimately metastasis of the cancer cell. Multiple genes involving cell cycle regulation, angiogenesis, immunoreactivity and cell adhesion are also involved. Since no single gene defect has been found to facilitate tumorigenesis for all cancer diseases, different mutation pathways may lead to the same end result. Consequently, the utilization of a single type of gene therapy may not be enough. Errors in gene regulation, transcription or translation can lead to morphologic and functional changes within the cell, whereafter the cancer phenotype may become apparent. There are several potential mechanisms by which gene therapy may achieve cancer control [Table 3].

Cytoreductive Therapies

Using this strategy, a gene (suicide gene) is injected into the tumor or specifically attached to it. After transfection, the gene that is expressed results in the production of a protein, usually an enzyme that is capable of converting an otherwise benign medication into a highly cytotoxic one. Obviously this will result in a high concentration of the cytotoxic agent in the tumor but without significant systemic concentrations. Active suicide genes enable not only destruction of the transfected cell, but the destruction of adjacent tumor cells (bystander effect). This means that not all tumor cells need to be directly transfected. Thus, one of the advantages of this approach is the need for less efficient transfection in comparison to other gene therapies. For example, the herpes simplex thymidine kinase gene (HSV-tk) is one of the most commonly used systems; another system uses cytosine deaminase [1]. Human thymidine kinase cannot phosphorylate certain pro-drugs like gancyclovir, while HSV-tk can phosphorylate gancyclovir to gancyclovir monophosphate. This is then converted to gancyclovir triphosphate by cellular kinases. The resulting triphosphate acts as

Table 1. Clinical trials for prostate cancer using molecular-based approaches

Basic principle	Principal investigators	Study center
Anti-EGF Ab	Belldegrun	UCLA
Liposomal IL-2	Belldegrun	UCLA
Adenovirus *P53*	Belldegrun	UCLA
Intradermal vaccinia–PSA	Chen	Naval Medical Academy
Autologous tumor cell vaccine + IFN/GM-CSF (phase II)	Dillman	Multicenter
Intramuscular Vaccinia virus–MUC1-IL2	Figlin	UCLA
Adenovirus gancyclovir/TK	Hall	Mount Sinai Hospital
PSA vaccine–Vaccinia virus (phase I)	Hamilton	NMOB
Adenovirus gancyclovir/TK	Kadmon	Baylor College of Medicine
Intradermal vaccinia–PSA	Kufe	Dana Farber Cancer Inst.
Adenovirus *P53*	Logothetis	MD Anderson
PSA vaccine–Vaccinia virus (phase I/II)	Sanda	University of Michigan
Adenovirus gancyclovir/TK	Scardino	Baylor College of Medicine
GM–CSF immunotherapy	Simons	John Hopkins
Autologous tumor cell vaccine + IFN/IL2	Slovin	MSKCC
Autologous tumor cell vaccine+ GM-CSF (phase I/II)	Small	UCSF
PSA vaccine–Lipid envelope (phase II)	Spitler (Chair, Ph)	Multicenter
Retrovirus antisense *c-myc*	Steiner	Vanderbildt

Adopted with changes from *http:/cancernet.nci.nih.gov/ and from Rodrigez R. et al, Urologic applications of gene therapy. *Urology* 1999;54:401–6.
MSKCC = Memorial Sloan-Kettering Cancer Center.

Table 2. Clinical trials for renal cell cancer using molecular-based approaches

Basic principle	Principal investigators	Study center
HLA-B7 and IL-2	Antonia	University of South Florida
Intra-tumoral injection of LEUVECTIN (phase II)	Belldegrun	UCLA
Liposome HLA-B7/β2 microglobulin	Chang	Multicenter
Autologous tumor cell vaccine+IFN/GM-CSF (phase II)	Dillman	Multicenter
TIL+INF+IL2	Economou	UCLA
Liposome IL-2	Figlin	UCLA
The HLA-B7 and IL-2 gene	Figlin	UCLA
HLA-B7/beta-2 microglobulin	Fox	Chiles Research Institute
IL-2 (allogeneic)	Gansbacher	MSKCC
Multi-antigen loaded dendritic cell vaccine (adoptive immunotherapy — phase I)	Gitlitz	UCLA
IL-4	Lotze	University of Pittsburgh
TNFV-	Rosenberg	NIH
IL-2	Rosenberg	NIH
GM-CSF	Simons	Johns Hopkins

Adopted with changes from *http://cancernet.nci.nih.gov and from Rodrigez R. et al, Urologic applications of gene therapy. *Urology* 1999;54:401–6.

a false base inhibiting DNA polymerase and DNA synthesis, ultimately leading to cell death. The bystander effect seen with this method is not clearly understood. Proposed explanations for this effect include the distribution of gancyclovir triphosphate via gap junctions into neighboring cells, a local inflammatory response due to direct injection into prostatic tissue, or a systemic immune response. The bystander effect and requirement for only transient and modest trans-gene expression has made suicide gene therapy very appealing and has contributed to its early approval for human trials. Other pro-drug systems include the cytosine deaminase gene and the *Escherichea coli* xanthine-guanine phosphoribosyltransferase. Cytosine deaminase is a bacterial gene that converts 5-fluorocytosine into the anti-metabolite 5-fluorouracil, which causes cell death by inhibiting the host cell DNA synthesis. *E. coli* xanthine-guanine phosphoribosyltransferase phosphorylates 6-thioxanthine to its cytotoxic monophosphate.

Corrective Gene Therapy

Many different genetic alterations have been recognized in urologic tumors. Most of them are distinguished by either the overexpression of an oncogene or the inactivation of a tumor-suppressor gene such as the *p53* gene. In diseases like cystic fibrosis where the disorder is caused by a single gene defect, gene replacement is particularly attractive. Unfortunately, these approaches have not proven successful in cancer patients. This may be due to the fact that there is no single oncogene or tumor-suppressor gene defect that can be definitely implicated in the formation of all tumors.

Table 3. Potential mechanisms by which molecular-based approaches may attain cancer control

Cytoreductive therapies
• Suicide gene (e.g., thimidine kinase gene therapy followed by gancyclovir administration).
• Drug activation of suicide genes.
• Oncolytic viruses (e.g., adenovirus that replicates in *p53*-deficient cells).
• Toxic gene therapy (e.g., diphtheria toxin that induces necrosis and apoptosis)
Corrective gene therapy
• Correction of defective tumor suppressor genes by insertion of wild type genes (e.g., p53, p16, p27).
• Growth factor modulation using antisense mRNA techniques (e.g., antisense bcl-2 and antisense TGF().
Immunotherapy/cancer (genetic) vaccine eliciting immune response
• Transfection of tumor cells with cytokines or growth factor genes. Secretion of the gene product (IL-2, GM-CSF (G-VAX), IL-12) for immune activation and effect on primary or systemic immunity. Lower toxicity than with direct injection of cytokines.
• Adoptive immunotherapy: expose cytotoxic lymphocytes or dendritic cells to cancer-specific antigens and instigate an immune response by returning cells to patient (e.g., PSMA).
Immunomodulation by administering cytokines directly (e.g., IL-4, GM-CSF and co-stimulation with B-7).

Thus, the simultaneous targeting of multiple gene defects will probably be a more appealing strategy in the future.

Immunotherapy

It has been hypothesized that tumor cells escape surveillance and destruction by the immune system through down-regulation of cell surface antigens, such as the major histocompatibility complex [2]. Tumor vaccine approaches involve the use of cytokine genes (interleukin-2, IL-6, tumor necrosis factor-, interferon gamma, granulocyte-macrophage colony-stimulating factor, IL-4, IL-12) transfected into tumor cells *in vitro* [3]. Cytokines originating in the transfected cells induce expression of cell surface proteins such as HLA class I and II that then augment their immunogenicity. Cells are thereafter irradiated to eliminate proliferative capability and are administered back to the patient as a vaccine in an attempt to generate an immune response against the remaining tumor burden. Efficacy of this approach depends on achieving a high level of cytokine expression in the tumor tissue and on the availability of tumor tissue for the process.

Second-generation tumor vaccines utilize retroviruses, lipid-packaged segments or naked DNA-encoding cytokine genes. These constructs are injected into the tumor. Transfection takes place *in vivo* and a chronic cytokine production results [4].

Molecular-based Approaches to Urologic Malignancy

Bringing prostate cancer into the framework of molecular-based therapy

The strategies for different gene and immune therapies in prostate cancer are illustrated in Figure 1. The expectation of a breakthrough in gene therapy for prostate cancer is based on the fact that cells in the prostate gland express tissue-specific molecules (prostate-specific antigen and prostate-specific membrane antigen). With the discovery and sequencing of additional genes encoding specific prostate antigens — such as the PSA enhancer promoter [5], specific oncogens, cell surface receptors and tumor suppressor genes [7] such as *p53*, Rb, p21, PML, BRCA1, *c-myc*, Bcl-2 and transforming growth factor-β [6] — the potential for prostate-specific targeting at the genetic level becomes apparent as the number of possible genetic targets increase. Phase I studies using PSA promoter-driven genes are now underway. The expression of these genes has been shown to be both androgen-responsive and -specific for prostate tissue [7]. These studies have combined the PSA gene enhancer or promoter with suicide genes like the HSV-tk gene or cytosine deaminase gene, or tumor suppressor genes. Studies utilizing intratumoral injection of adenovirus vector carrying the suicide gene HSV-tk alone have already been conducted and have shown it to be effective in the Dunning prostate cancer rat tumor model and nontoxic for both cancer patients and caregivers.

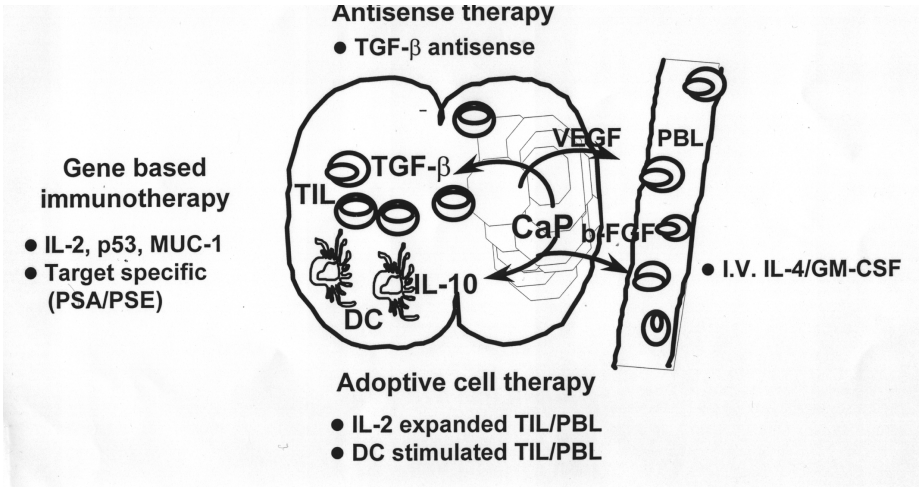

Figure 1. The strategies for different gene and immune-based therapies in prostate cancer. PBL = peripheral blood lymphocytes, VEGF = vascular endothelial growth factor, FGF = fibroblast growth factor

Cytoreductive therapy: Gene replacement and antisense strategies require highly efficient transfection of prostate cancer cells in order to be effective. Thus, suicide gene approaches that do not require as high levels of transfection seem appealing. Advances in prostate-specific suicide genes have been considerably promoted by the identification and cloning of the PSA promoter and enhancer. By using these sequences

PSA = prostate-specific antigen

to drive suicide gene expression, fairly high levels of prostate tissue specificity can be achieved while incurring minimal risk to non-prostatic tissues.

Direct intratumoral injections of suicide genes without the aid of the PSA promoter have also been tested and were found to be target-specific and effective. Intratumoral injection of the HSV-tk suicide gene in a Dunning rat prostate cancer model has shown a growth inhibition of subcutaneously implanted tumors with little systemic toxicity when compared to control animals [8]. The intratumoral injection route is used to minimize systemic effects, but investigators have recently demonstrated that the intratumoral injection of the HSV-tk gene also induces systemic anti-tumor effects. For example, one study showed growth inhibition of pulmonary metastasis in a mouse model of prostate cancer [1]. Moreover, when attenuated replication-deficient adenovirus carrying the HSV-tk gene was injected into the tumor in 18 patients, lower dose levels yielded no toxicity or clinical responses while higher doses showed 20–25% response rates.

The concept of suicide genes has led to new possibilities utilizing previously tested regulatory genes for prostate cancer to increase the effect of tumor cell death. Gene complexes that utilize promoter sequences requiring mutated *p53* for activity are being created. The suicide gene is placed under the control of a promoter. When the abnormal *p53* binds to it the suicide gene is expressed, leading to tumor cell death [9].

Another suicide gene system utilizes the human inducible heat shock protein 70 promoter sequence as a regulatory component. Thus, suicide gene expression is induced by a temperature elevation. A recent study using both cytosine deaminase and HSV-tk suicide genes under the control of this system employed a pro-drug. After appropriate heat induction and pro-drug administration, targeted PC-3 prostate cancer cell line growth was inhibited significantly. Additional suicide gene candidates for prostate cancer therapy have recently been identified and characterized. These include diphtheria toxin, which has significant *p53*-independent cytotoxic effects on the LnCaP cell line; and cerulenin, a fatty acid synthesis inhibitor that has demonstrated apoptotic effects on the TSU-Prl cell line. This gene system holds promise for future therapies.

Corrective gene therapy: Alternatively, correction of aberrant gene expression, such as through the replacement of malfunctioning tumor suppressor genes, is another route for gene therapy in prostate cancer.

- ***p53***: Abnormal expression of the *p53* has been implicated in a variety of tumor systems. The *p53* protein manifests control on cellular proliferation by blocking the binding of DNA polymerase to the DNA strand [10] if sufficient damage has been incurred. This results in the arrest of cell proliferation at the G1 checkpoint of the cell cycle. If the *p53* gene product is absent, cell proliferation will continue in the face of severe DNA damage, resulting in increased genetic instability and possible tumorigenic effects. Approximately 60% of prostate cancer cell lines have mutations in the *p53* gene [11]. Abnormal *p53* expression has been found in more aggressive tumors, and appears to be an independent predictor of cancer recurrence after radical prostatectomy. The transfection of normal wild type *p53* gene into *p53*-deficient prostate cancer cell line resulted in decreased tumorigenicity and decreased proliferation after being injected back into nude mice. Unfortunately, 80% of sporadic prostate cancers do not have an identifiable defect in the *p53* protein, but many other oncogenes and tumor suppressor genes have been identified as potential targets for gene therapy, including c-ras, H-ras, *c-myc*, TGF-β and bcl-2 [12].
- **p21**: This gene is thought to play a similar role as *p53* by inhibiting DNA replication when severely damaged. The p21 gene encodes for a protein that functions as an inducer of a cyclin-dependent kinase inhibitor, which may also play a role in DNA replication and repair [13]. Thus, lack of the wild type p21 protein results in perpetuation of the cell cycle in the presence of DNA damage. Recent studies comparing the transfection effects of *p53*-deficient prostate cancer cell lines with wild type adenovirus-mediated *p53* and p21 genes, both *in vitro* and *in vivo*, have shown greater growth suppression with p21 than p53 gene replacement. This has led to the assumption that certain subsets of *p53*-deficient prostate tumors may be more responsive to p21-directed gene replacement therapy than conventional therapy with *p53*.
- **PML gene (progressive multifocal leukoencephalopathy)**. Aberrant expression of this gene has been observed in various human cancers, including leukemia, breast cancer and prostate cancer. This gene encodes a protein that is involved in the suppression of growth and transformation of cells. Direct intratumoral injection of this vector into nude mice decreased tumor growth by more than 60%. These results suggest a promising role for the PML gene in future prostate cancer gene therapy.
- **BRCA1**: The breast cancer susceptibility gene (BRCA1), a tumor suppressor gene, has been implicated in hereditary prostate cancer. The BRCA1 gene is involved in transcriptional regulation, but its exact role is still poorly understood. *In vitro* experiments using wild type and mutant BRCA1 transfection into a low BRCA1-expressing prostate cancer cell line (DU145) have demonstrated increased tumor doubling time, increased susceptibility to drug-induced apoptosis, reduced capability to repair single strand DNA breaks, and alteration in regulatory genes such as p21 and Bcl-2. Phase I clinical trials using wild type BRCA1 incorporated into retrovirus vectors have shown encouraging results, and further studies are currently underway.

TGF-β = transforming growth factor-beta

Other prospective tumor suppressor genes that are currently being investigated for possible roles in prostate cancer and future gene therapy include the retinoblastoma gene, CDKN2, STEAP, KAI1, GSTP1 and various cellular adhesion molecule genes. These genes have all been implicated as having roles in different prostate cancer cell lines.

- **Antisense gene therapy** is an arm of corrective gene therapy [Table 3]. Antisense molecules use complimentary mRNA segments and oligonucleotides that bind to and inactivate target genes by inhibiting transcription or translation. This strategy has been proposed as a method to disarm the cancer cell by counteracting the overexpression of a particular oncogene. The antisense molecule can be delivered directly, or inside a vector as part of a gene consolidate, which will then transcribe antisense molecules in the presence of a promoter.
- ***c-myc*** is an oncogene whose mechanism of enhanced expression has yet to be explained. Its expression has been found in various prostate cancer cell lines [14]. Transduction of the LnCaP, PC3 and DU145 prostate cancer cell lines with antisense *c-myc* mRNA revealed dose-dependent reduction in DNA synthesis and cell viability [15]. Nude mice with established DU145 xenografts showed a significant decrease in tumor size and histological aggressiveness after intratumoral injection of antisense *c-myc* mRNA. This has led to Phase I clinical trials currently in progress.

Further proposed targets for antisense therapy include the Bcl-2 and TGF-β genes, both of which are overexpressed in certain aggressive prostate cancers.

- **Bcl-2**: The Bcl-2 protein inhibits cell apoptosis and has been implicated in the development of androgen-resistant prostate cancer cells. It is believed that Bcl-2, by blocking apoptosis, is responsible for the poor response to anti-neoplastic drugs and radiation therapy seen in tumors. *In vitro* studies using ribozymes to cleave the Bcl-2 RNA have demonstrated efficacy, and thus may be applicable for gene therapy.
- **TGF-β**: High levels of TGF-β enable prostate cancer cells to adhere to bone marrow stroma and, furthermore, to suppress local immune system responses against their tumoral antigens [16]. Recent experiments have also demonstrated the restoration of TGF-β growth inhibition function on prostate cancer cell lines via the overexpression of TGF-β1 type II receptors. Current experiments have also shown that TGF-β has a proliferative effect on the highly aggressive prostate cancer cell line, TSU-Pr1 [17]. Clearly the role of TGF-β in prostate cancer is a complex one. Work is currently underway to further delineate the function of this interesting substance in prostate cancer.

Numerous other genes are also being studied for their apoptotic effect in tumor cells in the hope of inducing cell death pathways by means of gene therapy. Possible targets being studied include Bax, Bcl-X-S, Bcl-X-L, E2F, DP, cytokines IL-1, tumor necrosis factor-α and viral proteins E1A and E1B.

Immunotherapy: Prostate cancer may escape immune response partly due to the lack of MHC class I expression, resulting in poor antigen presentation. Gene therapies have been constructed to overcome this lack of immunogenicity and loss of MHC-I expression by transfecting cytokine genes directly into prostate cancer cells. Because some segments of the PSA molecule are immunogenic and stimulate T and B cell immune responses [18,19], efforts have been made to transfect dendritic cells with PSM or PSA genes. The transfected dendritic cells will present the prostate antigens as foreign, thus stimulating an immune system-mediated, anti-tumor response [20]. Clinical trials are now underway in which patients are vaccinated against the PSA protein, with the hope that a systemic immune response against prostate cancer will develop. Local production of cytokines in the prostate can generate an immune response. The Dunning rat R3327-MatLyLu cell line was transfected with the IL-2 gene and then injected back to a site remote from the primary tumor. This procedure cured the rats and also protected them from future tumor cell challenges [20]. Similar work done with the GM-CSF gene formed the basis for current clinical trials using the GM-CSF gene in prostate cancer [25]. The long-term role that tumor vaccine therapies will play in the treatment of prostate cancer is yet to be determined.

Summary

Recent demonstrations that prostate tumors are immunogenic, coupled with new developments in gene delivery technology, and better understanding of prostate tissue-specific regulation of gene expression, have enhanced the prospects of gene therapy as a feasible approach to the treatment of advanced prostate cancer. Currently, phase I dose-escalation and safety-evaluation clinical trials are underway. These studies involve the use of PSA promoter-driven suicide genes, IL-2 gene transfection, PSA and GM-CSF tumor vaccines, and *p53* and anti *c-myc* gene replacement approaches. The biological efficacy of these approaches should become clear in the next few years. Some of these clinical trials are listed in Table 1.

From intravesical instillation of *Bacillus* calmette-guerin to molecular-based therapy for patients with transitional cell carcinoma

Although superficial papillary tumors are easily resected by trans-urethral resection of bladder tumor, there is a high recurrence rate after surgery. Risk factors for recurrence include the presence of multiple lesions, high grade tumors, and previous recurrences. Likewise, the presence of TCC *in*

MHC = major histocompatibility complex
PSM = prostate-specific membrane
GM-CSF = granulocyte macrophage colony-stimulating factor
TCC = transitional cell carcinoma

situ forecasts a worse outcome. Prevention of invasion and recurrence by superficial tumors and the amelioration of TCC *in situ* are the goals in the treatment of bladder cancer, thereby reducing the morbidity and mortality associated with advanced disease.

Intravesical instillation of *Bacillus calmette-guerin* mycobacteria strain is one of the earliest and most effective immunotherapies known. BCG plays an important role in the treatment of noninvasive bladder cancer and is recommended for prophylaxis against tumor recurrence after successful trans-urethral resection of bladder tumors. Its mechanism of action is currently unknown, although IVC-BCG is believed to induce anti-tumor effects through immune effector agents. IVC-BCG may initiate a tumor-specific immune response involving T cell-dependent agents, including T helper cells, macrophages, IL-2, IFN, and tumor necrosis factor. However, The degree of the local inflammatory/immune response correlates directly with the likelihood of a patient remaining disease free. Although IVC-BCG is generally well tolerated by 90–95% of patients, complications occasionally do occur and mortality is rarely reported [22]. Indeed, 2–5% of patients develop severe, potentially life-threatening complications that necessitate intensive treatment. Despite the impressive success of IVC-BCG treatment, an average of 20% of patients treated with combined trans-urethral and IVC-BCG fail to respond to therapy and will either develop recurrent tumors or progress to invasive disease [23]. For these patients, treatment options are limited. In the search for therapies as effective as IVC-BCG but with less toxicity, other immunotherapeutic agents have been investigated:

Cytoreductive therapy: Transfection with the HSV-tk suicide gene and administration of gancyclovir resulted in a tenfold decrease in tumor size in a murine model. One limitation to this approach is that the penetration of intravesical instillation of the transfecting agent is limited to the superficial cell layers.

Initial studies showed the safety and efficacy of a genetically modified BCG strain that also expresses the gene for IL-2. Even more promising results were reported in an animal model using the strategy of transfection with IL-2 plus B7, which is a potent T cell stimulator. The vector for the delivery of the transfecting agent is the rate-limiting factor. A recent report of a phase I study describes the intravesical administration of vaccinia virus to patients before radical cystectomy, with no apparent toxicity [24].

Adenovirus vectors appear to be promising as intravesical gene delivery vehicles with easy access and safety profile. Because they are not given systemically, pre-existing humoral blocking antibodies against adenovirus should not decrease the efficacy of treatment. Adenovirus vectors may be able to provide tumor-specific gene transfection because they seem to preferentially infect bladder tumor cells instead of normal mucosa [25]. Moreover, the administration of the naked IL-2 gene alone protected by a lipid envelope (liposomal IL-2) was reported to be very efficacious and a high concentration of post-transfection urinary IL-2 was demonstrated.

Corrective gene therapy: Gene therapy for TCC is appealing because the bladder urothelium is readily accessible for intravesical instillation of the genetic agent. The effect may be monitored with cystoscopy as well as cytology and other available tests (BTA, NMP-22 and CK-20). To date, the tumor suppressor genes *Rb*, *c-myc*, C-CAM 1, *Bdx-1* (cell apoptosis regulator) and *p53* have all been identified as potential targets for gene replacement therapy in TCC.

- ***Rb* gene**: Epidemiological evidence shows an increased incidence of TCC in retinoblastoma families [26]. Moreover, retinoblastoma gene activation has been observed in patients with sporadic bladder cancer. *Rb*-deficient TCC cell lines have been established. When these cell lines were transfected with the wild type *Rb*, a decreased tumorigenicity and slowed cellular proliferation were recorded both *in vitro* and *in vivo*. Phase I and Phase II clinical trials have already been initiated in humans, and some studies have shown that selected bladder cancer cell lines transfected by the wild type *Rb* gene still maintain some malignant features [26]. It was suggested that mutations in the *Rb* gene represent late events in carcinogenesis, leading to increased aggressiveness.
- ***c-myc***: Expression of this gene has been associated with tumor progression and chemotherapy resistance. Since the *c-myc* gene product confers resistance to cisplatin-induced DNA injury, cell replication continues despite DNA damage, leading to a severe genomic instability. Gene therapy against *c-myc* has been tried by adding *c-myc* antisense oligonucleotides to cell cultures containing human bladder tumor cell lines that are known to express *c-myc* and are resistant to cisplatin-based chemotherapy. The result was a significant decline in the translation of *c-myc* sense mRNA with an enhanced cytotoxic effect when used in combination with cisplatin [27]. The combination of *c-myc* antisense gene therapy and chemotherapy may significantly improve the outcome of patients with advanced TCC.
- ***p53***: Changes in *p53* are believed to be one of the first steps in bladder tumorigenesis. Initial studies using a wild type *p53* transfected into murine and human TCC cell lines and animal models are promising.

At this time a few clinical trials are applying gene therapy to human subjects with bladder cancer, and most of them are using adenoviral vectors. The genes tested are the *Rb* gene at the University of California in San Francisco, and the *p53* gene at the University of California in Los Angeles and the

IVC-BCG = intravesical instillation of Bacillus calmette-guerin
IFN = interferon

CAM = cell adhesion molecule

M.D. Anderson Cancer Institute [28]. The combination of the ability to directly examine and inject tumors intravesically, together with the accumulation of data on the genetic defects in bladder cancer make it only a matter of time before additional gene therapy clinical trials will be initiated.

Immunotherapy: The anti-tumor effect of IVC-BCG on bladder cancer is believed to result from increased endogenous cytokine secretion and the resulting stimulation of host defense and immune mechanisms. Therefore, many researchers have attempted to treat TCC by direct administration of cytokines.

- **IFN-α** was the first cytokine to be used. IFN has shown more promise as a prophylactic agent post-TURBT. In one study, a 79% disease-free rate at 98 weeks median follow-up was seen after TURBT. IFN has also tested well in comparison to chemotherapeutic agents. When compared to mitomycin-C, IFN was found to be equally effective in preventing the recurrence of superficial tumors [29]. The side effects of IFN are mild and consist mainly of low grade fever.
- **Bropirimine** is an oral agent shown to have various immunostimulatory properties, including the stimulation of endogenous IFN production. Sarosdy and associates in 1992 [30] reported a phase II trial of oral bropirimine for the treatment of superficial bladder cancer. In 20 (61%) of the 33 patients, malignant biopsies and bladder-wash cytology converted to negative, including 6 (50%) of 12 who failed prior to BCG immunotherapy. Median response duration exceeded 21 months. Four of the 20 responders had a papillary tumor recurrence at 3 to 15 months, all Staged Ta or T1 [30].
- **Interleukin 2** indirectly causes lysis of tumor cells by activating NK and LAK cells. Because IL-2 has a short half-life, systemic therapy requires high dosing, which results in significant toxicity. Intravesical administration of IL-2 dodges the complications of systemic infusion, and several studies have shown it to be effective. In one protocol, repeated intra- and paralesional injections of IL-2 resulted in a 50% complete response, a 33% partial response, and no side effects. In a different study, 33% of treated patients had complete tumor regression and an additional 33% had a partial response. *In vivo* intralesional IL-2 injections into murine bladder tumor reduced growth to a greater extent than with IVC-BCG. Less impressive results have been reported with topical intravesical IL-2 application, achieving no more than a 20% response rate with continuous IL-2 bladder irrigation after TURBT [31], even in combination with LAK cells in patients with metastatic bladder cancer.
- **Combining BCG and IL-2** has been suggested as a potentially effective treatment for superficial bladder cancer. Tumor-free responses of 81–85%, lasting an average of 13–28 months, have been reported [32]. Side effects were self-limited and minor, consisting of low grade fever, hematuria and bladder irritability lasting less than 24 hours.
- **Tumor necrosis factor** instilled intravesically for superficial bladder cancer demonstrated an 11% partial response rate in one clinical trial [33]. In another trial, recombinant TNF was given to 16 patients with refractory transitional cell bladder cancer. Two patients had complete responses, 9 had partial responses, 2 had minor responses, one was diagnosed with progressive disease, and 2 were not evaluated. Adverse effects were minimal. *In vivo* studies of TNF combined with IFN on cultured bladder cancer cells have also demonstrated powerful anti-proliferative effects.

Summary

Currently, combined TURBT and IVC-BCG therapy are the gold standard in the treatment for most patients with recurrent superficial bladder cancer. For the 20% that fail to respond to TURBT/IVC-BCG, therapeutic options have been limited, with most patients currently undergoing radical surgery. Immunotherapeutic agents other than IVC-BCG have demonstrated promise in the treatment of bladder cancer and are recommended as one approach to delay radical cystectomy in patients who fail IVC-BCG. The field of immunotherapy and gene therapy for bladder cancer is still evolving. Over the next decade it is anticipated that the introduction of novel advances such as tumor vaccines and gene therapy should significantly improve the prognosis and further decrease the mortality from bladder cancer.

Renal cell carcinoma — the future is here

Traditionally, there have been no other effective treatments for RCC aside from surgery. RCC is radiation- and chemoresistant. Metastatic RCC has a poor prognosis, with an average survival of only 6 to 12 months from the time of diagnosis and only a 6% objective response rate with conventional chemotherapy. Thus, the need for additional therapy, mainly for patients with advanced RCC, is strongly needed. The first advance in this direction occurred when IL-2 was isolated and identified. The molecular cloning of IL-2 revolutionized the field of cancer immunotherapy and significantly altered the treatment of metastatic RCC [34]. With the advent of recombinant DNA technology, the ability to produce large quantities of IL-2 has resulted in its widespread use. In a relatively short time, this agent was approved by the Food and Drug42 Administration as treatment for metastatic RCC. Since then, other immunostimulatory cytokines have been identified and purified. To date, most studies investigating the use of cytokines are focusing on IFN-α, IL-2, combinations of these cytokines, or adoptive immunotherapy with tumor infiltrating lymphocytes or LAK cells. The role of other cytokines (IL-4, IL-7,

TURBT = trans-urethral recombinant therapy

TNF = tumor necrosis factor

IL-12 and GM-CSF) is currently under investigation. Clinical trials conducted for RCC are listed in Table 2.

There has been much progress in understanding the genetic changes that are associated with the development of RCC. Thus gene therapy for RCC has advanced further than for any other urological organ system.

Cytoreductive therapy: A tumor marker for RCC has recently been identified. The function of this protein, named G250, is unclear. High levels of G250 antigen can be detected in up to 90% of all kidney cancer cells, with normal renal parenchyma showing no detectable G250 antigen. This antigen has been used as a target for monoclonal antibody immunohistochemical staining for diagnostic purposes and has also been used in radionuclide scans to localize tumor sites [35]. Because this antigen is found in a high proportion of RCCs, it has the potential to be a target for gene therapy. Recent studies have demonstrated that immune activation could be enhanced by administration of antibodies to G-250 in a cytokine-stimulated human RCC xenograft model. Further studies are currently underway to elucidate its role as a potential cancer vaccine. However, the field of tumor vaccines in RCC lags behind melanoma and no acceptable target is yet available. But, with its numerous tumor targets, restoration of the wild type von Hippel-Lindau gene product and the development of targeted therapy against the G250 protein hold significant promise for future trials.

Corrective gene therapy: From studies of familial RCC in patients with the VHL syndrome, the molecular basis for tumorigenesis of the kidney is becoming clearer: Loss of chromosome 3p in many sporadic and familial renal cell cancers has been noted [36] with restriction fragment length polymorphisms. The VHL gene was identified at 3p25.5 (VHL gene) of chromosome 3 [36]. It has been hypothesized that the VHL protein functions as a cell cycle regulator, controlling cellular proliferation by restricting gene transcription, translation, or repair. However, only 45–60% of all patients with sporadic RCC have a detectable mutation in the VHL gene. Furthermore, the phenotypic expression of VHL gene defect varies, with loss of the VHL gene product not always resulting in RCC [37]. Thus, the defect in the VHL gene is probably influenced by many other, yet to be defined epigenetic phenomena. Moreover, aberrations in chromosomes 5, 7, 14, and Y were also found to be associated with RCC. These loci may be able to act independently from the VHL locus, resulting in the development of RCC.

Despite the limitations of the VHL gene as a target for gene therapy, initial studies have tried to replace the defective tumor suppressor product in an attempt to reverse the cancer phenotype. Normal (wild type) VHL gene was transfected into RCC cell lines lacking the normal expression of the gene. The wild type VHL gene was attached to a constitutively activated cytomegalovirus promoter and placed into a liposome vehicle. Transfection of the wild type VHL gene had no effect on the transfected cell line growth *in vitro*, but the expression of the wild type VHL gene resulted in growth suppression of other RCC cell lines. This study showed that the suppression of cell growth was specific to RCC cell lines, which implied that the VHL protein has a role in controlling the proliferation of kidney cells. Thus, gene replacement therapy with the wild type VHL gene is promising for RCC patients, although the safety and efficacy of this treatment is yet to be defined.

In vitro attempts to replace the *p53* gene in RCC cell lines using liposome–*p53* gene complexes have resulted in decreased growth of tumor cells in culture. Transfection of the *p53* gene into a mouse xenograft model resulted in a decrease in the number of metastatic lung lesions [38]. The use of the *p53* wild gene by intratumoral injection may prove efficacy in the future.

Immunotherapy: It is well established that IL-2 has beneficial activity in patients with advanced RCC, thus a tumor vaccine seems appealing. By using a tumor vaccine, elevated cytokine concentrations can be achieved within the tumor, causing an increase in MHC expression. By increasing the surface MHC expression, especially of HLA-Cw7, an immune response is anticipated once the TIL are able to recognize the MHC-restricted tumor-related peptides. By achieving locally high cytokine concentrations, it is likely that the systemic toxicity that limits the efficacy of immunotherapy will be avoided.

Initial studies with tumor vaccines in animal models have shown that transfer of cytokine genes to tumor cells is feasible and can induce host anti-tumor effects [39]. IL-2-transfected RCC cells inhibit the growth of parental tumor cells in rats [39]. It was also shown that the production of IL-2 was more intense after intra-tumoral injection than after systemic administration of the transfecting agent. A synergistic anti-tumoral effect was reported for retroviral transduction of the IL-2 gene conjugated with systemic administration of IFN-α. This suggests that a synergistic, immunogenic effect can be obtained by using both local gene therapy and systemic, immune stimulation. IL-4, GM-CSF, HLA-137, and IFN-λ gene transfection are other immune system modulators that may have a role in future tumor vaccines for RCC [40].

Recent Phase I trials using tumor vaccines have been initiated in humans with metastatic RCC. Patients were given irradiated autologous tumor cells transfected *in vitro* with a retroviral vector carrying the GM-CSF gene. No significant toxicity was reported, and only one of 16 patients had a partial response [40]. In addition, studies using genetically modified dendritic cells and studies using the injection of cytokines into the tumor, with the HLA-B7 and IL-2 genes carried in a liposomal vector, have been performed at UCLA. In addition to these programs, at least three other tumor vaccine programs have been initiated

RCC = renal cell carcinoma
VHL = von Hippel-Lindau
TIL = tumor-infiltrating lymphocytes

using either intra-tumoral HLA-B7 or IL-2 gene transfection to enhance the immunogenicity of the tumor. At this time, although tumor vaccine-based gene therapy appears to be safe, its efficacy in metastatic RCC has yet to be proved.

Summary

The past two decades have witnessed impressive advances in the application of immunotherapy to the treatment of renal cell carcinoma. At UCLA we have seen a progressive increase in response to treatment, which has evolved from systemic IFN-α administration (16%), to combination IFN+IL-2 (25%), to the current method of bulk TIL (33%) and CD8/TIL (40%). Patient characteristics that predict improved responsiveness to therapy have been identified, and treatment protocols that decrease toxicity have been developed. The most encouraging results are the improved rates of complete clinical response, most of them durable and long-lasting. Further refinements in the treatment of renal cell disease with biologic and immunotherapeutic agents are still needed, yet there is no doubt that current immunotherapeutic protocols produce changes in the natural history of this disease and cause significant and lasting remissions in selected patients.

Conclusions

Although surgical management continues to be an effective treatment for organ-confined neoplastic disease, the treatment options for patients with disseminated cancer is limited. Immunotherapy has demonstrated significant success in the management of advanced renal cell cancer and localized transitional cell tumors. Recent exciting research has demonstrated that prostate cancer may also be susceptible to immunotherapeutic protocols, finally offering a glimmer of hope to physicians treating this very prevalent and morbid disease. Molecular-based therapy has many potential applications to the treatment of advanced genitourinary cancers. The reinsertion of inactivated tumor suppressor genes, the inactivation of oncogenes, the insertion of immunomodulatory genes, and the insertion of suicide genes have been used to treat genitourinary malignancies *in vitro* and in animal models. Progress is being made to better understand the genetic and cellular mechanisms that underlie tumorigenesis. Human clinical trials are already in Phase I testing in some tumor systems, including renal cell, transitional cell, and prostate cancer. However, limitations still have to be overcome. Safe and effective gene vectors will be needed to carry the therapeutic gene to the host cell, and treatments must be tailored so that the desired effects occur only in the tumor cells.

In conclusion, molecular-based therapy is appealing because of its ability to treat cancer at the level of the gene defect that causes the malignant phenotype, and it offers novel and exciting approaches for the treatment and ultimate eradication of cancer.

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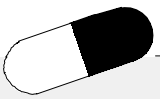
Capsule



Recurrence of bronchioloalveolar carcinoma in transplanted lungs

Bronchioloalveolar carcinoma has a high incidence of metastasis within the lung and a lower incidence of distant metastasis than adenocarcinoma of the lung. It accounts for 3–4% of cases of non-small-cell lung cancer. As conventional therapies for intrapulmonary metastatic bronchioloalveolar carcinoma are generally ineffective, seven patients who had intrapulmonary metastasis of bronchioloalveolar carcinoma received transplants of one or both lungs. The transplants were technically feasible, however recurrence of the original tumor within the donor lungs occurred within 4 years in three of seven patients.

Candidates for transplantation were required to have biopsy-proven bronchioloalveolar carcinoma and no evidence of extrapulmonary disease. PCR was used to detect recurrence of the tumor.

Of the three patients with recurrence, it appeared in a transbronchial biopsy specimen but has not been confirmed by a more extensive surgical biopsy. In the other two patients, treatment was resection with no evidence of recurrence at 18 months. One of the patients was treated by resection of the entire transplanted lung followed by a second lung transplantation. This patient died 9 months later from multiple pulmonary complications, including recurrence of the cancer. Histologic analysis of the recurrent tumors within the transplanted lungs showed that these tumors were similar to the original tumors in three of the patients with recurrence.

Survival rates for patients with stage 4 bronchioloalveolar cancer rarely exceed 5 years. For four of the seven patients, transplantation was followed by a lengthy period of disease-free survival. In all four cases of recurrence, the cancer was limited to the donor lung. The site from which the recurrent carcinoma arose within the donor lung remains unclear. It is possible that some bronchioloalveolar carcinoma cells present in the trachea and proximal mainstem bronchi of patients metastasized back into the transplanted portion(s) of lung tissue.

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