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Cytotoxic Immunotherapy Strategies for Cancer: Mechanisms and Clinical Development

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ABSTRACT

Traditional therapies for cancer include surgery, chemotherapy, and radiation. Chemotherapy has widespread systemic cytotoxic effects against tumor cells but also affects normal cells. Radiation has more targeted local cytotoxicity but is limited to killing cells in the radiation field. Immunotherapy has the potential for systemic, specific killing of tumor cells. However, if the immune response is specific to a single antigen, tumor evasion can occur by down-regulation of that antigen. An immunotherapy approach that induces polyvalent immunity to autologous tumor antigens can provide a personalized vaccine with less potential for immunologic escape. A cytotoxic immunotherapy strategy creates such a tumor vaccine in situ. Immunogenic tumor cell death provides tumor antigen targets for the adaptive immune response and stimulates innate immunity. Attraction and activation of antigen presenting cells such as dendritic cells is important to process and present tumor antigens to T cells. These include cytotoxic T cells that kill tumor cells and T cells which positively and negatively regulate immunity. Tipping the balance in favor of anti-tumor immunity is an important aspect of an effective strategy. Clinically, immunotherapies may be most effective when combined with standard therapies in a complimentary way. An example is gene-mediated cytotoxic immunotherapy (GMCI) which uses an adenoviral vector, AdV-tk, to deliver a cytotoxic and immunostimulatory gene to tumor cells in vivo in combination with standard therapies creating an immunostimulatory milieu. This approach, studied extensively in animal models and early stage clinical trials, is now entering a definitive Phase 3 trial for prostate cancer. J. Cell. Biochem. 112: 1969–1977, 2011.

KEY WORDS: CANCER IMMUNOTHERAPY; CANCER; ADENOVIRAL VECTOR

The immune system is important for defense against cancer. This is evidenced by the increased incidence of cancer in immune compromised hosts such as patients with HIV infection and in transplant recipients undergoing immune suppression who have an increased risk of cancer [Crum et al., 2004; Gutierrez-Dalmau and Campistol, 2007]. In mice, occult cancer grows out of control after suppression of T cells and IFNγ, suggesting that the immune system maintains cancer in an equilibrium state [Koebel et al., 2007]. Cancer cells themselves have been shown to offset this equilibrium through perturbation of immunity. For example, tumor cells have been shown to have decreased MHC class I expression and express soluble factors such as VEGF, IL-6, and IL-10 which inhibit maturation of dendritic cells [Gottfried et al., 2008]. Patients with aggressive tumors such as malignant brain and pancreatic cancer have altered serum cytokines, increased regulatory T cells (Treg), and decreased dendritic cell (DC) number and function [Liyanage et al., 2002; Ebrahimi et al., 2004; Facoetti et al., 2005; Yanagimoto et al., 2005; Fecci et al., 2006]. Effective cancer immunotherapy must overcome negative effects and tip the balance in favor of effective anti-tumor immunity.

INNATE AND ADAPTIVE IMMUNITY—BOTH ARE CRITICAL FOR AN EFFECTIVE CANCER IMMUNOTHERAPY

The innate immune response is critical for immediate response to a local insult and has specificity to the site of the tumor based on local soluble and cellular factors but is not antigen specific [Janeway and Medzhitov, 2002]. Systemic anti-tumor immunity depends on adaptive immunity mediated by antigen-specific immune cells, especially T cells. Danger signals that alert the innate immune system to react are critical to initiate a stimulatory response [Matzinger, 2002]. Stimulation of adaptive immunity requires presentation of tumor antigens to T cells by antigen presenting cells (APC) such as monocytes and dendritic cells (DC). APCs are activated in the milieu of an innate response. Antigen-specific T cells are stimulated to proliferate and generate anti-tumor effects through soluble factors, such as cytokines, and through direct killing by cytotoxic T lymphocytes (CTL). Like most biologic systems, regulatory factors exist to prevent unwanted or excessive immune

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reactions. These are also mediated by soluble factors such as IL-10 and TGFβ and often orchestrated by regulatory T cells (Treg). Overcoming immune inhibitory effects requires a multi-faceted immune stimulation to tilt the immune balance to the effector side. In addition, a polyvalent immune response is important to maintain targeting to the tumor which, through the selective pressure of therapy, can modulate antigen expression and become resistant to single antigen approaches.

STEP 1: IMMUNOGENIC CELL DEATH

Immunogenic cell death refers to cell death that ignites an innate immune response. Necrosis is the pathologic form of cell death, a messy process which is alarming to the immune system whereas apoptosis is a more stealth way in which normal cells die, avoiding immune stimulation under normal physiologic conditions. Both necrosis and apoptosis of tumor cells can provide tumor antigens for initiation of immune stimulation through multi-step processes. For example, the release of Toll-like receptor (TLR) ligands from dying cells induces migration and activation of DCs [Randolph et al., 2008]. Purine nucleotides, such as ATP, released from injured tissues bind to P2RX7 on the recruited DCs leading to IL-1β release which stimulates IFNγ secretion from T cells [Locher et al., 2010]. Exposure of calreticulin/ERp57 complexes on the cell surface of dying cells stimulates uptake by DCs [Locher et al., 2010]. Stimulation of APC expression of co-stimulatory molecules, such as occurs when DC are activated to become mature DC, is critical for stimulatory antigen presentation.

STEP 2: APC ANTIGEN PRESENTATION TO T CELLS—HAND OFF FROM INNATE TO ADAPTIVE IMMUNITY

An important factor for cell death to be immunogenic is processing of antigens into peptides for presentation to T cells [Friedman, 2002]. $CD4^+$ helper T cells require peptide antigen presentation on MHC class II molecules whereas $CD8^+$ CTL recognize peptide antigen on MHC class I. There are three general pathways for APC processing of antigens. Endogenous antigens from intracellular pathogens or self-derived proteins undergo proteosomal degradation into peptides, which are then loaded on MHC class I molecules in the endoplasmic reticulum and transported to the cell surface. Extracellular antigens taken up by phagocytosis or endocytosis are processed by endosomal proteases, loaded onto MHC class II molecules and transported to the cell surface. The third pathway called cross-priming or cross-presentation occurs when exogeneous antigens get loaded onto MHC class I. Heat-shock proteins (HSP) chaperone peptides onto MHC class I and are particularly important for cross-presentation such as through re-presentation of HSP-peptide complexes released from cells due to stress or cell death [Li et al., 2002]. Cross-presentation is particularly important for cytotoxic immunotherapy since it provides for tumor antigens released from dying tumor cells and taken up by APCs to be presented not only on MHC class II but also on MHC class I molecules; this is necessary for CTL stimulation.

The antigen specific T cell receptor mediates the binding of T cells to antigen presented as peptides in the groove of the MHC molecule on APC [Friedman, 2002]. T cell activation, rather than anergy, depends on a second signal delivered via binding of ligands on T cells to their respective co-stimulatory molecules on APCs. These co-stimulatory molecule-ligand pairs include CD80 and CD86 on APCs, which bind to CD28 on T cells and CD40 on APCs, which binds to CD154 (CD40L) on T cells. These interactions stimulate proliferation and functional activation of T cells. Recognition of an MHC-bound peptide without concurrent binding of co-stimulatory molecules is interpreted as processing of a self or nonpathologic peptide and can lead to T cell anergy. Co-stimulatory molecule expression on APCs is critical for maintaining a high avidity memory CTL response [Yang et al., 2005]. This circles back to the importance of the innate immune response and necrotic cell death which induce co-stimulatory molecule and MHC class II expression on APCs [Gottfried et al., 2008].

STEP 3: EFFECTOR T CELLS

Activated $CD4^+$ helper T cells include two subsets with distant cytokine patterns. Th1 cells stimulate CTL activation and expansion by secreting cytokines such as IL-2, IFNγ, TNFβ, IL-7, and Il-12. Th2 cells express cytokines including IL-2, IL-4, IL-5, and IL-10 and stimulate B cells to mature into plasma cells which secrete antigen-specific antibodies. The helper T cell response is down-regulated by expression of CD152 (CTLA-4) which binds to CD80 and CD86 on APCs sending a negative signal to the T cell. This is in contrast to the positive signal generated earlier in the T cell response when CD28 binds to CD80 and CD86.

$CD8^+$ CTL are the ultimate effectors of anti-tumor immunity. Tumor-antigen-specific activated CTL kill tumor cells through either perforin/granzyme-B or death receptor pathways [Friedman, 2002]. The frequency of tumor antigen-specific CTL in the peripheral blood has been shown to correlate with anti-tumor immune responses and clinical responses in tumor vaccine studies in melanoma, pancreas and prostate cancer [Thomas et al., 2004; Gulley et al., 2005; Sanderson et al., 2005]. Regulatory T cells (Treg, $CD4^+CD25^+Foxp3^+$) inhibit the activation and function of T cells and are critical for preventing autoimmunity and for down-regulating a response once a pathogen has been controlled. For tumor immunotherapy, overcoming suppressive factors such as Tregs may be critical to maintain anti-tumor immunity. For example, myeloid-derived suppressor cells (MDSC) are associated with defective dendritic cell function and suppress immunity in part by activating Treg cells [Serafini et al., 2008]. The frequency of circulating MDSCs is increased in some cancer patients and correlated with worse clinical cancer stage and metastatic tumor burden [Diaz-Montero et al., 2009]. The importance of danger signals in tipping the balance in favor of effector T cells is exemplified by a study in which a viral vaccine could break CD8 T cell tolerance in the presence of Treg whereas a DC-based vaccine required removal of Treg or co-administration of either a TLR ligand or an irrelevant virus [Yang et al., 2004]. Immune inhibitory factors elicited by tumors may be overcome by effectively and simultaneously stimulating innate and adaptive immune components (Fig. 1).

CYTOTOXIC IMMUNOTHERAPY STRATEGIES

CYTOTOXIC EFFECTS

Cytotoxicity is necessary for the release of tumor-associated antigens (TAAs). As discussed above, the method of cell death is

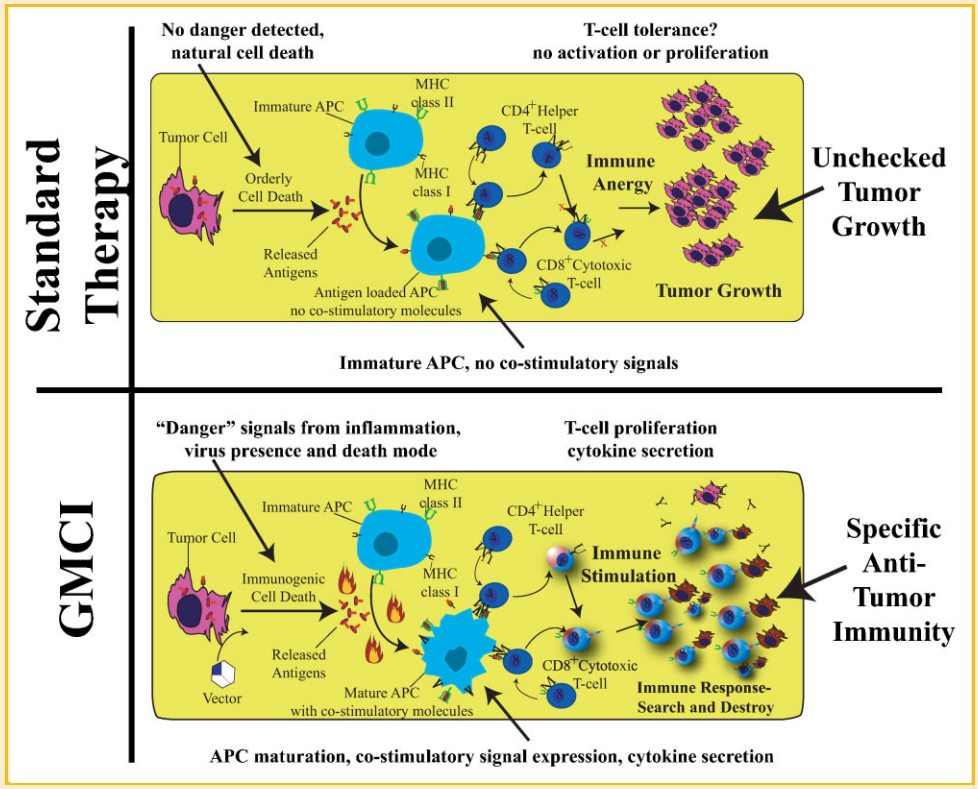

Fig. 1. Immune consequences of standard nonimmunogenic cytotoxic therapy versus gene-mediated cytotoxic immunotherapy (GMCI) using a viral vector. Immune stimulation requires positive signals in multiple steps to avoid immune anergy. Immunogenic cell death provides tumor antigens in a "danger" milieu with signals, such as heat shock proteins, Toll-like receptor ligands, and inflammatory mediators, that attract and activate antigen presenting cells (APC), including dendritic cells (DC). Mature APCs express costimulatory molecules that are required for T cell activation. In the absence of mature APCs, T cells may become anergic. Additional T cell stimulatory factors expand the tumor specific T cell army which can then attack tumor cells throughout the body.

an important determinant for how the released antigens will be handled. Immunogenic cell death can lead to T cell activation whereas normal, physiological cell death may result in T cell anergy. The gene-mediated cytotoxic immunotherapy (GMCI) approach, that will be highlighted here, consists of an adenoviral vector containing the Herpes virus thymidine-kinase gene (AdV-tk) delivered to tumor cells followed by systemic anti-herpetic prodrug administration in combination with the patient's chosen standard cancer therapy. AdV-tk is injected into the tumor site leading to local expression of HSV thymidine kinase (HSV-tk). Anti-herpetic prodrugs which are substrates for HSV-tk include valacyclovir (VCV), acyclovir, and ganciclovir (GCV). Valacyclovir, an oral formulation with very good bio-availability, is a valine-ester of acyclovir which is rapidly converted to acyclovir by first-pass intestinal and hepatic metabolism. Acyclovir is mono-phosphorylated by HSV-tk at the tumor site after which cellular kinases add the second and third phosphate groups to generate a nucleotide analog which is toxic to dividing or DNA-repairing cells [Fyfe et al., 1978; Eastham et al., 1996]. Normal, quiescent cells are less susceptible to this effect. DNA damaging agents, such as radiation and some chemotherapies, increase DNA repair activity and consequently increase susceptibility to AdV-tk/prodrug activity. The tumoricidal effect is expanded to nearby cells, even though not directly transduced by the injected AdV-tk, through spread of activated prodrug through GAP junctions, apoptotic vesicles and other mechanisms; this is known as the local bystander effect [Freeman et al., 1993; Fick et al., 1995].

An anti-angiogenic effect for AdV-tk/prodrug has also been described [Ram et al., 1994; Ayala et al., 2006]. This is likely due to the susceptibility of endothelial cells from growing tumor vessels to AdV-tk/prodrug-mediated cytotoxicity.

IMMUNE EFFECTS

In addition to the local bystander effect, a systemic bystander effect that generated protection against metastases and tumor re-challenge was observed [Perez-Cruet et al., 1994; Vile et al., 1994; Hall et al., 1997]. Three different groups in three different animal models, hepatocellular carcinoma, colon carcinoma, and melanoma, demonstrated efficacy in immunocompetent but not immunodeficient mice [Vile et al., 1994; Gagandeep et al., 1996; Kuriyama et al., 1999]. Thus, the systemic bystander effect was concluded to be immune-mediated. Furthermore, tumor growth inhibition could be adoptively transferred with splenocytes from animals bearing tumors treated with AdV-tk plus prodrug but not from controls treated with AdV-tk plus saline [Agard et al., 2001]. That study not only corroborated the immune nature of the response, but also showed that tumor cell killing was required to induce the response. The mode of cell death induced by AdV-tk/prodrug has been shown in animal models and clinical trials to include necrosis and apoptosis [Eastham et al., 1996; Ayala et al.,

2006]. In a pre-prostatectomy clinical trial, cytopathic effects were primarily observed in areas of carcinoma with little or no effect on benign glands or stroma; this is in contrast to radiation and hormonal ablation which induce changes in benign and malignant areas [Ayala et al., 2000, 2006]. Infiltration of APCs into tumors occurs after AdV-tk/prodrug treatment and is most abundant within tumor foci especially in areas of necrosis [Ayala et al., 2000]. Widespread and specific anti-tumor effects of AdV-tk/prodrug are immune-mediated and due to stimulation of each of the critical steps for an effective anti-tumor immune response.

INNATE IMMUNE STIMULATION

Danger signals expressed by AdV-tk/prodrug-treated tumors are important for stimulating APCs and include TLR ligands, heat shock proteins, adhesion molecules and cytokines. Increased expression of high-mobility-group-box-1 (HMGB1), a TLR2 and TLR4 agonist, was measured after AdV-tk/prodrug in a glioma mouse model [Curtin et al., 2009]. The Vile group discovered differential immunogenicity of HSV-tk expressing cell lines after prodrug treatment which correlated with the predominant type of cell death; B16tk cells primarily died by necrosis and were immunogenic whereas CMT93tk cells primarily underwent apoptosis and were poorly immunogenic [Melcher et al., 1998]. Another difference was that the B16tk cells showed high levels of hsp70 expression after GCV treatment whereas the CMT93tk cells did not [Melcher et al., 1998]. Tumors expressing hsp70 had infiltration of macrophages, T cells and predominantly DC [Todryk et al., 1999]. HSV-tk/prodrug-treated tumors have increased expression of the adhesion molecule ICAM-1 which mediates adhesion to endothelium and chemotaxis of leukocytes [Ramesh et al., 1996]. Supernatant or lysate from HSV-tk/prodrug-treated tumors induces expression of co-stimulatory molecules B7-1 (CD80) and B7-2 (CD86) on APCs which are critical for T cells to be stimulated, rather than anergized, to tumor antigens [Ramesh et al., 1996]. Thus, the evidence strongly suggest that AdV-tk/prodrug mediated cell death is a potent stimulator of an innate immune response.

T CELL FUNCTIONS

Significant evidence supports the critical importance of T cells in anti-tumor effects of AdV-tk/prodrug. For example, in HSV-tk/prodrug-treated tumors, infiltration of $CD4^+$ and $CD8^+$ T cells were found in animal models and human clinical trials following treatment including brain tumors and prostate cancer [Barba et al., 1994; Vile et al., 1997; Chhikara et al., 2001; Ayala et al., 2006; Chiocca et al., 2009]. Cytokines that stimulate APCs and T cells, including IL-2, IL-12, IFNγ, TNFα, and GM-CSF, were also increased in HSV-tk/prodrug-treated tumors whereas inhibitory cytokines IL-4, IL-6, and IL-10 were not [Vile et al., 1997]. In one of the prostate cancer clinical trials, serum IL-12 levels were measured and found to be significantly increased after AdV-tk/prodrug treatment whereas the inhibitory cytokine TGFβ was not increased [Ayala et al., 2006]. $CD8^+$ T cells were shown to be critical for local and distant anti-tumor effects based on comparisons of outcomes with and without anti-CD8 antibody depletion in preclincal studies [Agard et al., 2001].

The T cell response to HSV-tk/prodrug therapy has a specific tumor antigen associated component and a nonspecific TK modulatory effect. The specific response is illustrated by in vitro proliferation assays. For example, T cells from animals bearing HSV-tk/prodrug-treated tumors proliferated in response to parental tumor cells [Ramesh et al., 1996]. In addition, there is a nonantigen specific "superantigen-like" stimulation. In the presence of APCs expressing MHC class II, the HSV-tk protein, independent of its enzymatic function, stimulated T cells to proliferate and secrete IL-2 in a mixed lymphocyte reaction assay (unpublished results). This may be important for amplifying the T cell response in AdV-tk-mediated cytotoxic immunotherapy.

DELIVERY VEHICLE IS IMPORTANT COMPONENT

The convergence of released tumor antigens, danger-signals, APCs and stimulation of T-cell proliferation, provide the milieu for a potent anti-tumor immune response. In gene-mediated approaches, the vector type used to deliver the gene is also part of the equation. A previous Phase 3 study used a retroviral vector to deliver HSV-tk to malignant gliomas and failed due in large part to poor transduction [Ram et al., 1997; Harsh et al., 2000; Rainov, 2000]. Unlike the retroviral vectors that were applied in this study, adenoviral vectors can transduce either dividing or nondividing cells. In a small, controlled study in malignant gliomas, the combination of adenovirus with the HSV-tk gene showed a doubling of the mean survival compared to the retroviral delivery or the adenovirus with a marker gene [Sandmair et al., 2000]. Despite existing immunity to adenovirus, which is common in the general population, intratumoral delivery allows transduction of tumor cells before immune clearance and even permits repeat administration, with repeat tumor responses without added toxicity [Miles et al., 2001]. In animal models, improved suppression of tumor growth and increased survival has been demonstrated with three courses of AdV-tk/prodrug compared to one or two courses [Lambright et al., 2000]. Overall, the use of an adenoviral vector to deliver the HSV-tk gene has been fundamental to the positive results in human clinical studies.

COMBINATION WITH STANDARD OF CARE TREATMENTS

In the clinic, patients usually present with relatively large tumor burdens that have overwhelmed the immune system. Immunotherapy likely has the greatest potential for efficacy when used to combat minimal residual or small metastatic disease left after the debulking of the tumor with standard therapy. Preclinical studies demonstrated that AdV-tk/prodrug is synergistic with radiation, surgery, and some chemotherapeutic agents [Chhikara et al., 2001; Rainov et al., 2001; Sukin et al., 2001; Vlachaki et al., 2001; Nestler et al., 2004]. This likely results from increased incorporation of the nucleotide analogues during the DNA repair process, increased bystander effect due to radiation-induced cell membrane damage, and potentiation of the systemic anti-tumor effect of AdV-tk through enhanced immune stimulation by surgery or radiation-induced inflammation.

Synergy between AdV-tk/prodrug therapy and radiation was demonstrated in a murine prostate tumor model [Chhikara et al., 2001]. Subcutaneous tumors in the flank were generated with the

RM-1 cell line and lung metastases were generated by tail vein injection of RM-1 cells on the same day that they were injected subcutaneously. The subcutaneous tumors were treated by either intratumoral injection of saline, a control adenovirus AdV-βgal, AdV-tk followed by systemic GCV, local radiation therapy or the combination of AdV-tk/GCV and radiation therapy. The number of lung nodules was reduced by 37% following treatment with AdV-tk/GCV and by an additional 50% when combined with radiation to the primary tumor, whereas radiotherapy alone to the flank tumor had no effect on lung nodules. The fact that radiation alone did not have any effect on lung metastases indicates that the effect when combined with AdV-tk/GCV was synergistic rather than additive. Tumors treated with AdV-tk/GCV plus radiation had significantly increased T cell infiltration consistent with the immune-mediated mechanism for the synergistic effect [Chhikara et al., 2001]. Even in an orthotopic brain tumor model where inflammation within the limited space of the cranium could lead to toxicities, the combination of AdV-tk/GCV with radiation had better overall effects without overlapping toxicity [Nestler et al., 2004]. Combining AdV-tk/GCV with surgery showed similar results as with radiation in prostate and mammary tumor models; local administration of AdV-tk to the tumor bed following surgical resection not only delayed local recurrence but also had systemic anti-tumor effects [Sukin et al., 2001]. Thus, the combination of AdV-tk/prodrug with standard of care surgery and radiation therapy not only decreased the local tumor burden but also leads to synergistic immune-mediated systemic anti-tumor effects without added toxicity.

CLINICAL EXPERIENCE

Safety and evidence for efficacy have been demonstrated in numerous clinical trials of cytotoxic immunotherapy (Table I). For AdV-tk, early trials evaluated the approach as monotherapy. Phase 1 and 2 studies in recurrent prostate cancer demonstrated safety, PSA responses and immune stimulation [Herman et al., 1999; Miles et al., 2001]. Pre-prostatectomy studies also demonstrated safety and provided tissue for detailed analysis of the histologic and immunologic effects which were described above [van der Linden et al., 2005; Ayala et al., 2006]. In a Phase 1 trial for recurrent malignant gliomas involving 13 patients, three patients survived more than 25 months, one with stable disease and another with transient reduction in tumor dimensions; two had a post-treatment interval of reduced steroid requirement [Trask et al., 2000]. At autopsy, inflammation and necrosis were seen within tumors without extratumoral inflammation. Of particular interest, at autopsy from one patient, lymphocytic infiltrate was also observed in a contralateral, untreated tumor, suggesting induction of systemic anti-tumor immunity. Several groups have conducted Phase 1 studies with similar adenoviral-HSV-tk vectors in adult brain tumors and have reported comparable results with no significant toxicity [Germano et al., 2003; Smitt et al., 2003]. Other Phase 1 studies with adenoviral delivered HSV-tk/prodrug as a monotherapy have been performed in colorectal liver metastases, mesothelioma, and retinoblastoma [Sung et al., 2001; Chevez-Barrios et al., 2005; Sterman et al., 2005]. In the mesothelioma study, 34 patients were treated with vector ranging from 5×10^{10} vp to 5×10^{13} vp injected into the pleural space followed by ganciclovir [Sterman et al., 2005]. The approach was safe, acute inflammatory reactions were observed and antibody responses to tumor were consistently detected. Long-term durable clinical responses were observed in several patients without other therapy and two of the three patients at the highest dose level survived more than 8 years. Slow resolution of a right cardiophrenic tumor in one of these patients with eventual complete response at 3 years suggested a durable immune-mediated mechanism. Similar indications of possible efficacy with little or no toxicity have been reported for other indications [Sung et al., 2001; Chevez-Barrios et al., 2005].

Encouraging clinical results have also been observed with other cytotoxic immunotherapy approaches. One approach uses an oncolytic herpes simplex virus (JS1/34.5-/47-/GM-CSF; OncoVex) that leads to lytic replication after intra-tumoral injection of the agent and immunogenicity is enhanced by the expression of GM-CSF [Harrington et al., 2010]. In a single-arm Phase 2 study in patients with stage III and IV malignant melanoma, intriguing evidence of efficacy was reported with a 26% objective response rate affecting both treated and distant lesions demonstrating a systemic in situ vaccine effect generated from a locally administered therapy [Senzer et al., 2009]. Phase 3 clinical trials have been initiated with this agent in melanoma and squamous cell carcinoma of the head and neck. Another similar approach using a replicating adenoviral vector expressing HSV-tk and cytosine deaminase delivered intratumorally has positive results from Phase 1 trials for prostate cancer and a Phase 2 trial has been initiated [Freytag et al., 2007a,b].

Immunologic effects of standard cancer treatments that may affect the results of immunotherapy approaches are important to

TABLE I. Examples of Cytotoxic Immunotherapy Strategies in the Clinic and the Current Phase of Clinical Development

Intervention	Indication	Phase	Refs.
AdV-tk/GCV	Recurrent malignant glioma	1	Trask et al. [2000]
AdV-tk/GCV	Colorectal liver metastases	1	Sung et al. [2001]
Adeno-HSV-tk vectors/GCV	Mesothelioma	1	Sterman et al. [2005]
AdV-tk/GCV	Retinoblastoma	1	Chevez-Barrios et al. [2005]
AdV-tk/VCV + topotecan	Recurrent ovarian cancer	1	Hasenburg et al. [2001]
AdV-tk/VCV + surgery or chemoradiation	Upfront Pancreatic cancer	1	Bloomston et al. [2011]
AdV-tk/GCV	Recurrent prostate cancer	2	Herman et al. [1999], Miles et al. [2001]
AdV-tk/GCV	Pre-prostatectomy	2	Ayala et al. [2006], van der Linden et al. [2005]
AdV-tk/VCV + surgery and chemoradiation	Upfront malignant glioma	2	Chiocca et al. [2009]
Ad5-CD/TKrep + GCV/5-FC + radiation	Upfront prostate cancer	2	Freytag et al. [2007a]
AdV-tk/VCV + radiation ± ADT	Upfront prostate cancer	3	Teh et al. [2004], Aguilar et al. [2006]
JS1/34.5-/47-/GM-CSF	Metastatic melanoma	3	Senzer et al. [2009]
JS1/34.5-/47-/GM-CSF + chemoradiation	Upfront squamous cell carcinoma head and neck	3	Harrington et al. [2010]

consider in the clinical development strategy. Although radiation and chemotherapy primarily kill tumor cells through apoptosis and also have the potential to kill immune effector cells, positive immune modulating effects may help potentiate novel cytotoxic immunotherapy. For example, radiation upregulates expression in tumors of MHC class I, adhesion molecules, heat shock proteins, inflammatory mediators, and immunostimulatory cytokines [Friedman, 2002]. Chemotherapy such as cyclophosphamide and temozolomide has been shown to decrease the number and function of regulatory T cells [Lutsiak et al., 2005; Heimberger et al., 2008; Jordan et al., 2008; Emens et al., 2009; Greten et al., 2010]. Another approach being developed is to inhibit the CTLA4 regulatory pathway in T cells with an anti-CTLA4 antibody (e.g., ipilimumab) which was recently shown to improve survival in patients with metastatic melanoma but also with the risk of autoimmunity [Hodi et al., 2010].

Combining AdV-tk with standard therapy including radiation, surgery, and chemotherapy has proven to be safe and potentially effective in clinical trials including ovarian, brain, pancreatic, and prostate cancer [Hasenburg et al., 2001; Teh et al., 2004]. In malignant gliomas, a European group reported increased survival results from a randomized Phase 2 study in conjunction with surgery compared to surgery alone [Immonen et al., 2004]. We have recently conducted a Phase 1b/2a study to evaluate AdV-tk therapy with surgery and chemoradiation in newly diagnosed malignant gliomas with similarly encouraging safety and efficacy results [Chiocca et al., 2009]. In ovarian and pancreatic cancer, combining AdV-tk/prodrug with topotecan or 5-fluorouracil, respectively, was well tolerated [Hasenburg et al., 2001; Bloomston et al., 2011]. A 71 patient Phase 2 study for newly diagnosed prostate cancer as an upfront adjuvant to radiation therapy found a statistically significant improvement in disease free survival, compared to historical controls, with no agent-specific toxicity [Aguilar et al., 2004, 2006; Teh et al., 2004]. In this trial, more so than in the previous prostate cancer trials with AdV-tk/prodrug alone, the percentage of activated $CD4^+$ and $CD8^+$ T cells in the peripheral blood increased significantly after treatment and was sustained for 8–12 months [Satoh et al., 2004; Fujita et al., 2006]. These studies support the hypothesis that adjuvant up-front use of AdV-tk/prodrug in combination with standard treatments can tip the balance in favor of the effector immune response to prevent disease progression or recurrence and have led to the development of a randomized-controlled, definitive Phase 3 trial for prostate cancer.

CONCLUSION

Clinical development of cytotoxic immunotherapy has entered the definitive clinical testing stage, Phase 3. The field has evolved as it proceeded through the stages of safety and early efficacy analysis. Lessons learned from mechanistic studies in both preclinical and clinical stages as well as from other immunotherapy strategies have led to improvements. The realization that standard cancer treatments have positive immunologic effects has been an important contribution. Incorporating novel agents such as AdV-tk/prodrug into the standard treatment armamentarium for cancer early in the disease process has the potential to prevent recurrence or metastases without added toxicity. This approach has been well received by patients and clinicians since it adds to the well-known treatment modalities, is simple to administer and has the potential to improve quality of life long-term. The results of definitive Phase 3 trials in the coming years will be highly anticipated.

REFERENCES

Agard C, Ligeza C, Dupas B, Izembart A, El Kouri C, Moullier P, Ferry N. 2001. Immune-dependent distant bystander effect after adenovirus-mediated suicide gene transfer in a rat model of liver colorectal metastasis. Cancer Gene Ther 8:128–136.

Aguilar LK, Teh BS, Ayala G, Vlachaki MT, Wheeler T, Kadmon D, Thompson T, Miles B, Aguilar-Cordova E, Butler EB. 2004. PSA nadir and 24 month biopsy interim analysis of AdV-tk/valacyclovir gene therapy in combination with radiotherapy vs radiotherapy alone for prostate cancer. Proc Am Soc Clin Oncol Annu Meet 22:399s.

Aguilar LK, Teh BS, Mai W, Caillouet J, Ayala G, Aguilar-Cordova E, Butler EB. 2006. Five year follow up of a phase II study of cytotoxic immunotherapy combined with radiation in newly diagnosed prostate cancer. Proc Am Soc Clin Oncol Annu Meet 24:250.

Ayala G, Wheeler TM, Shalev M, Thompson TC, Miles B, Aguilar-Cordova E, Chakraborty S, Kadmon D. 2000. Cytopathic effect of in situ gene therapy in prostate cancer. Hum Pathol 31:866–870.

Ayala G, Satoh T, Li R, Shalev M, Gdor Y, Aguilar-Cordova E, Frolov A, Wheeler TM, Miles BJ, Rauen K, Teh BS, Butler EB, Thompson TC, Kadmon D. 2006. Biological response determinants in HSV-tk + ganciclovir gene therapy for prostate cancer. Mol Ther 13:716–728.

Barba D, Hardin J, Sadelain M, Gage FH. 1994. Development of anti-tumor immunity following thymidine kinase-mediated killing of experimental brain tumors. Proc Natl Acad Sci USA 91:4348–4352.

Bloomston M, Marsh C, Walker J, Coyle W, Marx H, Tahiri S, Moran Cruz C, Aguilar LK, Aguilar-Cordova E, Chung VM. 2011. Phase I trial of Gene Mediated Cytotoxic Immunotherapy in combination with chemoradiation for locally advanced pancreatic cancer. J Clin Oncol 29(Suppl 4): abstr 195.

Chevez-Barrios P, Chintagumpala M, Mieler W, Paysse E, Boniuk M, Kozinetz C, Hurwitz MY, Hurwitz RL. 2005. Response of retinoblastoma with vitreous tumor seeding to adenovirus-mediated delivery of thymidine kinase followed by ganciclovir. J Clin Oncol 23:7927–7935.

Chhikara M, Huang H, Vlachaki MT, Zhu X, Teh B, Chiu KJ, Woo S, Berner B, Smith EO, Oberg KC, Aguilar LK, Thompson TC, Butler EB, Aguilar-Cordova E. 2001. Enhanced therapeutic effect of HSV-tk + GCV gene therapy and ionizing radiation for prostate cancer. Mol Ther 3:536–542.

Chiocca EA, Kaur B, Hardcastle J, Bell S, Cavaliere R, Lo S, Badie B, Portnow J, Monterroso C, Aguilar LK, Aguilar-Cordova E, Baskin DS, Trask TW, New PZ. 2009. Phase I/II results of AdV-tk gene-mediated cytotoxic immunotherapy adjuvant to standard upfront surgery and chemoradiation for malignant gliomas. Neuro-Oncology 11:693.

Crum NF, Spencer CR, Amling CL. 2004. Prostate carcinoma among men with human immunodeficiency virus infection. Cancer 101:294–299.

Curtin JF, Liu N, Candolfi M, Xiong W, Assi H, Yagiz K, Edwards MR, Michelsen KS, Kroeger KM, Liu C, Muhammad AK, Clark MC, Arditi M, Comin-Anduix B, Ribas A, Lowenstein PR, Castro MG. 2009. HMGB1 mediates endogenous TLR2 activation and brain tumor regression. PLoS Med 6:e10.

Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. 2009. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 58:49–59.

Eastham JA, Chen SH, Sehgal I, Yang G, Timme TL, Hall SJ, Woo SL, Thompson TC. 1996. Prostate cancer gene therapy: Herpes simplex virus thymidine kinase gene transduction followed by ganciclovir in mouse and human prostate cancer models. Hum Gene Ther 7:515–523.

Ebrahimi B, Tucker SL, Li D, Abbruzzese JL, Kurzrock R. 2004. Cytokines in pancreatic carcinoma: Correlation with phenotypic characteristics and prognosis. Cancer 101:2727–2736.

Emens LA, Asquith JM, Leatherman JM, Kobrin BJ, Petrik S, Laiko M, Levi J, Daphtary MM, Biedrzycki B, Wolff AC, Stearns V, Disis ML, Ye X, Piantadosi S, Fetting JH, Davidson NE, Jaffee EM. 2009. Timed sequential treatment with cyclophosphamide, doxorubicin, and an allogeneic granulocyte-macrophage colony-stimulating factor-secreting breast tumor vaccine: A chemotherapy dose-ranging factorial study of safety and immune activation. J Clin Oncol 27:5911–5918.

Facoetti A, Nano R, Zelini P, Morbini P, Benericetti E, Ceroni M, Campoli M, Ferrone S. 2005. Human leukocyte antigen and antigen processing machinery component defects in astrocytic tumors. Clin Cancer Res 11:8304–8311.

Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, Herndon JE II, Bigner DD, Dranoff G, Sampson JH. 2006. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res 66:3294–3302.

Fick J, Barker FG II, Dazin P, Westphale EM, Beyer EC, Israel MA. 1995. The extent of heterocellular communication mediated by gap junctions is predictive of bystander tumor cytotoxicity in vitro. Proc Natl Acad Sci USA 92:11071–11075.

Freeman SM, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, Abraham GN. 1993. The "bystander effect": Tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 53:5274–5283.

Freytag SO, Movsas B, Aref I, Stricker H, Peabody J, Pegg J, Zhang Y, Barton KN, Brown SL, Lu M, Savera A, Kim JH. 2007a. Phase I trial of replication-competent adenovirus-mediated suicide gene therapy combined with IMRT for prostate cancer. Mol Ther 15:1016–1023.

Freytag SO, Stricker H, Peabody J, Pegg J, Paielli D, Movsas B, Barton KN, Brown SL, Lu M, Kim JH. 2007b. Five-year follow-up of trial of replication-competent adenovirus-mediated suicide gene therapy for treatment of prostate cancer. Mol Ther 15:636–642.

Friedman EJ. 2002. Immune modulation by ionizing radiation and its implications for cancer immunotherapy. Curr Pharm Des 8:1765–1780.

Fujita T, Teh BS, Timme TL, Mai WY, Satoh T, Kusaka N, Naruishi K, Fattah EA, Aguilar-Cordova E, Butler EB, Thompson TC. 2006. Sustained long-term immune responses after in situ gene therapy combined with radiotherapy and hormonal therapy in prostate cancer patients. Int J Radiat Oncol Biol Phys 65:84–90.

Fyfe JA, Keller PM, Furman PA, Miller RL, Elion GB. 1978. Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound, 9-(2-hydroxyethoxymethyl)guanine. J Biol Chem 253:8721–8727.

Gagandeep S, Brew R, Green B, Christmas SE, Klatzmann D, Poston GJ, Kinsella AR. 1996. Prodrug-activated gene therapy: Involvement of an immunological component in the "bystander effect". Cancer Gene Ther 3:83–88.

Germano IM, Fable J, Gultekin SH, Silvers A. 2003. Adenovirus/herpes simplex-thymidine kinase/ganciclovir complex: Preliminary results of a phase I trial in patients with recurrent malignant gliomas. J Neurooncol 65:279–289.

Gottfried E, Kreutz M, Mackensen A. 2008. Tumor-induced modulation of dendritic cell function. Cytokine Growth Factor Rev 19:65–77.

Greten TF, Ormandy LA, Fikuart A, Hochst B, Henschen S, Horning M, Manns MP, Korangy F. 2010. Low-dose cyclophosphamide treatment impairs regulatory T cells and unmasks AFP-specific CD4+ T-cell responses in patients with advanced HCC. J Immunother 33:211–218.

Gulley JL, Arlen PM, Bastian A, Morin S, Marte J, Beetham P, Tsang KY, Yokokawa J, Hodge JW, Menard C, Camphausen K, Coleman CN, Sullivan F, Steinberg SM, Schlom J, Dahut W. 2005. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. Clin Cancer Res 11:3353–3362.

Gutierrez-Dalmau A, Campistol JM. 2007. Immunosuppressive therapy and malignancy in organ transplant recipients: A systematic review. Drugs 67: 1167–1198.

Hall SJ, Mutchnik SE, Chen SH, Woo SL, Thompson TC. 1997. Adenovirus-mediated herpes simplex virus thymidine kinase gene and ganciclovir therapy leads to systemic activity against spontaneous and induced metastasis in an orthotopic mouse model of prostate cancer. Int J Cancer 70:183–187.

Harrington KJ, Hingorani M, Tanay MA, Hickey J, Bhide SA, Clarke PM, Renouf LC, Thway K, Sibtain A, McNeish IA, Newbold KL, Goldsweig H, Coffin R, Nutting CM. 2010. Phase I/II study of oncolytic HSV GM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. Clin Cancer Res 16:4005–4015.

Harsh GR, Deisboeck TS, Louis DN, Hilton J, Colvin M, Silver JS, Qureshi NH, Kracher J, Finkelstein D, Chiocca EA, Hochberg FH. 2000. Thymidine kinase activation of ganciclovir in recurrent malignant gliomas: A gene-marking and neuropathological study. J Neurosurg 92:804–811.

Hasenburg A, Tong XW, Fischer DC, Rojas-Martinez A, Nyberg-Hoffman C, Kaplan AL, Kaufman RH, Ramzy I, Aguilar-Cordova E, Kieback DG. 2001. Adenovirus-mediated thymidine kinase gene therapy in combination with topotecan for patients with recurrent ovarian cancer: 2.5-year follow-up. Gynecol Oncol 83:549–554.

Heimberger AB, Sun W, Hussain SF, Dey M, Crutcher L, Aldape K, Gilbert M, Hassenbusch SJ, Sawaya R, Schmittling B, Archer GE, Mitchell DA, Bigner DD, Sampson JH. 2008. Immunological responses in a patient with glioblastoma multiforme treated with sequential courses of temozolomide and immunotherapy: Case study. Neuro Oncol 10:98–103.

Herman JR, Adler HL, Aguilar-Cordova E, Rojas-Martinez A, Woo S, Timme TL, Wheeler TM, Thompson TC, Scardino PT. 1999. In situ gene therapy for adenocarcinoma of the prostate: A phase I clinical trial. Hum Gene Ther 10:1239–1249.

Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. 2010. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711–723.

Immonen A, Vapalahti M, Tyynela K, Hurskainen H, Sandmair A, Vanninen R, Langford G, Murray N, Yla-Herttuala S. 2004. AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: A randomised, controlled study. Mol Ther 10:967–972.

Janeway CA Jr, Medzhitov R. 2002. Innate immune recognition. Annu Rev Immunol 20:197–216.

Jordan JT, Sun W, Hussain SF, DeAngulo G, Prabhu SS, Heimberger AB. 2008. Preferential migration of regulatory T cells mediated by glioma-secreted chemokines can be blocked with chemotherapy. Cancer Immunol Immunother 57:123–131.

Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, Smyth MJ, Schreiber RD. 2007. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 450:903–907.

Kuriyama S, Kikukawa M, Masui K, Okuda H, Nakatani T, Akahane T, Mitoro A, Tominaga K, Tsujinoue H, Yoshiji H, Okamoto S, Fukui H, Ikenaka K. 1999. Cancer gene therapy with HSV-tk/GCV system depends on T-cell-mediated immune responses and causes apoptotic death of tumor cells in vivo. Int J Cancer 83:374–380.

Lambright ES, Force SD, Lanuti ME, Wasfi DS, Amin KM, Albelda SM, Kaiser LR. 2000. Efficacy of repeated adenoviral suicide gene therapy in a localized murine tumor model. Ann Thorac Surg 70:1865–1870; discussion 1870–1.

Li Z, Menoret A, Srivastava P. 2002. Roles of heat-shock proteins in antigen presentation and cross-presentation. Curr Opin Immunol 14:45–51.

Liyanage UK, Moore TT, Joo HG, Tanaka Y, Herrmann V, Doherty G, Drebin JA, Strasberg SM, Eberlein TJ, Goedegebuure PS, Linehan DC. 2002. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol 169:2756–2761.

Locher C, Conforti R, Aymeric L, Ma Y, Yamazaki T, Rusakiewicz S, Tesniere A, Ghiringhelli F, Apetoh L, Morel Y, Girard JP, Kroemer G, Zitvogel L. 2010. Desirable cell death during anticancer chemotherapy. Ann N Y Acad Sci 1209:99–108.

Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. 2005. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood 105: 2862–2868.

Matzinger P. 2002. The danger model: A renewed sense of self. Science 296:301–305.

Melcher A, Todryk S, Hardwick N, Ford M, Jacobson M, Vile RG. 1998. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. Nat Med 4:581–587.

Miles BJ, Shalev M, Aguilar-Cordova E, Timme TL, Lee HM, Yang G, Adler HL, Kernen K, Pramudji CK, Satoh T, Gdor Y, Ren C, Ayala G, Wheeler TM, Butler EB, Kadmon D, Thompson TC. 2001. Prostate-specific antigen response and systemic T cell activation after in situ gene therapy in prostate cancer patients failing radiotherapy. Hum Gene Ther 12:1955–1967.

Nestler U, Wakimoto H, Siller-Lopez F, Aguilar LK, Chakravarti A, Muzikansky A, Stemmer-Rachamimov A, Chiocca EA, Aguilar-Cordova E, Hochberg FH. 2004. The combination of adenoviral HSV TK gene therapy and radiation is effective in athymic mouse glioblastoma xenografts without increasing toxic side effects. J Neurooncol 67:177–188.

Perez-Cruet MJ, Trask TW, Chen SH, Goodman JC, Woo SL, Grossman RG, Shine HD. 1994. Adenovirus-mediated gene therapy of experimental gliomas. J Neurosci Res 39:506–511.

Rainov NG. 2000. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 11:2389–2401.

Rainov NG, Fels C, Droege JW, Schafer C, Kramm CM, Chou TC. 2001. Temozolomide enhances herpes simplex virus thymidine kinase/ganciclovir therapy of malignant glioma. Cancer Gene Ther 8:662–668.

Ram Z, Walbridge S, Shawker T, Culver KW, Blaese RM, Oldfield EH. 1994. The effect of thymidine kinase transduction and ganciclovir therapy on tumor vasculature and growth of 9L gliomas in rats. J Neurosurg 81:256–260.

Ram Z, Culver KW, Oshiro EM, Viola JJ, DeVroom HL, Otto E, Long Z, Chiang Y, McGarrity GJ, Muul LM, Katz D, Blaese RM, Oldfield EH. 1997. Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. Nat Med 3:1354–1361.

Ramesh R, Munshi A, Abboud CN, Marrogi AJ, Freeman SM. 1996. Expression of costimulatory molecules: B7 and ICAM up-regulation after treatment with a suicide gene. Cancer Gene Ther 3:373–384.

Randolph GJ, Ochando J, Partida-Sanchez S. 2008. Migration of dendritic cell subsets and their precursors. Annu Rev Immunol 26:293–316.

Sanderson K, Scotland R, Lee P, Liu D, Groshen S, Snively J, Sian S, Nichol G, Davis T, Keler T, Yellin M, Weber J. 2005. Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and montanide ISA 51 for patients with resected stages III and IV melanoma. J Clin Oncol 23:741–750.

Sandmair AM, Loimas S, Puranen P, Immonen A, Kossila M, Puranen M, Hurskainen H, Tyynela K, Turunen M, Vanninen R, Lehtolainen P, Paljarvi L, Johansson R, Vapalahti M, Yla-Herttuala S. 2000. Thymidine kinase gene therapy for human malignant glioma, using replication-deficient retroviruses or adenoviruses. Hum Gene Ther 11:2197–2205.

Satoh T, Teh BS, Timme TL, Mai WY, Gdor Y, Kusaka N, Fujita T, Pramudji CK, Vlachaki MT, Ayala G, Wheeler T, Amato R, Miles BJ, Kadmon D, Butler EB, Thompson TC. 2004. Enhanced systemic T-cell activation after in situ gene therapy with radiotherapy in prostate cancer patients. Int J Radiat Oncol Biol Phys 59:562–571.

Senzer NN, Kaufman HL, Amatruda T, Nemunaitis M, Reid T, Daniels G, Gonzalez R, Glaspy J, Whitman E, Harrington K, Goldsweig H, Marshall T, Love C, Coffin R, Nemunaitis JJ. 2009. Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. J Clin Oncol 27:5763–5771.

Serafini P, Mgebroff S, Noonan K, Borrello I. 2008. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. Cancer Res 68:5439–5449.

Smitt PS, Driesse M, Wolbers J, Kros M, Avezaat C. 2003. Treatment of relapsed malignant glioma with an adenoviral vector containing the herpes simplex thymidine kinase gene followed by ganciclovir. Mol Ther 7:851–858.

Sterman DH, Recio A, Vachani A, Sun J, Cheung L, DeLong P, Amin KM, Litzky LA, Wilson JM, Kaiser LR, Albelda SM. 2005. Long-term follow-up of patients with malignant pleural mesothelioma receiving high-dose adenovirus herpes simplex thymidine kinase/ganciclovir suicide gene therapy. Clin Cancer Res 11:7444–7453.

Sukin SW, Chhikara M, Zhu X, Ayala G, Aguilar LK, O'Brian Smith E, Miles BJ, Thompson TC, Kadmon D, Aguilar-Cordova E. 2001. In vivo surgical resection plus adjuvant gene therapy in the treatment of mammary and prostate cancer. Mol Ther 3:500–506.

Sung MW, Yeh HC, Thung SN, Schwartz ME, Mandeli JP, Chen SH, Woo SL. 2001. Intratumoral adenovirus-mediated suicide gene transfer for hepatic metastases from colorectal adenocarcinoma: Results of a phase I clinical trial. Mol Ther 4:182–191.

Teh BS, Ayala G, Aguilar L, Mai WY, Timme TL, Vlachaki MT, Miles B, Kadmon D, Wheeler T, Caillouet J, Davis M, Carpenter LS, Lu HH, Chiu JK, Woo SY, Thompson T, Aguilar-Cordova E, Butler EB. 2004. Phase I-II trial evaluating combined intensity-modulated radiotherapy and in situ gene therapy with or without hormonal therapy in treatment of prostate cancer-interim report on PSA response and biopsy data. Int J Radiat Oncol Biol Phys 58:1520–1529.

Thomas AM, Santarsiero LM, Lutz ER, Armstrong TD, Chen YC, Huang LQ, Laheru DA, Goggins M, Hruban RH, Jaffee EM. 2004. Mesothelin-specific CD8(+) T cell responses provide evidence of in vivo cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. J Exp Med 200:297–306.

Todryk S, Melcher AA, Hardwick N, Linardakis E, Bateman A, Colombo MP, Stoppacciaro A, Vile RG. 1999. Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake. J Immunol 163:1398–1408.

Trask TW, Trask RP, Aguilar-Cordova E, Shine HD, Wyde PR, Goodman JC, Hamilton WJ, Rojas-Martinez A, Chen SH, Woo SL, Grossman RG. 2000. Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. Mol Ther 1:195–203.

van der Linden RR, Haagmans BL, Mongiat-Artus P, van Doornum GJ, Kraaij R, Kadmon D, Aguilar-Cordova E, Osterhaus AD, van der Kwast TH, Bangma CH. 2005. Virus specific immune responses after human neoadjuvant adenovirus-mediated suicide gene therapy for prostate cancer. Eur Urol 48:153–161.

Vile RG, Nelson JA, Castleden S, Chong H, Hart IR. 1994. Systemic gene therapy of murine melanoma using tissue specific expression of the HSVtk gene involves an immune component. Cancer Res 54:6228–6234.

Vile RG, Castleden S, Marshall J, Camplejohn R, Upton C, Chong H. 1997. Generation of an anti-tumour immune response in a non-immunogenic tumour: HSVtk killing in vivo stimulates a mononuclear cell infiltrate and a Th1-like profile of intratumoural cytokine expression. Int J Cancer 71:267–274.

Vlachaki MT, Chhikara M, Aguilar L, Zhu X, Chiu KJ, Woo S, Teh BS, Thompson TC, Butler EB, Aguilar-Cordova E. 2001. Enhanced therapeutic effect of multiple injections of HSV-TK+GCV gene therapy in combination with ionizing radiation in a mouse mammary tumor model. Int J Radiat Oncol Biol Phys 51:1008–1017.

Yanagimoto H, Takai S, Satoi S, Toyokawa H, Takahashi K, Terakawa N, Kwon AH, Kamiyama Y. 2005. Impaired function of circulating dendritic cells in patients with pancreatic cancer. Clin Immunol 114:52–60.

Yang Y, Huang CT, Huang X, Pardoll DM. 2004. Persistent Toll-like receptor signals are required for reversal of regulatory T cell-mediated CD8 tolerance. Nat Immunol 5:508–515.

Yang S, Hodge JW, Grosenbach DW, Schlom J. 2005. Vaccines with enhanced costimulation maintain high avidity memory CTL. J Immunol 175:3715–3723.