

Cancer Immunogene Therapy: A Review

Ian F. Parney^a Lung-Ji Chang^b

^aNeuro-Oncology Service, Department of Neurological Surgery, University of California, San Francisco, Calif.,

^bDepartment of Molecular Genetics and Microbiology, Powell Gene Therapy Center, and McKnight Brain Institute, University of Florida, Gainesville, Fla., USA

Key Words

Gene therapy · Immunotherapy · Cancer · Vaccine

Abstract

Although immunotherapy has long held out promise as a specific, potent approach to cancer therapy, clinical applications have been unrewarding to date. However, advances in gene transfer technology and basic immunology have opened new avenues to stimulate antitumor immune responses including immunogene therapy. Many different approaches to immunogene therapy have been identified. These include transferring genes encoding proinflammatory proteins to tumor cells, suppressing immunosuppressive gene expression, and transferring proinflammatory genes and/or tumor antigen genes to professional antigen-presenting cells. In some cases, genes are transferred to tumor or antigen-presenting cells in situ. In others, gene transfer is performed *ex vivo* as part of preparing an anticancer vaccine. We discuss the underlying approach, relative success, and clinical application of various cancer immunogene therapy strategies, paying particular attention to immunogene therapy vaccines. Large numbers of preclinical studies have been reported, but only scattered clinical trial results have appeared in the literature. Although very successful preclinically, the ideal cancer immunogene therapy approach remains to be determined and will likely vary with tumor type. Clinical impact may be improved in the future as treatment protocols are refined.

Introduction

Although significant advances have been made for some subtypes, many cancers remain resistant to conventional therapy. Immunotherapy, stimulating the immune system to attack tumor cells, has long been investigated as an alternative to conventional therapy. It is attractive as the specificity of the immune system gives it the potential to target tumor cells while leaving normal cells unharmed. However, many early attempts to apply immunotherapy to the immune system were unrewarding or showed only modest benefits. Most of these early studies relied on vaccination with preparations derived from either autologous or allogeneic tumor cells, sometimes supplemented by nonspecific immunoadjuvants such as BCG [31, 42, 43]. While these approaches represented the state of the art when they were employed, there has been an explosion of knowledge concerning both molecular biology and basic immunology since then. This has led to a renaissance in cancer immunotherapy. In this review, we discuss one aspect of this renaissance: immunogene therapy.

Cancer immunogene therapy can be defined as genetically manipulating human cells in order to stimulate antitumor immunity. Many methods are available for genetic manipulation, including viral-vector-mediated gene transfer (e.g. retrovirus, adenovirus, adenoassociated virus, Canary-Pox-based virus), nonviral-vector-mediated gene transfer (e.g. naked DNA, liposomes, biolistics, polymer gene delivery systems, protein gene delivery systems), and antisense oligonucleotide strategies. These have been reviewed elsewhere [53, 62, 83]. Cancer immunogene

Copyright © 2003 National Science Council, ROC and S. Karger AG, Basel

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2003 National Science Council, ROC
S. Karger AG, Basel
1021-7770/03/0101-0037\$19.50/0
Accessible online at:
www.karger.com/jbs

Dr. I.F. Parney
400 Parnassus Avenue, Room A808, Neuro-Oncology Service
Department of Neurological Surgery, University of California
San Francisco, CA 94143-0372 (USA)
Tel. +1 415 353 2302, Fax +1 415 353 2167, E-Mail parneyi@neurosurg.ucsf.edu

therapy can involve transferring proinflammatory genes, inhibiting immunosuppressive gene expression, or transferring tumor antigen genes to antigen-presenting cells [47, 48, 59, 77]. Genetic manipulation may occur either *in situ* or *ex vivo*. These strategies can be either preventative or therapeutic [39]. Immunogene therapy strategies are summarized in figure 1.

Preclinical Studies

Transferring proinflammatory genes to tumor cells to try and create a microenvironment conducive to stimulating antitumor immune responses is, by far, the most common cancer immunogene therapy strategy. One of the earliest approaches studied was transferring class I major histocompatibility complex (MHC) genes to tumors known to be class I MHC deficient, such as melanomas [21, 45]. Even though these MHC genes were allogeneic, this may have stimulated antitumor immunity against cells expressing tumor-associated antigens in the context of their own MHC molecules [45]. Much subsequent work has focussed on transferring genes encoding proinflammatory cytokines. One of the earliest cytokine genes to show promise in this regard was interleukin-2 (IL-2) [20, 24, 58]. In many ways, these early IL-2 immunogene therapy studies came about as a means to maintain the benefits but avoid the side effects associated with systemic IL-2 therapy in cancer patients [57, 60].

These early efforts were refined over time. In a seminal work, Dranoff et al. [17] found that vaccinating with irradiated tumor cells transduced with granulocyte-macrophage colony-stimulating factor (GM-CSF) induced specific and long-lasting antitumor immunity and in a number of animal cancer models the effects of GM-CSF effects were more potent than those of several other cytokines. It has been postulated that these effects may be due to the ability of GM-CSF to induce Th1 immune responses and stimulate dendritic cells [29, 38]. Subsequent to the pioneering work of Dranoff et al., other groups have demonstrated efficacy for GM-CSF immunogene therapy in many animal and human tumor preclinical models [38, 51, 54, 61, 81]. Other cytokine genes have also yielded impressive preclinical results. IL-12 immunogene therapy has been particularly effective in animal tumor models [72, 82]. This is thought to be due to the potent ability of IL-2 to stimulate Th1 responses [23, 63].

In addition to proinflammatory cytokines, T cell co-stimulatory molecules (such as B7-1 and B7-2) have been popular targets in immunogene therapy studies [7, 30, 54,

80]. Additional B7 molecule binding (through CD28) is necessary to prevent anergy when T cells recognize peptides in the context of MHC molecules [10]. Whether B7-1 or B7-2 is a better choice to stimulate antitumor immunity remains controversial, and both molecules have shown benefit in preclinical studies [3, 28].

Immunogene therapy is not limited to proinflammatory gene transfer. Several antisense strategies designed to inhibit immunosuppressive gene expression in tumor cells have been studied. Vaccination with tumor cells treated with antisense genes or oligonucleotides directed against insulin-like growth factor-1 (IGF-1) and transforming growth factor- β (TGF- β) have resulted in immune-mediated tumor regression in a number of animal models [19, 35, 40, 75]. TGF- β is a known immunosuppressive cytokine [22] and promoting antitumor immunity by blocking TGF- β expression makes logical sense. The impact of IGF-1 on the immune system is more cryptic, but blocking IGF-1 expression appears to result in increased class I MHC and B7-1 expression [35, 74]. Interestingly, efforts are now being made to combine antisense therapies with proinflammatory gene transfer [15, 40]. This may lead to even further antitumor immune stimulation.

Clinical Studies

Although immunogene therapy is still in its relative infancy, some clinical results are now available. The earliest clinical trial results were, not surprisingly, based on the first strategies examined in preclinical models. For example, direct intratumoral HLA-B7 (MHC class I) gene transfer via liposome/plasmid DNA complex injection has been studied relatively extensively in phase I clinical trials in metastatic melanoma, colonic adenocarcinoma, and renal cell carcinoma patients [11, 45, 55, 69]. All of these trials demonstrated safety and most included at least some patients that appeared to have clinical responses to treatment. One study was able to document *in vitro* evidence for specific antitumor activity with cytotoxic T lymphocyte (CTL) assays in 2 patients (of 5 receiving treatment) where autologous tumor cell lines could be established [45]. In one phase II trial, 52 patients with metastatic melanoma received this treatment [70]. The injected lesion regressed in 18% and a partial systemic response was seen in 4%. A further 18% had objectively stable disease for at least 11 weeks. This study did not attempt to document antitumor immunity *per se* and was not able to identify clinical factors associated with response to treatment.

Several IL-2 immunogene therapy case reports and clinical trials have been published. In 1995, Sobol et al. [66] reported a complete response to IL-2 immunogene therapy in a glioblastoma multiforme patient who had received concurrent stereotactic radiosurgery. They demonstrated increased antitumor CTL activity in this patient after vaccination. IL-2 immunogene therapy has led to similarly measurable antitumor immune responses (with some clinical response) in melanoma and renal cell carcinoma patients [9, 78]. In 1999, Sobol et al. [67] followed up their initial report with a phase I immunogene therapy study in colorectal cancer patients using autologous irradiated tumor cells and fibroblasts engineered to secrete IL-2. They demonstrated that this treatment was safe and was associated with an increased frequency of antitumor cytotoxic T cell precursors in peripheral blood. One patient (of 10 treated) had objectively stable disease for 3 months, but all others had progressive disease despite treatment.

In another trial, Trudel et al. [76] evaluated IL-2 immunogene therapy in 8 multiple myeloma patients. Autologous plasma cells (i.e. tumor cells) were engineered to express IL-2 by adenoviral gene transfer. Vaccinations with these irradiated cells were well tolerated, but did not result in measurable increases in antitumor immunity or in clinical responses. The same group has published more promising results with direct injection of adenoviral encoding IL-2 into subcutaneous deposits of metastatic melanoma and breast carcinoma [68]. This protocol was also well tolerated, but produced local inflammation in 60%, incomplete local tumor regression in 24%, and evidence for CD3- and CD8-positive lymphocyte infiltration into tumors following injection. No partial or complete systemic responses were noted.

There are fewer clinical trial results for other immunogene therapy strategies. Vaccination with irradiated autologous GM-CSF-transduced tumor cells was first reported by Ellem et al. [18] in a patient with metastatic melanoma in 1997. This patient tolerated treatment well (although he/she did develop increased cerebral edema around brain metastases), had strong laboratory evidence of enhanced antitumor immunity, and had a partial clinical response. GM-CSF immunogene therapy phase I clinical trials have now been reported for renal cell carcinoma, metastatic melanoma, and prostate carcinoma [14, 56, 64, 65]. These trials have demonstrated safety, with toxicities being limited to erythema and pruritus at vaccination sites. Treatment resulted in delayed-type hypersensitivity conversion to challenge with irradiated, autologous tumor cells, suggesting that T-cell-mediated immune responses

were generated. Furthermore, a distinct pattern of inflammatory cell infiltrates (T cells, dendritic cells, macrophages, and eosinophils) was seen at vaccination sites. Clinical responses have been less conclusive, but one study (published in abstract form) reported 20% of melanoma patients receiving three vaccinations were still alive at 3–5 years [14].

Clinical trial results for other immunogene therapy strategies are even less common. A Medline search failed to document any antisense immunogene therapy clinical trials to date. Only one IL-12 immunogene therapy clinical trial has been published [71]. In this trial, 6 melanoma patients received vaccinations of irradiated autologous tumor cells modified to express IL-12. Minimal toxicity was seen. Three patients had disease stabilization and one had regression of some skin lesions. Clear evidence of increased antitumor immune activity was not seen. Our own pilot clinical trial of combined B7-2/GM-CSF immunogene therapy for gliomas and melanomas appears to be the first clinical study available for T cell costimulatory molecule gene transfer [50]. This trial demonstrated safety. We demonstrated clear increases in cytotoxic T cell activity versus autologous tumor after vaccination (at least for melanomas). Three of 6 treated patients had prolonged periods of objectively stable disease.

Problems and Solutions

The first immunogene therapy clinical studies have made a number of issues apparent. The first is that most reports have not shown clear clinical improvement. In interpreting these trials, it must be remembered that phase I and II trials are restricted to the sickest patients. These patients have already failed standard therapies. It may be unrealistic to expect any treatment to have a significant impact on the clinical course of end-stage patients. Therefore, the lack of major clinical responses to pro-inflammatory gene transfer so far is not surprising. As long as immunogene therapy trials are limited to end stage patients, clinical response cannot be used as a major indicator of a treatment strategies potential benefit [16]. Like other cancer treatments, evaluation of clinical efficacy will have to wait until phase III trials can be performed with patients at an earlier stage of their disease.

With this in mind, what criteria can be used to decide what small phase I/II trials show sufficient promise to warrant further evaluation with phase III trials? Clearly, safety is paramount. Strategies resulting in significant

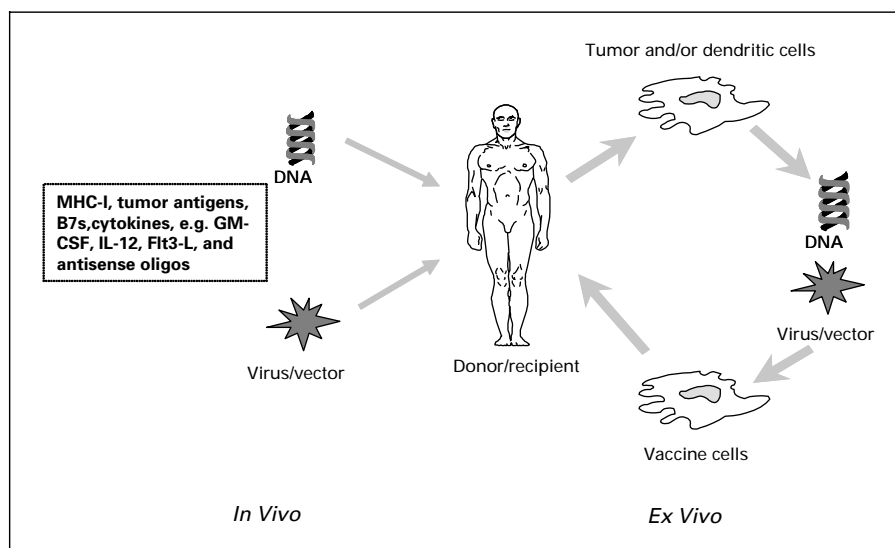


Fig. 1. Schematic cancer immunogene therapy overview showing possible therapeutic genes, gene transfer methods, and gene delivery (ex vivo, in situ) options.

treatment-related toxicity are not good candidates for further testing, but toxicities have been relatively minor to date. In this situation, laboratory confirmation of increased antitumor immunity after treatment may be the most important indicator that a particular proinflammatory gene transfer strategy warrants further clinical testing.

Unfortunately, there is no consensus on what laboratory assays represent a true clinical antitumor immune response [16]. Intuitively, tumor-specific T cell cytotoxicity seems a clear indicator of antitumor immunity. However, post-treatment increases in CTL activity versus autologous tumor have been sporadic in clinical trials to date [45, 50, 66]. In several studies, CTL assays were limited due to technical difficulties in culturing sufficient target, stimulator, and effector cells for a given patient [50, 76]. Other assays have been investigated. Adoptive T cell transfer studies have suggested that tumor-induced cytokine production by T cells may be an effective predictor of effective immune responses [5, 46]. Peptide/MHC tetramers have recently proven sensitive tools for detecting T-cell-specific responses to specific antigens [2]. Unfortunately, peptide/MHC tetramers require knowledge of specific tumor antigens and do not differentiate activated from anergic T cells [36]. These limitations may be overcome by focussing instead on T cell interferon- γ (IFN- γ) production through IFN- γ capture assays [8]. Incorporating these refined T cell functional assays will no doubt improve the sensitivity of future immunogene therapy trials to detect increased antitumor immunity.

Notwithstanding the above discussion, it could be argued that the failure to demonstrate clear clinical and immunological responses to proinflammatory gene transfer in cancer patients simply reflects an inability of current strategies to stimulate strong antitumor immunity. In a recent insightful review, Parmiani et al. [49] suggested a number of ways to improve the efficacy of future immunogene therapy trials. In order to achieve a maximal immune stimulus, a larger amount of tumor antigen must be provided. Parmiani et al. suggest that this may be best achieved by using large numbers of tumor cells preselected for high expression of known tumor antigens. Alternatively, tumor antigen genes may be transduced/transfected directly. This second strategy has already been investigated at a preclinical stage with promising results by Wan et al. [79]. These authors also advocate introducing genes encoding T cell costimulatory molecules in addition to proinflammatory cytokines. This strategy has also been investigated extensively preclinically [3] and our own study represents the first clinical report combining cytokine and T cell costimulatory molecule immunogene therapy [50]. Finally, Parmiani et al. suggest improvement is needed to increase the amount of cytokine released locally by irradiated cells [1]. They further advocate co-administering adjuvant cytokines (IL-2, IL-12) systemically in order to expand the T cell pool activated by vaccines.

Another issue has been made clear by early immunogene therapy clinical trials. While preparing vaccines from established tumor cell lines in animal tumor models is relatively straightforward, preparing vaccines from

operative specimens for a given patient can be difficult. This reflects difficulties in establishing and genetically modifying rapidly growing cell cultures from any given tumor [32, 52]. If immunogene therapy is to become a viable treatment option for the majority of cancer patients, these problems need to be circumvented. One option may be to prepare immunogene therapy vaccines from allogeneic tumor cell lines. Despite concerns that resulting allo-MHC responses would interfere with antitumor immunity, promising results have been seen in pre-clinical models [4, 6, 12, 73]. Indeed, Jaffee et al. [33] have now reported results from a phase I clinical trial using allogeneic irradiated GM-CSF-secreting pancreatic carcinoma cells as a vaccine in 14 patients with pancreatic carcinoma. This approach appeared safe in these patients. Furthermore, patients who received the highest dose per vaccine ($>10^8$ cells) showed a trend to increased postvaccination delayed tumor hypersensitivity responses against autologous tumor. This apparent dose response underlies the importance of using larger cell numbers for each vaccine [49], something which is often difficult to achieve with autologous tumor cells [50, 52]. If effective, using allogeneic cell lines would greatly simplify vaccine development, characterization, and safety testing.

There are other cancer immunogene therapy options that avoid autologous tumor cell culture in addition to allogeneic tumor cell vaccines. For example, more easily cultured fibroblasts can be used as carrier cells for immunostimulatory genes and mixed with fresh autologous tumor cell suspensions [25, 26]. This has already been examined in a clinical trial of IL-2 immunogene therapy in colorectal carcinoma with promising results [67]. Im-

munogene therapy can also be combined with dendritic cell therapy, another popular immunotherapeutic approach [27, 37, 41]. Immunomodulatory genes and/or tumor antigen genes may be transferred to professional antigen-presenting cells such as dendritic cells, combining the strengths of two immunotherapeutic approaches and avoiding the need for autologous tumor cell culture [34, 44, 79]. Finally, focussing on in situ gene transfer methods may avoid cell culture altogether. This last approach is attractive and has been used in some clinical trials [68, 70]. However, it is limited both by physical access to tumors and by low levels of gene transfer achieved in situ to date [13].

Conclusions

Numerous preclinical studies have made the potential of cancer immunogene therapy clear. Despite this, early clinical trials have yet to replicate these impressive pre-clinical results. This may partly reflect the nature of conducting clinical trials with end-stage patients, uncertainty concerning T cell functional assays, and pre-clinical reliance on murine models that may not adequately reflect human immune parameters. Avenues exist to improve the potency of immunogene therapy, but protocols must be streamlined if this strategy is to become universally available. Despite these concerns, immunogene therapy remains a promising modality for cancer treatment. As early clinical trials lead to refinements, significant immunological and clinical benefits may be seen.

References

- Allione A, Consalvo M, Nanni P, Lollini PL, Cavallo F, Giovarelli M, Forni M, Gulino A, Colombo MP, Dellabona P, Hock H, Blankenstein T, Rosenthal FM, Gansbacher B, Colombo MC, Musso T, Gusella L, Forni G. Immunizing and curative potential of replicating and non-replicating murine mammary adenocarcinoma cells engineered with interleukin (IL)-2, IL-4, IL-6, IL-7, IL-10, TNF-alpha, GM-CSF, and IFN-gamma or admixed with conventional adjuvants. *Cancer Res* 54:6022-6026;1994.
- Altman J, Moss P, Goulder P, Barouch D, McHeyzer-Williams M, Bell J, McMichael A, Davis M. Direct visualization and phenotypic analysis of virus-specific T-lymphocytes in HIV-infected individuals. *Science* 274:94-96; 1996.
- Antonia SJ. B7-1 gene-modified tumor cell vaccines. *Curr Opin Molec Therap* 1:50-56;1999.
- Arienti F, Sule-Suso J, Melani C, Maccalli C, Belli F, Illeni MT, Anichini A, Cascinelli N, Colombo MP, Parmiani G. Interleukin-2 gene-transduced human melanoma cells efficiently stimulate MHC-unrestricted and MHC-restricted autologous lymphocytes. *Hum Gene Ther* 5:1139-1150;1994.
- Aruga A, Shu SY, nd Chang AE. Tumor-specific granulocyte-macrophage colony-stimulating factor and interferon gamma secretion is associated with in vivo therapeutic efficacy of activated tumor-draining lymph node cells. *Cancer Immunol Immunother* 41:317-324; 1995.
- Ashley DM, Sampson JH, Archer GE, Batra SK, Bigner DD, Hale LP. A genetically modified allogeneic cellular vaccine generates MHC class I-restricted cytotoxic responses against tumor-associated antigens and protects against CNS tumors in vivo. *J Neuroimmunol* 78:34-46;1997.
- Baskar S, Glimcher L, Nabavi N, Jones R, Ostrand-Rosenberg S. Major histocompatibility complex class II + B7-1+ tumor cells are potent vaccines for stimulating tumor rejection in tumor-bearing mice. *J Exp Med* 181:619-629;1995.
- Becker C, Pohla H, Frankenberger B, Schuler T, Assenmacher M, Schendel DJ, Blankenstein T. Adoptive tumor therapy with T lymphocytes enriched through an interferon-gamma capture assay. *Nature Med* 7:1159-1162;2001.

- 9 Belli F, Arienti F, Sule-Suso J, Clemente C, Mascheroni L, Cattelan A, Santantonio C, Galino GF, Melani C, Rao S, Colombo MP, Maio M, Cascinelli N, Parmiani G, Sanantonio C. Active immunization of metastatic melanoma patients with interleukin-2 transduced allogeneic melanoma cells: Evaluation of efficacy and tolerability. *Cancer Immunol Immunother* 44:197-203;1997.
- 10 Chen C, Nabavi N. In vitro induction of T cell anergy by blocking B7 and early T cell costimulatory molecule ETC-1/B7-2. *Immunity* 1:147-154;1994.
- 11 Clark PR, Stopeck AT, Ferrari M, Parker SE, Hersh EM. Studies of direct intratumoral gene transfer using cationic lipid-complexed plasmid DNA. *Cancer Gene Ther* 7:853-860;2000.
- 12 Crowley NJ, Slingluff CL, Darrow TL, Seigler HF. Generation of human autologous melanoma-specific cytotoxic T cells using HLA-A2-matched allogeneic melanomas. *Cancer Res* 50:492-498;1990.
- 13 Curiel DT, Gerritsen WR, Krul MRL. Progress in cancer gene therapy. *Cancer Gene Ther* 7:1197-1199;2000.
- 14 De Gast GC, Gallee MPW, Spits H. Immunological, pathological, and long-term clinical data of vaccination with autologous granulocyte-macrophage colony-stimulating factor-transduced tumor cells in metastatic melanoma. *Cancer Gene Ther* 7:1204;2000.
- 15 Dorigo O, Shawler DL, Royston I, Sobol RE, Berek JS, Fakhrai H. Combination of transforming growth factor-beta antisense and interleukin-2 gene therapy in the murine ovarian teratoma model. *Gynecol Oncol* 71:204-210;1998.
- 16 Dranoff G. Interpreting cancer vaccine clinical trials. *J Gene Med* 1:80-83;1999.
- 17 Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Haurada H, Pardoll D, Mulligan R. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific and long lasting anti-tumor immunity. *Proc Natl Acad Sci USA* 90:3539-3543;1993.
- 18 Ellem KAO, O'Rourke MGE, Johnson GR, Parry G, Misko I S, Schmidt CW, Parsons PG, Burrows SR, Cross S, Fell A, Li CL, Bell JR, Dubois PJ, Moss DJ, Good MF, Kelso A, Cohen LK, Dranoff G, Mulligan RC. A case report: Immune responses and clinical course of the first human use of granulocyte-macrophage colony-stimulating factor-transduced autologous melanoma cells for immunotherapy. *Cancer Immunol Immunother* 44:10-20;1997.
- 19 Fakhrai H, Dorigo O, Shawler DL, Lin H, Mercola D, Black KL, Royston I, Sobol RE. Eradication of established intracranial rat gliomas by transforming growth factor beta antisense gene therapy. *Proc Natl Acad Sci USA* 93:2909-2914;1996.
- 20 Fearon ER, Pardoll DM, Itaya T, Golumbek P, Levitsky HI, Simons JW, Karasuyama H, Vogelstein B, Frost P. Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* 60:397-403;1990.
- 21 Ferrone S, Marincola FM. Loss of HLA class I antigens by melanoma cells: Molecular mechanisms, functional significance, and clinical relevance. *Immunol Today* 16:487-494;1995.
- 22 Fontana A, Constam DB, Frei K, Malipiero U, Pfister HW. Modulation of the immune response by transforming growth factor beta. *Int Arch Allergy Immunol* 99:1-7;1992.
- 23 Gajewski TF, Renaud JC, Van Pel A, Boon T. Costimulation with B7-1, IL-6, and IL-12 is sufficient for primary generation of murine antitumor cytolytic T lymphocytes in vitro. *J Immunol* 154:5637-5648;1995.
- 24 Gansbacher B, Zier K, Daniels B, Cronin K, Bannerji R, Gilboa E. Interleukin 2 gene transfer into tumor cells abrogates tumorigenicity and induces protective immunity. *J Exp Med* 172:1217-1224;1990.
- 25 Glick RP, Lichtor T, Kim TS, Ilangovan S, Cohen EP. Fibroblasts genetically engineered to secrete cytokines suppress tumor growth and induce antitumor immunity to a murine glioma in vivo. *Neurosurgery* 36:548-555;1995.
- 26 Glick RP, Lichtor T, Mogharbel A, Taylor CA, Cohen EP. Intracerebral versus subcutaneous immunization with allogeneic fibroblasts genetically engineered to secrete interleukin-2 in the treatment of central nervous system glioma and melanoma. *Neurosurgery* 41:898-907;1997.
- 27 Grabbe S, Beissert S, Schwarz T, Granstein RD. Dendritic cells as initiators of tumor immune responses: A possible strategy for tumor immunotherapy? *Immunol Today* 16:117-121;1995.
- 28 Greenfield EA, Nguyen KA, Kuchroo VK. CD28/B7 costimulation: A review. *Crit Rev Immunol* 18:389-418;1998.
- 29 Hanada K, Tsunoda R, Hamada H. GM-CSF-induced in vivo expansion of splenic dendritic cells and their strong costimulation activity. *J Leukocyte Biol* 60:181-190;1996.
- 30 Hodge J, Abrams S, Schlom J, Kantor J. Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules. *Cancer Res* 54:5552-5555;1994.
- 31 Hoover HC, Surdyke MG, Brandhorst JS. Five year follow-up of a controlled trial of active immunotherapy in colorectal cancer. *Proc Am Soc Clin Oncol* 9:106;1990.
- 32 Jaffee E, Dranoff G, Cohen L, Hauda K, Clift S, Marshall F, Mulligan R, Pardoll D. High efficiency gene transfer into primary human tumor explants without cell selection. *Cancer Res* 53:2221-2226;1993.
- 33 Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, Lillemore KD, O'Reilly S, Abrams RA, Pardoll DM, Cameron JL, Yeo CJ. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: A phase I trial of safety and immune activation. *J Clin Oncol* 19:145-156;2001.
- 34 Kirk CJ, Mule JJ. Gene-modified dendritic cells for use in tumor vaccines. *Hum Gene Ther* 11:797-806;2000.
- 35 Lafarge-Frayssinet C, Duc HT, Frayssinet C, Sarasin A, Anthony D, Guo Y, Trojan J. Antisense insulin-like growth factor-1 transferred into a rat hepatoma cell line inhibits tumorigenesis by modulating major histocompatibility complex 1 cell surface expression. *Cancer Gene Ther* 4:276-285;1997.
- 36 Lee PP, Yee C, Savage PA, Fong L, Brockstedt D, Weber JS, Johnson D, Swetter S, Thompson J, Greenberg PD, Roederer M, Davis MM. Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients. *Nature Med* 5:677-685;1999.
- 37 Liao LM, Black KL, Prins RM, Sykes SN, DiPatre P-L, Cloughesy TF, Becker DP, Bronstein JM. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *J Neurosurg* 90:1115-1124;1999.
- 38 Lim M, Simons JW. Emerging concepts in GM-CSF gene-transduced tumor vaccines for human prostate cancer. *Curr Opin Mol Ther* 1:64-71;1999.
- 39 Ling M, Kanayama M, Roden R, Wu TC. Preventive and therapeutic vaccines for human papillomavirus-associated cervical cancers. *J Biomed Sci* 7:341-356;2000.
- 40 Liu Y, Wang H, Zhao J, Ma J, Wei L, Wu S, Xie T, Shen F, Trojan J, Habib N, Anthony DD, Wu M, Guo Y. Enhancement of immunogenicity of tumor cells by cotransfection with genes encoding antisense insulin-like growth factor-1 and B7-1 molecules. *Cancer Gene Ther* 7:456-465;2000.
- 41 Lotze MT. Getting to the source: Dendritic cells as therapeutic reagents for the treatment of patients with cancer. *Ann Surg* 226:1-5;1997.
- 42 Mahaley MS, Bigner DD, Dudka LF, Wilds PR, Williams DH, Bouldin TW, Whitaker JN, Bynum JM. Immunobiology of primary intracranial tumors. 7. Active immunization of patients with anaplastic human glioma cells: A pilot study. *J Neurosurg* 59:201-207;1983.
- 43 McCune CS, O'Donnell RW, Marquis DM, Sahasrabudhe DM. Renal cell carcinoma treated by vaccines for active specific immunotherapy: Correlation of survival with skin testing by autologous tumor cells. *Cancer Immunol Immunother* 32:62-66;1990.
- 44 Mitchell DA, Nair SK. RNA transfected dendritic cells as cancer vaccines. *Curr Opin Mol Ther* 2:176-181;2000.
- 45 Nabel GJ, Nabel EG, Yang Z-Y, Fox BA, Plautz GE, Gao X, Huang L, Shu S, Gordon D, Chang AE. Direct gene transfer with DNA-liposome complexes in melanoma: Expression, biologic activity, and lack of toxicity in humans. *Proc Natl Acad Sci USA* 90:11307-11311;1993.
- 46 Nagoshi M, Goedegebuure P, Burger U, Sadanaga N, Chang M, Eberlein T. Successful adoptive cellular immunotherapy is dependent on induction of a host immune response triggered by cytokine (interferon-gamma and granulocyte-macrophage colony-stimulating factor) producing donor tumor-infiltrating lymphocytes. *J Immunol* 160:334-344;1998.

- 47 Ockert D, Schmitz M, Hampl M, Reiber EP. Advances in cancer immunotherapy. *Immunol Today* 20:63–65;1999.
- 48 Pardoll DM. Paracrine cytokine adjuvants in cancer immunotherapy. *Annu Rev Immunol* 13:399–415;1995.
- 49 Parmiani G, Rodolfo M, Melani C. Immunological gene therapy with ex vivo gene-modified tumor cells: A critique and a reappraisal. *Hum Gene Ther* 11:1269–1275;2000.
- 50 Parney IF, Chang L-J, Farr-Jones MA, Vandenhoven H, Urlacher V, Kane K, Hao C, Gainer A, Solano E, Smylie M, Fulton D, Urtasun R, Petruk KC. Combination B7-2 and GM-CSF immunogene therapy for gliomas and melanomas: Pilot clinical trial. Submitted.
- 51 Parney IF, Farr-Jones MA, Kane K, Chang L-J, Petruk KC. Human autologous in vitro models of glioma immunogene therapy using B7-2, GM-CSF, and IL12. *Can J Neurol Sci*, in press.
- 52 Parney IF, Farr-Jones MA, Petruk KC. Improved technique for establishing short term human brain tumor cultures. *J Neurooncol* 43: 1–10;1999.
- 53 Parney IF, Hao C, Petruk KC. Glioma immunology and immunotherapy: A review. *Neurosurgery* 46:778–792;2000.
- 54 Parney IF, Petruk KC, Zhang C, Farr-Jones MA, Sykes DB, Chang L-J. GM-CSF and B7-2 combination immunogene therapy in an allogeneic hu-PBL-SCID/beige mouse – human glioblastoma multiforme model. *Hum Gene Ther* 8:1073–1085;1997.
- 55 Rini BI, Selk LM, Vogelzang NJ. Phase I study of direct intralesional gene transfer of HLA-B7 into metastatic renal carcinoma lesions. *Clin Cancer Res* 5:2766–2772;1999.
- 56 Robert Soiffer TL, Mihn M, Jung K, Rhuda C, Jan C, Schmollinger F, Hodi S, Liebster L, Lan P, Mentzer S, Singer S, Jenneth K, Cosimi B, Duda R, Sober A, Bhan A, Daley J, Neuberger D, Pa G. Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. *Proc Natl Acad Sci USA* 95:13141–13146;1998.
- 57 Rosenberg S, Abersold P, Cornetta K, Kand A, Morgar RA, Moen K, Karson EM, Lotze MT, Yang JC, Topalian SL. Gene transfer into humans – immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med* 323:570–578;1990.
- 58 Rosenberg S, Lotze M, Mule J. New approaches to the immunotherapy of cancer using interleukin-2. *Ann Intern Med* 108:853–864; 1988.
- 59 Rosenberg SA. A new era for cancer immunotherapy based on genes that encode tumor antigens. *Immunity* 10:281–287;1999.
- 60 Rosenberg SA, Lotze MD, Muul LM, Chang AE, Avis FP, Leitman S, Linehan WM, Robertson CN, Lee RE, Rubin JT. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N Engl J Med* 16:889–897;1987.
- 61 Sampson JH, Archer GE, Ashley DM, Fuchs HE, Hale LP, Dranoff G, Bigner DD. Subcutaneous vaccination with irradiated, cytokine-producing tumor cells stimulates CD8+ cell-mediated immunity against tumors located in the ‘immunologically privileged’ central nervous system. *Proc Natl Acad Sci USA* 93: 10399–10404;1996.
- 62 Schatzlein AG. Non-viral vectors in cancer gene therapy: Principles and progress. *Anti-Cancer Drugs* 12:275–304;2001.
- 63 Scott P. IL-12: Initiation cytokine for cell-mediated immunity. *Science* 260:496–497; 1993.
- 64 Simons JW, Jaffee EM, Weber CE, Levitsky HI, Nelson WG, Carducci MA, Lazenby AJ, Cohen LK, Finn CC, Clift SM, Hauda KM, Beck LA, Leiferman KM, Owens AH, Piantadosi S, Dranoff G, Mulligan RC, Pardoll DM, Marshall FF. Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. *Cancer Res* 57: 1537–1546;1997.
- 65 Simons JW, Mikhak B, Chang J-F, De Marzo AM, Carducci MA, Lim M, Weber CE, Baccala AA, Goemann MA, Clift SM, Ando DG, Levitsky HI, Cohen LK, Sanda MG, Mulligan RC, Partin AW, Carter HB, Piantadosi S, Marshall FF, Nelson WG. Induction of immunity to prostate cancer antigens: Results of a clinical trial of vaccination with irradiated autologous prostate cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer. *Cancer Res* 59:195–204;1999.
- 66 Sobol RE, Fakhrai H, Shawler D, Gjerset R, Dorigo O, Carson C, Khaleghi T, Koziol J, Shiftan TA, Royston I. Interleukin-2 gene therapy in a patient with glioblastoma. *Gene Ther* 2:164–167;1995.
- 67 Sobol RE, Shawler DL, Carson C, Van Beveren C, Mercola D, Fakhrai H, Garrett MA, Barone R, Goldfarb P, Bartholomew RM, Brostoff S, Carlo DJ, Royston I, Gold DP. Interleukin 2 gene therapy of colorectal carcinoma with autologous irradiated tumor cells and genetically engineered fibroblasts: A phase I study. *Clin Cancer Res* 5:2359–2365;1999.
- 68 Stewart AK, Lassam NJ, Quirt IC, Bailey DJ, Rotstein LE, Krajden M, Dessureault S, Gallinger S, Cappe D, Wan Y, Addison CL, Moen RC, Gaudie J, Graham FL. Adenoviral-mediated gene delivery of IL-2 in metastatic breast cancer and melanoma: Results of a phase I clinical trial. *Gene Ther* 6:350–363;1999.
- 69 Stopeck AT, Hersh EM, Akporiaye ET, Harris DT, Grogan T, Unger E, Warneke J, Schluter SF, Stahl S. Phase I study of direct gene transfer of an allogeneic histocompatibility antigen, HLA-B7, in patients with metastatic melanoma. *J Clin Oncol* 15:341–349;1997.
- 70 Stopeck AT, Jones A, Hersh EM, Thompson JA, Finucane DM, Gutheil JC, Gonzalez R. Phase II study of direct intralesional gene transfer of allovectin-7, an HLA-B7/beta-2 microglobulin DNA-liposome complex, in patients with metastatic melanoma. *Clin Cancer Res* 7:2285–2291;2001.
- 71 Sun Y, Jurgovsky K, Moller P, Alijagic S, Dorbic T, Georgieva J, Wittig B, Schandendorf D. Vaccination with IL-12 gene-modified autologous melanoma cells: Preclinical results and a first clinical phase I study. *Gene Ther* 5:481–490;1998.
- 72 Tahara H, Lotze MT. Antitumor effects of interleukin-12 (IL-12): Applications for the immunotherapy and gene therapy of cancer. *Gene Ther* 2:96–106;1995.
- 73 Thomas MC, Greten TF, Pardoll DM, Jaffee EM. Enhanced tumor protection by granulocyte-macrophage colony-stimulating factor expression at the site of an allogeneic vaccine. *Hum Gene Ther* 9:835–843;1998.
- 74 Trojan J, Duc HT, Upegui-Gonzalez LC, Hor F, Guo Y, Anthony D, Ilan J. Presence of MHC-I and B-7 molecules in rat and human glioma cells expressing antisense IGF-I mRNA. *Neurosci Lett* 212:9–12;1996.
- 75 Trojan J, Johnson TR, Rudin SD, Ilan J, Tydocinski ML, Ilan J. Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin-like growth factor I-RNA. *Science* 259:94–96;1993.
- 76 Trudel S, Li Z, Dodgson C, Nanji S, Wan Y, Voralia M, Hitt M, Gaudie J, Graham FL, Stewart AK. Adenovector engineered interleukin-2 expressing autologous plasma cell vaccination after high-dose chemotherapy for multiple myeloma – a phase I study. *Leukemia* 15:846–854;2001.
- 77 Tuting T, Storkus WJ, Lotze MT. Gene-based strategies for immunotherapy of cancer. *J Mol Med* 75:478–491;1997.
- 78 Veelken H, Mackensen A, Lahn M, Kohler G, Becker D, Franke B, Brennscheidt U, Kulmburg P, Rosenthal FM, Keller H, Hasse J, Schultze-Seeman W, Farthmann EH, Mertelsmann R, Lindemann A. A phase I clinical study of autologous tumor cells plus interleukin-2 gene-transfected allogeneic fibroblasts as a vaccine in patients with cancer. *Int J Cancer* 70: 269–277;1997.
- 79 Wan Y, Emtage P, Zhu Q, Foley R, Pilon A, Roberts B, Gaudie J. Enhanced immune response to the melanoma antigen gp100 using recombinant adenovirus-transduced dendritic cells. *Cell Immunol* 198:131–138;1999.
- 80 Yang G, Hellstrom KE, Hellstrom I, Chen L. Antitumor immunity elicited by tumor cells transfected with B7-2, a second ligand for CD28/CTLA-4 costimulatory molecules. *J Immunol* 154:2794–2800;1995.
- 81 Yu JS, Burwick JA, Dranoff G, Breakefield XO. Gene therapy for metastatic brain tumors by vaccination with granulocyte-macrophage colony-stimulating factor-transduced tumor cells. *Hum Gene Ther* 8:1065–72;1997.
- 82 Zitvogel L, Tahara H, Robbins PD. Cancer immunotherapy of established tumors with IL-12: Effective delivery by genetically engineered fibroblasts. *J Immunol* 155:1393–1403;1995.
- 83 Zlokovic BV, Apuzzo MLJ. Cellular and molecular neurosurgery: Pathways from concept to reality. 2. Vector systems and delivery methodologies for gene therapy of the central nervous system. *Neurosurgery* 40:805–812;1997.

Copyright: S. Karger AG, Basel 2003. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.