

Cancer vaccine for brain tumors and brain tumor antigens

Review Article

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Key Words: glioma, CNS, HSV, G207, DC, tumor antigen

Abbreviations: antigen-presenting cells, (APCs); blood-brain barrier, (BBB); Cancer-testis, (CT); central nervous system, (CNS); cytotoxic T lymphocytes, (CTLs); dendritic cells, (DCs); herpes simplex virus type-1, (HSV-1); high mobility group, (HMG); major histocompatibility complex, (MHC); natural killer, (NK); peripheral blood mononuclear cells, (PBMCs); PHD finger protein 3, (PHF3)

Received: 27 February 2004; Accepted: 15 March 2004; electronically published: March 2004

Summary

Although treatment modalities for malignant gliomas have advanced remarkably, the prognosis remains poor. This has led to an intensive search for effective treatment alternatives. Recently, T cells activated by antigens from brain tumors were shown to migrate across the blood-brain barrier into the central nervous system (CNS) and selectively attack brain tumors. Then, various vaccination strategies against cancer have been attempted to induce specific immune responses against gliomas in the body outside the CNS. Encouraging results of preclinical studies of cancer vaccines against CNS tumors have led to clinical trials of these vaccines for the treatment of patients with malignant gliomas. In this review, recent progress in the use of cancer vaccines for the treatment of malignant gliomas is described, followed by a description of brain tumor antigens recognized by the immune system.

I. Introduction

The gliomas are the most common malignant tumors of the brain, and extensive invasion into the surrounding normal brain tissue is often seen because of their infiltrating nature. Despite surgical and technological progress in the treatment of central nervous system (CNS) diseases, the prognosis of patients with malignant gliomas still remains poor. With the current treatment modalities for malignant gliomas, which consist of surgical resection followed by radiation therapy and/or chemotherapy, the median survival is still less than 1 year (Prados et al, 1992). Thus, the development of new therapeutic approaches for gliomas is essential.

Vaccination against cancer using either tumor cells or tumor antigens is an active immunotherapeutic strategy that induces and/or enhances anti-tumor immunity in the patient's body. This therapeutic strategy differs from passive immunotherapy, in which immune cells having antitumor activity, such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, are prepared *in vitro* and administered to cancer patients. Furthermore, specific immune responses against tumor antigens induced by cancer vaccines have been shown to be effective in the treatment of cancer patients.

The concept of the CNS being an "immune privileged site" was developed from classical studies, which showed that the brain is more permissive to transplantation of allografts than other organs of the body. In fact, antigen-presenting cells (APCs), such as dendritic cells (DCs), do not work efficiently within the CNS. Therefore, it would be theoretically difficult to present antigens within the CNS to the immune system. However, it was demonstrated in some studies that activated T cells can migrate across the blood-brain barrier (BBB) and infiltrate the brain (Wekerle, 1993; Fabry et al, 1994). Therefore, immunotherapy has been targeted at inducing specific immune responses against brain tumors within the body outside the CNS. Recently, clinical trials of a cancer vaccine containing DCs were performed in glioma patients and the vaccine was reported to be effective in some patients (Yu et al, 2001). Although the effectiveness of this DC therapy still needs to be evaluated in future clinical trials, including Phase II trials, it is considered significant that no marked adverse effects were recognized and the safety of the vaccine for the induction of tumor-specific immunity in glioma patients has been proven. In this review, the recent advances in cancer vaccine therapy

against gliomas are described, followed by a discussion on the glioma antigens.

II. Cancer vaccines using tumor cells

One of the rational strategies for the treatment of cancer is the stimulation of specific immune responses against the tumor antigens *in vivo*. Successful cancer vaccination to induce immunity against tumor antigens could lead to tumor cell destruction and prolong the survival of cancer patients. A variety of strategies have been used to enhance the antigenicity of the tumor cells, including genetically modifying the cells to secrete cytokines involved in antitumor immunity, and initiating a viral infection for the 'xenogenization' of the tumor cells. A major advantage of these methods is that identification of the tumor antigens is not required, and theoretically, immunization with multiple tumor antigens, including tumor antigens specific for individual tumors, is possible.

A. Cancer vaccines using cytokines

Transduction of genes encoding cytokines into tumor cells has been shown to result in augmentation of the immunogenic properties of brain tumors. In a large preclinical study, irradiated B16 murine melanoma cells producing murine IL-2, IL-3, IL-4, IL-6, IFN- γ , or GM-CSF, were administered subcutaneously as a cancer vaccine against tumors of the brain (Sampson et al, 1996). Of the cytokine-based vaccines examined, the GM-CSF-producing cells were found to be the most effective for increasing the survival of mice with established brain tumors. A major concern with cytokine-based vaccines for CNS tumors is that they can potentially induce cerebral edema, because a high dose of IL-2 administered systemically can cause an increase in the vascular permeability, which in turn, could lead to cerebral edema (Merchant et al, 1990). In fact, severe cerebral edema was reported in animals injected intracranially with syngeneic cytokine-secreting cells (Tjuvajev et al, 1995). These findings indicate that subcutaneously administered cancer vaccines containing cytokines can be safe and effective in the treatment of CNS tumors.

While the promising preclinical results mentioned above prompted several clinical trials, to date, only isolated case reports have been published. In one case report, a patient with malignant glioma received subcutaneous (s.c.) immunization with autologous tumor cells and fibroblasts transduced with IL-2 (Sobol et al, 1995). Enhanced CD8⁺ CTL responses against the autologous tumor in peripheral blood mononuclear cells (PBMCs) were seen after the vaccination. The patient survived for 10 months after the first vaccination. In another case report, a patient with metastatic melanoma with brain metastasis received a vaccine of autologous melanoma cells transduced with GM-CSF (Ellem et al, 1997). In this patient, both increased anti-melanoma delayed-type hypersensitivity reactions and increased CTL responses against the tumor were seen. After the vaccination, axillary lymph node metastases regressed and an increase in cerebral edema surrounding the brain

metastasis was observed. These two reports showed the beneficial clinical effects of cytokine-based vaccines against CNS tumors. However, further study will be required to define the safety and efficacy of this therapeutic strategy.

B. Cancer vaccines using viruses

When a tumor was infected with a leukemia virus and transplanted into syngeneic rats, the tumor grew for a while but regressed subsequently (Pelner et al, 1958). Furthermore, when native tumor cells were transplanted into rats that had rejected the tumor, these tumor cells were eliminated. Based on this experimental evidence, the concept of tumor xenogenization by viruses was proposed. In fact, a number of clinical trials were performed between the 1950s and 1970s, in which patients with advanced malignancies were treated with lytic viruses (Moore, 1960; Asada, 1974). However, these trials were not well controlled and the results were highly variable. One critical problem that was observed in some cases was viral toxicity. In an attempt to overcome this problem, replication-conditional mutant viruses, such as the herpes simplex virus type-1 (HSV-1) mutant G207 (Mineta et al, 1995; Markert et al, 2000), and the adenovirus mutant ONYX-015 (Bischoff et al, 1996; Khuri et al, 2000; Nemunaitis et al, 2000), were developed. These viruses could replicate within the tumor and selectively destroy only the tumor cells, and had no local or systemic toxicity, because they failed to grow within normal tissues.

Using the conditionally replicating HSV-1 mutant G207, we developed an approach for the treatment of metastatic brain tumors using a combination of viral therapy with immunotherapy. G207 replicates selectively within tumor cells and causes tumor cell destruction without local or systemic toxicity (Toda et al, 1998). Furthermore, inoculation with G207 into tumors outside the CNS induces systemic immune responses against not only HSV, but also against the tumor antigens (Toda et al, 1999). However, the antitumor effect of inoculation with G207 into s.c. tumors as a cancer vaccine has been shown to be less effective against brain tumors than against liver or skin tumors, even though systemic immune responses to the tumor antigen were induced (Endo et al, 2002). Similarly, it has been reported that immunization with CT26 cells expressing the hemagglutination antigen of influenza virus produces systemic antitumor immunity in various tissues, but not in the brain (Schackert et al, 1989). These observations suggest that modification of the brain tumor and/or the immunological environment in the CNS is needed for effective immunotherapy of brain tumors. Thus, we developed an approach for the treatment of metastatic brain tumors using a combination of oncolytic viral therapy and a cancer vaccine using G207 (Toda, 2002, 2003).

An experimental model of brain metastasis was developed using immunocompetent mice harboring both intracranial (i.c.) and s.c. syngeneic tumors (Toda et al, 2002). Intratumoral injections of G207 into both the i.c. and s.c. tumors was associated with a significant antitumor effect on the metastatic brain tumors. This therapeutic effect was absent in athymic mice, indicating that it was

mediated by T cells. CTL responses against HSV as well as the tumor antigen were seen in mice given the combined treatment. These results suggest that with our strategy, in which both the metastatic brain tumor and the primary tumor outside the CNS are inoculated with G207, HSV-infected brain tumors may be eliminated by the combined effects of the direct oncolysis and the induced anti-HSV and anti-tumor T cells.

For the clinical application of this therapeutic approach, various host-virus interactions, particularly immune responses, need to first be considered. By adulthood, 60-90% of the human population is seropositive for HSV-1. Pre-existing and therapeutically elicited immune responses to the virus may cooperate to enhance the efficacy of the combined treatment. G207 is currently being used in a clinical trial for the treatment of recurrent glioma, and its safety has been proven (Martuza, 2000). This reassurance opens up the possibility of using G207 for the treatment of metastatic brain tumors.

C. Cancer vaccines using dendritic cells

DCs are the most potent APCs and are the only cells capable of priming naïve T-cells. Cancer vaccines containing DCs can be applied to cases in which specific tumor antigens are not used, such as tumor cell lysate, acid-eluted peptides from tumor cells, tumor cell-derived RNA, or fused DCs and tumor cells (Gong et al, 1997), as well as to those in which identified tumor antigens are used. Since single large-scale isolation and expansion of DCs in culture has become feasible, DC-based therapy has been successfully employed in several clinical trials for cancer, including melanoma (Thurner et al, 1999), renal cell carcinoma (Kugler et al, 2000), and prostate cancer (Lodge et al, 2000).

The first clinical trial using a DC-based cancer vaccine for glioma patients was reported by Yu et al, (2001). DCs pulsed with acid-eluted peptides from cultured autologous tumor cells were injected intradermally into the deltoid region three times biweekly. The DC vaccination was associated with significantly increased survival, and no significant side effects or autoimmune toxicity was observed. A clinical trial using fused DC and glioma cells also showed partial responses and no serious adverse effects (Kikuchi et al, 2001). To enhance the therapeutic efficacy, another clinical trial using these fused DC cells with recombinant human IL-12 is currently under way. So far, DC-based cancer vaccines have proved to be safe. However, it is difficult to precisely determine the efficacy of the DC vaccines, because of differences in the protocol design among studies. The efficacy of the vaccines, therefore, remains to be further evaluated in randomized and controlled clinical trials.

III. Cancer vaccines using identified tumor antigens

Since human tumor antigens recognized by T cells were identified, manipulation of immune responses against a tumor target became possible. Furthermore, T cells, which are capable of antigen-specific propagation and

have a memory mechanism, have been shown to be important in tumor rejection in not only mouse tumor models, but also in human cancer patients. Thus, T cells are considered to play a central role in cancer vaccine therapies. So far, mainly the major histocompatibility complex (MHC)-class I binding peptides that can activate CD8-positive CTLs have been identified as tumor antigens. However, it is also necessary to identify MHC-class II binding antigen peptides that activate helper T cells for the enhancement of antitumor immune responses.

For cancer vaccines using identified tumor antigens, various forms of antigens are available, including peptides, proteins, and genes, which are concurrently used with various adjuvants. The advantage of antigen peptides is the ease with which they can be synthesized and used. However, identification of peptides binding to a variety of MHCs is necessary. Although immunization with recombinant antigenic proteins has also been considered, quality control for clinical applications is not easy. In addition, clinical trials of cancer vaccines containing virus vectors expressing antigenic genes have been performed, based on the potential for their preparation in large quantities and induction of strong antitumor immune responses. However, the results of clinical trials have revealed certain problems, including the finding that repeated administration induces anti-virus neutralizing antibodies, which attenuates the immune response to tumor antigens. Thus, it is necessary to further evaluate which forms of tumor antigens would be appropriate for the induction of antitumor immune responses for successful treatment of cancer patients.

Since the identification, for the first time, of the MAGE-1 gene as a human tumor antigen recognized by CTLs (van der Bruggen et al, 1991), numerous human melanoma antigen genes have been identified. These antigens can be grossly classified into the following categories.

1. Cancer-testis (CT) antigens:

Cancer-testis (CT) antigens are a group of antigens that are expressed in various cancer tissues, but not in normal tissues except for the testis. The most representative of these antigens is the MAGE gene family (Boon et al, 1994). Expression of MHC molecules is extremely limited in cells of the reproductive system. Therefore, CTLs against CT antigens do not attack reproductive system cells and instead selectively attack cancer cells. Their expression patterns make them an ideal target, and in fact, a number of clinical trials are in progress.

2. Differentiation antigens

Differentiation antigens, whose expression is enhanced in tumors, although they are also expressed in the normal tissue of origin of the tumor, are recognized by CTLs. Such antigens as tyrosinase, MART-1, and gp100 that are expressed in both normal melanocytes and melanomas have been identified (Kawakami and Rosenberg, 1997). Because they are autoantigens, normal tissue can also be a target for the CTLs. These antigens are used in tumor vaccine therapies for the treatment of

melanomas, and their potential usefulness has been reported (Rosenberg, 1999).

3. Mutated antigens:

A multitude of gene mutations are accumulated within tumor cells. Mutant peptides derived from tumor-specific genetic mutations are recognized by CTLs as tumor antigens. The mutated peptides of CDK4 and β -catenin have been identified as CTL-recognized antigens (Kawakami and Rosenberg, 1997).

IV. Glioma antigens

Only a few reports have been published so far concerning glioma antigens that are recognized by the immune system. Until recently, cloning of tumor antigens was mainly performed using tumor-specific CTLs. However, an attempt has been made to identify glioma antigens by SEREX (serological identification of antigens by recombinant expression cloning) (Sahin et al, 1995, 1997, 2000; Fischer et al, 2001; Okada et al, 2001; Struss et al, 2001; Behrends et al, 2003; Ueda et al, 2004).

A. Human glioma antigens identified by the SEREX method

The TEGT gene was the first gene to be identified as a human glioma antigen by the SEREX method (Sahin et

al, 1995). The expression level of the TEGT gene, which is controlled during the process of sperm development, has been found to be high in gliomas. Although the number of analyzed cases is small, IgG responses in the serum against the TEGT antigen have been detected only in glioma patients, and not in patients with other cancers or healthy donors. Another report showed positive IgG responses in the serum to PHD finger protein 3 (PHF3) in 24 of 39 glioma patients, but not in 14 healthy donors (Struss et al, 2001). However, the reasons for the more frequent positive IgG responses to the PHF3 antigen in glioma patients than in healthy donors still remain to be clarified, because neither expression specificity nor genetic mutations have been recognized in relation to the PHF3 antigen.

The SEREX method fundamentally uses a combination of a cDNA library constructed from tumor tissue and the serum of the same patient (autoserum). However, in order to identify CT antigens, we performed a modified SEREX method using a testis cDNA library and the sera of multiple glioma patients (allosera) (**Figure 1**) and identified a glioma antigen, SOX6 (Ueda et al, 2004).

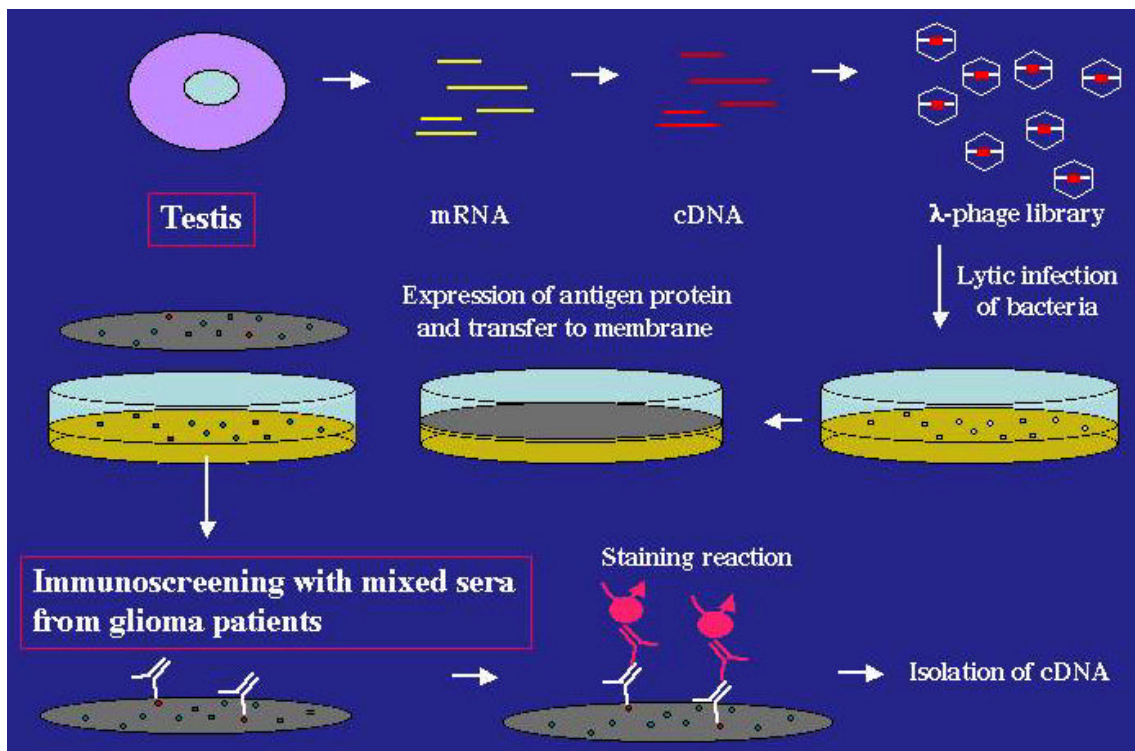


Figure 1. SEREX (serological identification of antigens by recombinant expression cloning) with multiple sera from glioma patients. A testis cDNA library was constructed with the Poly (A)⁺ RNA of adult human testis. The cDNA fragments were directionally inserted into the bacteriophage expression vector and packaged into phage particles. The phage vector was expressed in *E. coli*, and the colonies were transferred to nitrocellulose membranes. Mixed sera from four glioma patients were preabsorbed with transformed *E. coli* lysates and *E. coli* infected with the lambda phage, and prepared to a final dilution of 1:400 for each serum. The membranes were incubated in the diluted sera, followed by incubation with antihuman IgG (Fc) antibody. Positive plaques were picked from the plates and purified through secondary and tertiary rounds of additional screening.

SOX6, a Sry-related HMG (high mobility group) box-containing gene, is specifically expressed in the developing central nervous system and in the early stages of chondrogenesis in mouse embryos. Our study revealed that IgG antibodies against SOX6 were present in the sera of 12 out of 36 glioma patients (33.3%), 0 out of 14 patients with other brain disease (0%), and 1 out of 54 patients with other cancer (1.9%). No IgG responses to SOX6 were identified in the sera of any of 37 healthy individuals, except in one elderly female. RT-PCR and Northern blot analysis showed that the SOX6 gene was more highly expressed in glioma tissues than in normal adult tissues, except the testis. Furthermore, immunohistochemical analysis with anti-SOX6 antibody showed that SOX6-positive cells were detected in all the glioma tissues analyzed, but only a few positive cells were detected in nonneoplastic tissue samples from the cerebral cortex. These results indicate that the developmentally regulated transcription factor SOX6 is aberrantly expressed in gliomas and is specifically recognized by the IgGs in the sera of glioma patients.

The fact that glioma antigens recognized by IgG were identified in the patients' sera suggests antigen-specific activation of T cells. To apply them to tumor vaccine therapies in the future, it would be necessary to first determine whether these identified antigens can induce or enhance glioma-specific immunity.

B. Glioma antigens recognized by T lymphocytes

So far, no glioma-specific antigen recognized by T lymphocytes has been identified. However, it has been reported that SART1 and SART3, tumor rejection antigens against epithelial cancers, are expressed in gliomas, and that CTLs specific for the SART1 and SART3 antigens destroyed glioma cells (Imaizumi et al, 1999; Murayama et al, 2000).

We have tried to identify glioma antigens recognized by T lymphocytes by a method of induction of tumor-specific immune responses using HSV (Iizuka et al, 2004). We transplanted a mouse glioma cell line in syngeneic mice and administered HSV into the tumor tissue to induce CTLs specific for the mouse glioma cells. We then screened for antigenic genes using the established CTLs that specifically destroy gliomas in a MHC-restrictive fashion and identified a new mouse glioma antigen (unpublished data). The function of this molecule is not yet known, but sequence analysis revealed that genetic mutations exist in the gene isolated from the mouse glioma. A mutated peptide including one of these gene mutations has been shown to be recognized by the CTLs as a T cell epitope of a glioma antigen. We propose to analyze whether this antigen peptide can induce specific immune responses useful for the treatment of gliomas.

V. Conclusion

The encouraging results from preclinical studies of immunotherapy against brain tumors have led to clinical

trials of cancer vaccines for the treatment of malignant gliomas. So far, cancer vaccine strategies appear to be safe for the treatment of brain tumors and no severe side effects have been reported. Although further feasibility studies are required, the immunotherapeutic approach is a potent strategy for specifically targeting invasive malignant gliomas within normal brain tissue.

References

- Asada T. (1974) Treatment of human cancer with mumps virus. **Cancer** 34, 1907-1928
- Behrends U, Schneider I, Rossler S, et al. (2003) Novel tumor antigens identified by autologous antibody screening of childhood medulloblastoma cDNA libraries. **Int J Cancer** 106, 244-251
- Bischoff JR, Kim DH, Williams A, et al. (1996) An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. **Science** 274, 373-376
- Boon T, Cerottini JC, Van den Eynde B, et al. (1994) Tumor antigens recognized by T lymphocytes. **Annu Rev Immunol** 12, 337-365
- Ellem KA, O'Rourke MG, Johnson GR, et al. (1997) 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
- Endo T, Toda M, Watanabe M, et al. (2002) In situ cancer vaccination with a replication-conditional HSV for the treatment of liver metastasis of colon cancer. **Cancer Gene Ther** 9, 142-148
- Fabry Z, Raine CS, Hart MN. (1994) Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. **Immunol Today** 15, 218-224
- Fischer U, Hemmer D, Heckel D, et al. (2001) KUB3 amplification and overexpression in human gliomas. **Glia** 36, 1-10
- Gong J, Chen D, Kashiwaba M, et al. (1997) Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. **Nat Med** 3, 558-561
- Iizuka Y, Suzuki A, Kawakami Y, et al. (2004) Augmentation of Antitumor Immune Responses by Multiple Intratumoral Inoculations of Replication-Conditional HSV and Interleukin-12. **J Immunother** 27, 92-98
- Imaizumi T, Kuramoto T, Matsunaga K, et al. (1999) Expression of the tumor-rejection antigen SART1 in brain tumors. **Int J Cancer** 83, 760-764
- Kawakami Y, Rosenberg SA. (1997) Human tumor antigens recognized by T-cells. **Immunol Res** 16, 313-339
- Khuri FR, Nemunaitis J, Ganly I, et al. (2000) a controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. **Nat Med** 6, 879-885
- Kikuchi T, Akasaki Y, Irie M, et al. (2001) Results of a phase I clinical trial of vaccination of glioma patients with fusions of dendritic and glioma cells. **Cancer Immunol Immunother** 50, 337-344
- Kugler A, Stuhler G, Walden P, et al. (2000) Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. **Nat Med** 6, 332-336
- Lodge PA, Jones LA, Bader RA, et al. (2000) Dendritic cell-based immunotherapy of prostate cancer: immune monitoring of a phase II clinical trial. **Cancer Res** 60, 829-833
- Markert JM, Medlock MD, Rabkin SD, et al. (2000) Conditionally replicating herpes simplex virus mutant, G207

- for the treatment of malignant glioma: results of a phase I trial. **Gene Ther** 7, 867-874
- Martuza RL. (2000) Conditionally replicating herpes vectors for cancer therapy. **J Clin Invest** 105, 841-846
- Merchant RE, Ellison MD, Young HF. (1990) Immunotherapy for malignant glioma using human recombinant interleukin-2 and activated autologous lymphocytes. A review of pre-clinical and clinical investigations. **J Neurooncol** 8, 173-188
- Mineta T, Rabkin SD, Yazaki T, et al. (1995) Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. **Nat Med** 1, 938-943
- Moore AE. (1960) The oncolytic viruses. **Prog. Exp. Tumor Res.** 1, 411-439
- Murayama K, Kobayashi T, Imaizumi T, et al. (2000) Expression of the SART3 tumor-rejection antigen in brain tumors and induction of cytotoxic T lymphocytes by its peptides. **J Immunother** 23, 511-518
- Nemunaitis J, Ganly I, Khuri F, et al. (2000) Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. **Cancer Res** 60, 6359-6366
- Okada H, Attanucci J, Giezeman-Smits KM, et al. (2001) Immunization with an antigen identified by cytokine tumor vaccine-assisted SEREX (CAS) suppressed growth of the rat 9L glioma in vivo. **Cancer Res** 61, 2625-2631
- Pelner L, Fowler GA, Hauts HC. (1958) Effects of concurrent infections and their toxins on the course of leukemia. **Acta. Med. Scand.** 338 (suppl.), 1-47
- Prados M, Gutin P, Philips T. (1992) Highly anaplastic astrocytoma: review of 357 patients treated between 1977 and 1989. **Int J Radiat Oncol Biol Phys** 23, 3-8
- Rosenberg SA. (1999) A new era for cancer immunotherapy based on the genes that encode cancer antigens. **Immunity** 10, 281-287
- Sahin U, Koslowski M, Tureci O, et al. (2000) Expression of cancer testis genes in human brain tumors. **Clin Cancer Res** 6, 3916-3922
- Sahin U, Tureci O, Pfreundschuh M. (1997) Serological identification of human tumor antigens. **Curr Opin Immunol** 9, 709-716
- Sahin U, Tureci O, Schmitt H, et al. (1995) Human neoplasms elicit multiple specific immune responses in the autologous host. **Proc Natl Acad Sci U S A** 92, 11810-11813
- Sampson JH, Archer GE, Ashley DM, et al. (1996) 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 U S A** 93, 10399-10404
- Schackert HK, Itaya T, Schackert G, et al. (1989) Systemic immunity against a murine colon tumor (CT-26) produced by immunization with syngeneic cells expressing a transfected viral gene product. **Int J Cancer** 43, 823-827
- Sobol RE, Fakhrai H, Shawler D, et al. (1995) Interleukin-2 gene therapy in a patient with glioblastoma. **Gene Ther** 2, 164-167
- Struss AK, Romeike BF, Munnia A, et al. (2001) PHF3-specific antibody responses in over 60% of patients with glioblastoma multiforme. **Oncogene** 20, 4107-4114
- Thurner B, Haendle I, Roder C, et al. (1999) Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. **J Exp Med** 190, 1669-1678
- Tjuvajev J, Gansbacher B, Desai R, et al. (1995) RG-2 glioma growth attenuation and severe brain edema caused by local production of interleukin-2 and interferon-gamma. **Cancer Res** 55, 1902-1910
- Toda M, Rabkin SD, Martuza RL. (1998) Treatment of human breast cancer in a brain metastatic model by G207, a replication-competent multimutated herpes simplex virus 1. **Hum. Gene Ther.** 9, 2177-2185
- Toda M, Rabkin SD, Kojima H, et al. (1999) Herpes simplex virus as an in situ cancer vaccine for the induction of specific anti-tumor immunity. **Hum. Gene Ther.** 10, 385-393
- Toda M, Iizuka Y, Kawase T, et al. (2002) Immuno-viral therapy of brain tumors by combination of viral therapy with cancer vaccination using a replication-conditional HSV. **Cancer Gene Ther** 9, 356-364
- Toda M. (2003) Immuno-viral therapy as a new approach for the treatment of brain tumors. **Drug News Perspect** 16, 223-229
- Ueda R, Iizuka Y, Yoshida K, et al. (2004) Identification of a human glioma antigen, SOX6, recognized by patients' sera. **Oncogene** 23, 1420-1427
- van der Bruggen P, Traversari C, Chomez P, et al. (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. **Science** 254, 1643-1647
- Wekerle H. (1993) T-cell autoimmunity in the central nervous system. **Intervirology** 35, 95-100
- Yu JS, Wheeler CJ, Zeltzer PM, et al. (2001) Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. **Cancer Res** 61, 842-847