J HK Coll Radiol 2002;5:219-226

REVIEW ARTICLE

Cellular Immunotherapy for Cancer

B Chan, W Lee

Biological Therapy Unit, Hong Kong Sanatorium & Hospital, Hong Kong

ABSTRACT

Cancer immunotherapy is a major branch of biological therapy that utilises living cells and their products. Advances in molecular biology and immunology have led to the development of novel therapeutic strategies. This paper is an introductory review of the present status of this new field, including the use of cancer cells as a source of antigen, the use of dendritic cells (a type of antigen presenting cell) to augment the immune response, the activation of cytotoxic T cells and memory T cells, and other targeted strategies in the cellular immunotherapy for cancer.

Key Words: *Immunotherapy, Lymphocytes, Tumor cells, Vaccines*

INTRODUCTION

Background

Cancer immunotherapy is a major branch of biological therapy which may be defined as the therapeutic use of living cells and their products. During the past 2 decades, advances in molecular and cell biology have led to the identification of large numbers of antigens, signal transducers, cytokines, and receptors and their ligands, which are potential targets in cancer therapy.

Modern biotechnology has made possible the cloning of the genes that specify these molecules; and to produce large quantities of these molecules using recombinant DNA technology in bacteria (for example, *Escherichia coli*), yeast, or mammalian cell systems, in transgenic animals, and in the case of monoclonal antibodies using hybridoma technology.

As a result, many new biological agents have entered the therapeutic armamentarium with more precisely targeted actions than the traditional drugs used in cancer treatment. Traditional drugs are mostly empirically derived, consist of imprecise or unknown mechanisms of action, and are often toxic compounds such as chemical dyes or heavy metals (for example, platinum). However, the use of these new drugs, in part because of the precision of their actions, presents new challenges. Generally, their precise mechanisms call for molecule-to-molecule interactions, usually across cell membranes and within an activation cascade. These have to be sensitively modulated and counter-modulated, so that the response is accurate — neither too weak nor too strong. This precision is in stark contrast to the crudeness of conventional drug delivery systems, for example, intravenous infusions of trillions of biologically active molecules are sometimes given in the hope that a small fraction may land on the correct and desired receptors.

To use these biological drugs rationally, it is necessary to devise strategies based on a new type of delivery vehicle, which, fortunately, has existed for millennia: the living cell. This paper is an introductory review of the present status of the cellular immunotherapy for cancer.

Scientific Basis of Cancer Immunotherapy

The effector branch of the immune system comprises cytokines, antibodies and T cells, which have been well characterised in many studies of the immune response against bacteria, viruses, and allogeneic cells.[1] These cells are all recognised by the immune system as targets for rejection responses. For many years, the conundrum in cancer research has been the observed lack or relative lack of specific response against cancer antigens, often formulated as "the lack of immunogenicity" of cancer cells.

Correspondence: *Dr. Bernard Chan, MD, FRCP, Biological Therapy Unit, Room 400, Central Block, Hong Kong Sanatorium & Hospital, 2 Village Road, Happy Valley, Hong Kong.*
Tel: (852) 2849 4459; Fax: (852) 2849 4814;
E-mail: bc@netvigator.com

Submitted: 7 August 2002; Accepted: 5 November 2002.

However, modern immunology has unveiled mechanisms in the host defence against cancer. Relatively recently, researchers thought that the immune system constantly patrolled for cancer cells, that is, actively prevented cancer. According to this 'immune surveillance' hypothesis, cancer represented a breakdown of the immune defence against cancer.[2] This is now known to be too simplistic.

To maintain health, the immune system must be able to 'tolerate' large numbers of antigens, including many that are not of host origin but which nevertheless do not evoke a 'danger signal'. According to this new theory, called the 'Danger Model',[3-4] the immune system only mounts a specific rejection response when it recognises danger such as inflammation and necrotic cell death caused by bacterial or viral infection. In most cancers, the cancer cell is different in only small ways from a healthy cell, and does not provoke a danger response.

The sentinel cell, which senses the danger signal, is usually the dendritic cell, a specialised antigen-presenting cell. Under normal circumstances, necrotic cell death triggers the danger signal via the dendritic cells, whereas apoptotic cell death does not evoke this signal. One mechanism involves specialised proteins, which 'chaperone' antigens to be presented to T cells. Heat shock proteins, which are synthesised when cells are in danger, for example, subject to heat shock, (which may lead to necrosis) is one such class of chaperone proteins.

An important new understanding is that, apart from the antigen itself, a second signal called the co-stimulation signal, is necessary to activate T cells. In the absence of the co-stimulation signal, tolerance may be induced instead. Co-stimulation and its opposite, counter co-stimulation, are highly sensitive mechanisms modulated by cascades of activation and response, which have major implications for cancer, auto-immunity, and transplantation.

In summary, this new knowledge of antigen presentation by dendritic cells forms the basis of the modern approach to immunotherapy. These dendritic cells must first mature, and then express co-stimulation molecules, before presenting cancer antigens to T cells in a highly precise manner. This process also involves major histocompatibility complex (MHC) and T cell receptor molecules.

The Living Cell as a Delivery System

An attractive and readily available delivery system for biotherapeutics is the living cell, often, but not necessarily, from the patient being treated. Thus, the treatment agent(s) could be cells mixed with biological drugs, or simply cells themselves. These cells may be selected according to biological characteristics, for example, with magnetic bead systems, ex vivo expanded, modified by activation of surface receptors or the insertion of genes.

Cells may be given with other cells, either fused together, for example, by the passage of an electric current, or separately. This development introduces a new class of therapeutic agents that are self-delivered, although someone still has to hang the bag labelled 'lymphocytes' by the bedside.

Current cell therapy strategies that are promising and within the capabilities of many teams such as those engaged in stem cell transplantation often utilise biopharmaceuticals, which are already available. These include granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-2 (IL-2), anti-CD3 monoclonal antibody, and bacille Calmette-Guerin (BCG). These biopharmaceuticals are all approved biological drugs in the major countries of Europe, Asia, and North America, albeit for other specific indications, that is, they are approved in biologically massive doses given in conventional delivery systems.

Similarly the blood cell separators and processors that are used by cell therapy teams have been approved by regulatory agencies, in some cases more than a quarter of a century ago. This paper will focus on the following strategies:

- irradiated autologous tumour cell vaccines unmodified except for the addition of BCG
- irradiated autologous tumour cells fused with dendritic cells as vaccines, which may be used with GM-CSF
- combining the above tumour cell vaccine approach with activation and ex vivo expansion of T lymphocytes
- autologous lymphocyte therapy based on ex vivo activation of memory T cells
- lymphokine activated killer (LAK) cells, tumour infiltrating lymphocytes (TILs), and related approaches
- targeted cellular therapy for Epstein-Barr virus (EBV)-associated malignancies;
- the use of allogeneic T lymphocytes in a stem cell transplantation setting.

CANCER VACCINES

Principles of Cancer Vaccines

Vaccination is the intentional introduction of antigen(s) to evoke an immune response. Although the familiar model of vaccination for viral diseases, for example, is that of prophylaxis, in oncology the term tumour vaccine refers to therapeutic vaccines. Therapeutic vaccines are those used to treat patients who already have cancer, by preventing recurrence or to control disease. Tumour vaccines and vaccine-based immunotherapy comprise a large and active field of research and clinical practice.[5-8]

A notable difference between the familiar prophylactic model for viral diseases and therapeutic tumour vaccines is that, in the former, the individual to be vaccinated has not been exposed to the antigens (the viruses). Once exposed to antigens such as modified viruses, the immune system, alerted by the 'danger signals', mobilises the dendritic cells. Co-stimulation and chaperone molecules are expressed and antigen presentation to T cells then occurs in the normal manner. In the case of cancer, the patient has already been exposed to the tumour antigens but the response has been inadequate due to the lack of dendritic cell mobilisation, the absence of appropriate danger signals, and other factors. Thus the focus is not on the tumour antigens themselves, but on other critical secondary processes that are prerequisites to the activation of T cells.

Autologous Tumour Cell Vaccines

The use of tumour cells from patients themselves is the simplest way to produce a vaccine, although this approach is limited to patients who have biopsy accessible lesions or, better still, have tissue stored at the time of primary surgery. The use of autologous tumour overcomes most of the difficulties associated with MHC restriction during the process of antigen-presentation and a large number of preclinical and clinical studies have been reported using such cells.[9-12]

For the sake of brevity, this paper will first focus on the use of autologous vaccines in early stage colon cancer, in which there is substantial evidence for clinical efficacy. A subsequent section will discuss the use of dendritic cell vaccines for renal cell cancer.

Early stage colon cancer has been the subject of at least 2 phase III randomised controlled clinical trials, with long periods of follow up. In the American study, Hoover et al randomised 94 patients with Duke's stage B2 to C3 colorectal cancer to surgery alone or surgery followed by autologous tumour cell vaccination.[13] For patients with rectal cancer, radiation therapy was also given. The results for 6.5 years of follow-up showed that, for colon cancer, the recurrence rate for vaccinated patients was 25% compared with 56.5% for the control group. The number of deaths among vaccinated patients was 16.7% compared with 47.8% in the control group.

The study of Hoover et al, however, has been criticised for technical as well as statistical flaws.[13] Only a proportion of patients had adequate vaccination as judged by the delayed type hypersensitivity response. There was a relatively small number of patients and the results did not reach statistical significance. Patients with rectal cancer did not enjoy clinical benefit, a result the authors attributed to the use of radiation soon after vaccination. These observations were therefore regarded as preliminary and only suggestive of clinical benefit.

More definitive results were obtained in another phase III prospective randomised controlled trial conducted by the Netherlands National Cancer Institute, with a median follow up of 5.3 years.[14] This study used a booster dose of vaccine, for a total of 4 doses, and patients with rectal cancer were excluded. 254 patients were randomised to treatment with surgery only or surgery followed by autologous tumour cell vaccination.

For the whole group (patients with stage II and III disease), there was a 41% risk reduction in tumour recurrence in favour of vaccination ($p < 0.023$). However, this benefit appears to be restricted to patients with stage II disease in this study. In this subset of patients, the risk reduction for recurrence was 61% ($p < 0.011$), a very notable outcome.

Both these studies utilised autologous tumour cells obtained at the time of surgery, made into single cell suspensions, and then irradiated. The production of a vaccine utilises enzyme digestion to process the tumour tissue. This is a skilled technique as excessive enzyme may damage cell surface antigens. Alternatively, a mechanical processor can be used to produce the cell suspension. The irradiated cells may be stored frozen. When required for use, the cell suspension is thawed and then mixed with BCG before injection. BCG recruits antigen-presenting cells to the injection sites but has the disadvantage of causing local reactions, including small superficial ulcers, which usually heal spontaneously. All groups using this simple technique

report no other significant side effects. It is possible to use GM-CSF instead of BCG and this substitution has the advantage of avoiding the skin ulcerations.

Dendritic Cell Vaccines

The relatively straightforward approach described above showed efficacy in early stage colon cancer, and a similar vaccine is reported to be efficacious for early stage renal cell cancer.[15] However, for many clinical situations, a more powerful vaccine is desirable. The pivotal role of dendritic cells in the presentation of tumour antigens has already been discussed. Much has now been learned about these specialised cells that account for less than 0.2% of human mononuclear cells in the peripheral blood.

Although the first successful culture of human dendritic cells was reported in 1992,[16] it was not until 1994 that a breakthrough occurred.[17,18] This was the development of a relatively simple method to generate large numbers of dendritic cells from blood monocytes by culture with GM-CSF and IL-4. Further research on the use of pro-inflammatory cytokines (IL-1, IL-6, and tumour necrosis factor [TNF]-α) as maturation stimuli in 1997[19] led to reliable methods of producing ex vivo generated and matured dendritic cells, which have become widely used in the design of immunotherapeutic strategies.

Dendritic cell-based immunotherapy was successfully tested in preclinical studies.[19,20] More recently, regression of established metastatic tumour was achieved — a first for vaccine therapy in human cancer. This was reported in 2000 by Kugler et al who used allogeneic dendritic cells fused with autologous tumour cells from 17 patients with metastatic renal cell cancer.[21] There were 4 complete responses (CRs) and 2 partial responses.

The dendritic cells and tumour cells can be fused by aligning them in a cuvette and passing an electric current, which enlarges the pores on the cell membrane, allowing the smaller cells to enter. Frequently the resulting hybrid cell has 2 or more tumour cells within a larger dendritic cell (Figure 1). As with the standard autologous tumour vaccine described above for colon cancer, the dendritic cell fusion vaccine can also be injected intradermally and mixed with GM-CSF. The dendritic cell fusion vaccine also produces delayed type hypersensitivity (DTH) responses in the majority of patients. Side effects are also mild and are mainly the side effects of GM-CSF in the vaccines.

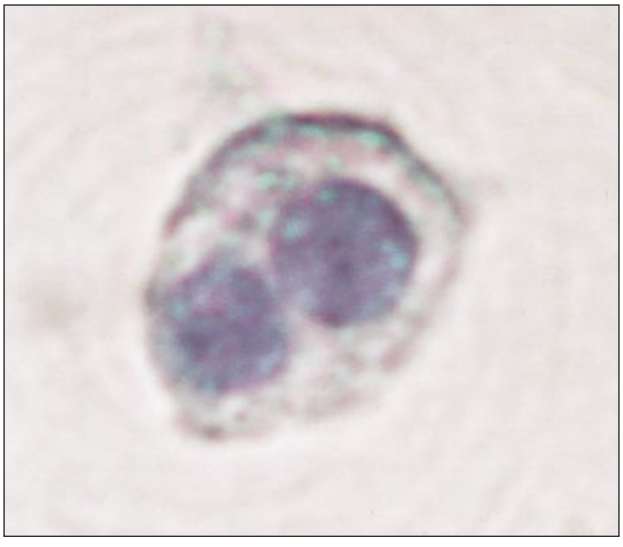

Figure 1. A dendritic cell containing 2 tumour cells.

CELL TRANSFER THERAPY

Historical Perspectives

Lymphokine activated killer (LAK) cells were discovered by Rosenberg's group at the National Cancer Institute in the USA in the 1970s.[22] Following preclinical studies that showed tumour response in animals, a number of pilot trials as well as randomised trials comparing LAK cells plus IL-2 with IL-2 alone, totalling more than 600 patients, were reported, mostly during the 1980s.[22-24]

In a recent summary of 679 patients with metastatic renal cell cancer treated with LAK cells plus IL-2 during this period, there were 169 overall responses (25%) with 55 CRs (8%), although the use of LAK cells plus IL-2 was not found to be statistically superior to high-dose IL-2 alone.[25]

Rosenberg's group went on to study tumour-infiltrating lymphocytes (TILs) that were prepared from tumour obtained at the time of surgery.[22] In a recent summary of 115 patients from various cooperative groups treated with TILs, there were 26 (23.2%) overall responses with 7 CRs (6%),[25] that is, no better than LAK cells or even high-dose IL-2 alone.

These studies, however, established that reproducible results, with many of the CRs being durable, could be obtained for metastatic human cancer using an immunotherapeutic strategy.

From the perspective of the year 2002, the studies of IL-2, LAK cells, and TILs in the 1980s had a number of defects. The molecular structure of IL-2 receptors

was not yet known, leading to the use of relatively high doses of IL-2 in infusions and in cultures. Thus, cells with low- and intermediate-affinity receptors were activated as well as cells with high-affinity receptors. The culture methodology was complex and the time of culture quite long, making the production process highly intensive. The LAK cells were mostly polyclonal T cells, with only a small percentage of cells with the natural killer (NK) cell phenotype.

However, interest in NK cells has recently been revived, with new understanding of their receptor biology and their possible role in mediating the graft versus tumour response.[26] With the newly developed technologies for cell purification and ex vivo expansion, NK cell-based therapies hold much promise. There are 2 potential advantages. First, unlike cytotoxic T cells they are not subject to the requirement of MHC restriction in order to produce cytotoxicity against tumour cells. Second, they can provide a source of Fc-γ receptors (CD16). This may be important especially in conjunction with monoclonal antibodies such as those already approved for clinical use, for example, anti-Heu for breast cancer and anti-CD20 for malignant lymphoma, which may require interaction between the Fc portion of the antibody and Fc-γ receptors to accomplish antibody-dependent cellular cytotoxicity (ADCC). Recently the allogeneic cell line NK-92 has entered phase I trials in Europe and the USA.

The autolymphocyte therapy (ALT) approach described below may also provide a supply of Fc-γ receptors, as the cell culture product using the ALT method is relatively rich in NK cells, which can be selected and expanded in an ex vivo system. Thus, although LAK cells are no longer used, many important lessons were learned that are likely to find applications in the future.

Vaccines Primed Ex Vivo Expanded T Cells

Another approach to developing a more powerful response to vaccination is to collect lymphocytes after in vivo activation by vaccine and to culture the lymphocytes using the mitogenic monoclonal antibody to CD3. CD3 forms a complex with the T cell receptor, and the antibody in the complement-free laboratory system activates the T cell receptor, but without cell lysis. It is then possible to use IL-2 to stimulate T cell proliferation. This ex vivo expansion procedure can generate large numbers of T cells that can be re-infused into patients for antitumour cytotoxicity and to enhance endogenous immune responses.

Preclinical studies showed that this combination of vaccine priming and the ex vivo expansion of lymphocytes from draining lymph nodes[27] and blood[28] is a feasible strategy, resulting in immune tumour rejection in the majority of treated animals. In phase I/II studies this strategy has been used to treat patients with end-stage refractory malignant glioma, with 2/9 durable long-term complete remissions reported.[29,30] The treatments were well tolerated with no long-term side effects.

Autolymphocyte Therapy

The term ALT refers to an adoptive cellular transfer strategy based on the ex vivo activation of memory T cells. The assumption is that because patients with metastatic cancer have been previously exposed to tumour antigens, memory T cells exist which can be collected and activated ex vivo. This results in autologous cytokines that can be used to activate other T cells. The method is simpler than it sounds and basically consists of 2 cell collections using a cell separator and 2 cell cultures. The supernatant from the first culture, which contains the desired cytokines, is used to activate the second cohort of T cells.

ALT has been studied extensively in renal cell carcinoma. In a phase II clinical trial, Krane et al showed a delayed progression of disease in 33% of patients with metastatic renal cell carcinoma.[31] The 1-year survival was 56% and 2-year survival 36% in a group of patients with a poor prognosis. In a multicentre phase III study, ALT plus cimetidine (which decreases suppressor T cell activity) was compared with cimetidine alone.[32] Initial results showed a survival of 21 months for patients receiving ALT plus cimetidine compared with 8.5 months for cimetidine alone. Updated results continued to show a survival advantage for the patients who received ALT plus cimetidine. These results led to the use of ALT in the clinical practice setting in Boston, Atlanta, and Orange County, California, USA, with continued reports of favourable results.[33,34] Patients tolerated the treatment well with virtually no side effects apart from fever at the time of re-infusion of activated T cells.

Epstein-Barr Virus-associated Malignancies

EBV plays an important role in a number of human cancers, including Burkitt lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, and others. EBV-associated lymphoproliferative disease (EBV-LPD) can be particularly devastating for immunocompromised patients.

Successful prevention and treatment of EBV-LPD, particularly in the post-transplant setting, is a major triumph of cellular immunotherapy.

The incidence of EBV-LPD following stem cell transplants is correlated with the degree of immunosuppression. The highest incidence is found in patients receiving partially mismatched related transplants, and those receiving matched unrelated donor transplants. This is particularly evident when T cell depletion is used, and the lowest incidence is among autotransplant recipients.

Conventional therapy for EBV-LPD is generally ineffective. In 1994, Papadopoulos et al from the Memorial Sloan Kettering Cancer Center in the USA, reported on the use of unmanipulated donor T cells to treat 5 patients with EBV-LPD.[35] These researchers based their treatment on the assumption that the donor T cell population would contain EBV specific T cells, although the number of such cells would be very small.

All 5 patients responded but 3 of the patients developed graft-versus-host disease (GVHD) and 2 patients died from respiratory failure. This report demonstrated that even a small number of specific T cells could undergo large-scale in vivo expansion in a suitable setting. However, the price to be paid in terms of GVHD-related morbidity and mortality is too high for the use of non-selected donor T cells to be recommended. This remains a concern for applications involving allogeneic T cells in general.

Brenner et al, Heslop et al, and Rooney et al undertook an extensive programme of targeted adoptive cellular immunotherapy using virus specific cytotoxic T lymphocytes (CTLs).[36-39] These researchers established EBV-specific CTL cell lines from more than 120 healthy donors. The CTLs were infused into patients post-stem cell transplant for the prevention of EBV-LPD.

An interesting feature of this phase I study was that, instead of the originally planned dose escalation, dose modification with progressively decreasing cell dose was eventually performed due to the discovery that a single dose of 2×10^7 cells was effective. This again showed that a minute dose of T cells could be expanded in vivo for a major increase in host immunity, and that the maximum tolerated dose (MTD) paradigm may not necessarily apply to cancer immunotherapy. None of the 54 patients who received the CTLs developed EBV-LPD compared with 6 of 52 controls or 11% ($p < 0.03$).[36] This is the first report of successful cellular prophylaxis of a human malignancy.

Brenner et al also treated 4 patients with established EBV-LPD using virus specific CTLs.[36] Three of the patients attained complete remission. The cause of failure in the fourth patient due to a mutation in the virus was also elucidated.[40] These pioneering studies brought forward many insights and showed new directions in developmental cellular therapeutics, with possible future applications for other types of EBV-associated malignancies.

Allogeneic T Cells as Cancer Immunotherapy

The use of allogeneic T cells to evoke a powerful graft-versus-tumour response has now become a routine practice for most stem cell transplant teams. This can take the form of non-myeloablative transplants ('mini transplants') or as donor lymphocyte infusions (DLI) in patients who relapse after conventional bone marrow transplantation for haematological malignancies.[41-43]

These graft-versus-tumour responses led to the development of mini transplants for solid tumours. In an update of 47 patients with renal cell carcinoma, the group at the US National Institutes of Health reported 18 partial and 4 complete responses for a total response rate of 47%.[44] Many of the responses occur late, usually after withdrawal of cyclosporin or other treatment for GVHD; and typically only after T cell chimerism has transitioned from mixed to predominantly donor (usually 6 months or longer).

CONCLUSIONS

This paper is an introductory review of a large field. To the busy clinical oncologist, cancer immunotherapy may at first appear unfamiliar. However, just as the main body of biology, having focused for half a century on the gene has gradually moved on from genomics to proteomics, so recently a new term 'cellomics' was coined to describe a renewed focus on the living cell. The key to the understanding of cancer immunotherapy may be stated simply: "Which cell?"

Despite an apparently complex nomenclature, essentially only 2 types of cells are involved: cancer cells and lymphocytes. Cancer cells are the invader cells and lymphocytes are the defender cells. These 2 cell types are the protagonists in the drama of cancer therapy.

By utilising the invader cells as the source of antigens, and by developing different sub-populations of the defender cells, normal in vivo constraints may be circumvented. The engineering of these immune cells may be achieved through innovation in the collection, selection, and culture in precisely regulated laboratory systems. Desirable characteristics may be enhanced in the laboratory and such enhanced cells may be returned to the patient, not only as a passive treatment, but also as an active immunisation strategy.

The old paradigm of the MTD and defining dose by toxicity is increasingly being questioned in the new era of cancer treatment, which is based on the precise understanding of cellular mechanisms. Similarly, the classical developmental therapeutic model of incremental 'cell kill' is being supplemented by new models based on the host defence repertoire of immune cells.

REFERENCES

1. Roitt I. Immunity to infection. In: essential Immunology. Part 5. Oxford: Blackwell Science Ltd; 1997:251-307.
2. Thomas L. On immunosurveillance in human cancer. Yale J Biol Med 1982;55:329-335.
3. Matzinger P. An innate sense of danger. Semin Immunol 1998; 10:399-413.
4. Matzinger P. Tolerance, danger, and the extended family. Ann Rev Immunol 1994;12:991-1045.
5. Stern PL, Beverley PLC, Carroll MW, editors. Cancer vaccines and immunotherapy. Cambridge: Cambridge University Press; 2000.
6. Morton DL, Foshag LJ, Hoon DS, et al. Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. Ann Surg 1992;216:463-482.
7. Adler A, Gillon G, Lurie H, et al. Active specific immunotherapy of renal cell carcinoma patients: a prospective randomized study of hormono-immuno-versus hormonotherapy. Preliminary report of immunological and clinical aspects. J Biol Res Mod 1987; 6:610-624.
8. Hudson CN, McHardy JE, Curling OM, et al. Active specific immunotherapy for ovarian cancer. Lancet 1976;2:877-879.
9. Schulof RS, Mai D, Nelson MA, et al. Active specific immunotherapy with an autologous tumor cell vaccine in patients with resected non-small-cell lung cancer. Mol Biother 1988;1: 30-36.
10. Berd D, Kairys J, Dunton C, et al. Autologous, hapten-modified vaccine as a treatment for human cancers. Semin Oncol 1998;25:646-653.
11. Schirrmacher V, Ahlert T, Probstle T, et al. Immunization with virus-modified tumor cells. Semin Oncol 1998;25:677-696.
12. Simons JW, Mikhak B. Ex vivo gene therapy employing cytokine-transduced tumor vaccines: molecular and clinical pharmacology. Semin Oncol 1998;25(6):661-676.
13. Hoover HC Jr, Brandhorst JS, Peters LC, et al. Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase II prospectively randomized trial. J Clin Oncol 1993;11:390-399.
14. Vermorken JB, Claessen AME, van Tinteren H, et al. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. Lancet 1999;353:345-350.
15. Dillman RO, Beutel LD, Cornforth AN, Nayak SK. Short-term cell lines from renal cell carcinoma for use as autologous tumor cell vaccines in the treatment of kidney cancer. Cancer Biother Radiopharm 2000;15:161-168.
16. Caux C, Dezutter DC, Schmitt D, et al. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature 1992;360:258-261.
17. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and down regulated by tumour necrosis factor alpha. J Exp Med 1994;179:1109-1118.
18. Romani N,Gruner S, Brang D, et al. Proliferating dendritic cell progenitors in human blood. J Exp Med 1994;180:83-93.
19. Jonuleit H, Kuhn U, Muller G, et al. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum free condition. Eur J Immunol 1997;27:3135-3142.
20. Hart I, Colaco C. Fusion induces tumor rejection. Nature 1997; 388:627-628.
21. Kugler A, Stuhler G, Walden P, et al. Regression of human metastatic renal cell carcinoma after vaccination with tumour cell-dendritic cell hybrids. Nat Med 2000;6:332-336.
22. Rosenberg SA. Karnofsky Memorial Lecture: The immunotherapy and gene therapy of cancer. J Clin Oncol 1992;75: 1578-1583.
23. Rosenberg SA, Lotze MT. Cancer immunotherapy using interleukin 2-actived lymphocytes. Annu Rev Immunol 1986; 4:681-709.
24. Mulé JJ, Shu S, Schwarz SL, et al. Adoptive immunotherapy of established pulmonary metastases with LAK cells and recombinant interleukin-2. Science 1984;225:1487-1489.
25. Belldegrun AS, Figlin RA, Patel B. Cell transfer therapy: clinical applications: Renal cell carcinoma. Principles and practice of the biologic therapy of cancer. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2000:333-345.
26. Farag SS, Fehniger TA, Ruggeri L, et al. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood 2002;100:1935-1947.
27. Plautz GE, Toualisky JE, Shu S. Treatment of murine gliomas by adoptive transfer of ex vivo activated tumor draining lymph node cells. Cell Immunol 1997;178:101-107.
28. Wood GW, Turner T, Wang YY, et al. Immune rejection of intracerebral gliomas using lymphocytes from glioma-bearing rats. J Immunother 1999;22(6):497-505.
29. Holladay FP, Heitz-Turner T, Bayer WL, Wood GW. Autologous tumor cell vaccination combined with adoptive cellular immunotherapy in patients with grade III/IV astrocytoma. J Neuro-Oncol 1996;27:179-189.
30. Wood GW, Holladay FP, Turner T, et al. A pilot study of autologous cancer cell vaccination and adoptive cellular immunotherapy using Anti-CD3 stimulated lymphocytes in patients with recurrent grade III/IV astrocytoma. J Neuro-Oncol 2000; 48:113-120.
31. Krane RJ, Carpinito GA, Ross SD, et al. Treatment of metastatic renal cell carcinoma with autolymphocyte therapy. Urol 1990;35:417-422.
32. Osband ME, Lavin PT, Babayan RK, et al. Effect of autolymphocyte therapy on survival and quality of life in patients with metastatic renal cell carcinoma. Lancet 1990;335: 994-998.

33. Lanvin PT, Maar R, Franklin M, et al. Autolymphocyte therapy for metastatic renal cell carcinoma — initial clinical results from 335 patients treated in a multisite clinical practice. Transpl Proc 1992;24:3057-3062.
34. Graham S, Babayan RK, Lamm DL, et al. The use of ex vivo activated memory T cells (autolymphocyte therapy) in the treatment of metastatic renal cell carcinoma — final results from a randomized controlled multisite study. Semin Urol 1993;11:27-34.
35. Papadopoulos EB, Ladanyi M, Emanuel D, et al. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. N Engl J Med 1994;330:1185-1191.
36. Brenner MK, Heslop HE, Rooney CM. Adoptive cellular immunotherapy for Epstein-Barr virus-associated malignancies. Principles and practice of the biologic therapy of cancer. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2000:357-367.
37. Rooney CM, Smith CA, Ng CYC, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood 1998;92:1549-1555.
38. Heslop HE, Ng CYC, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. Nat Med 1996;2:551-555.
39. Rooney CM, Roskrow MA, Smith CA, et al. Immunotherapy of EBV lymphoma. J Natl Cancer Inst Monogr 1998;23:89-93.
40. Burrows SR, Khanna R, Burrows JM, et al. An allo response in humans is dominated by cytotoxic T lymphocytes (CTL) cross-reactive with a single Epstein-Barr virus CTL epitope: implications for graft-versus-host disease. J Exp Med 1994;179: 1155-1161.
41. Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 1995;86:2041-2050.
42. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 1998;91:756-763.
43. Collins RH, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 1997;15: 433-444.
44. Childs R. Investigating the graft versus tumor effect in renal cell carcinoma following non-myeloablative allogenic peripheral blood transplant. Biol BM Transpl 2002;8:341.