Review Article

Current status of immunotherapy for the treatment of lung cancer

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ABSTRACT Immunotherapy is a novel approach for the treatment of systemic malignancies. Passive and adaptive immunotherapy have been applied to the treatment of a wide variety of solid tumors such as malignant melanoma (1), renal cell carcinoma (2) and ovarian cancer (3). Several early clinical trials of immune based therapy for both non-small (NSCLC) and small cell lung cancer (SCLC) have demonstrated limited or no success (3,4) but recent trials of antigen-specific cancer immunotherapy have shown early therapeutic potential and are now being rigorously evaluated on a larger scale (5). In this communication we briefly review the historic aspects of immune based therapy for solid cancer, describe therapeutic strategies aimed at targeting lung cancer, and discuss limitations of current therapy and future directions of this field.

Key Words: lung cancer; immunotherapy

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Does the immune system influence the development and progression of cancer?

Clinical data from immunosuppressed organ transplant recipients or patients with HIV clearly demonstrates an increase in viral mediated cancers such as cervical carcinoma (Human Papilloma Virus), hepatocellular carcinoma (Hepatitis B and C), as well as Burkitt's Lymphoma (Epstein Barr Virus) (6-8). While such data maybe understandable in light of the defined role that the immune system plays in elimination of oncogenic viruses, large population based studies of solid organ transplant recipients also demonstrate an increase in multiple types of malignancies with no known viral etiology, such as malignant melanoma, renal cell carcinoma and non-Burkitt's lymphoma (9,10). Such "experiments of nature" form the basis for the theory of cancer immunosurveillance, which proposes that a healthy immune system can protect an individual from the development and uncontrolled growth of malignancies (11). The theory of cancer immunoediting furthers the immunosurveillance theory and suggests that the immune system can both control the growth as well as shape the phenotype of cancer. It is thus possible that once a clinically evident tumor develops in an immunocompetent individual it has been sculpted to avoid immunorecognition, akin to bacterial drug resistance that develops in those treated with long-term antibiotics (12). Many studies in small animals with genetically engineered immune defects support observational human data and provide an experimental platform for studying the role of the tumor immune response.

Unfortunately few human or animal studies have focused on the role of the immune system in surveillance for lung cancer. Such lack of data leads to controversy in this field. A recently published European study of immunosuppressed patients demonstrated no increase in lung cancer in kidney or liver transplant recipients but an increase in those receiving a heart allograft (13). Based on this data it is possible to conclude that in the population as a whole immunosurveillance for lung cancer may not occur. Alternatively one could conceive that differences in cancer incidence may be unmasked only in patients with a significant smoking history, such as those requiring heart transplantation for coronary artery disease. Other observational studies, however, do not demonstrate an increase in lung cancer in heart transplant recipients, despite an increase in other cancers such as malignant melanoma and cervical carcinoma (14). Similar controversy exists in small animal models of lung cancer. Kobayashi and colleagues, for example, demonstrated that the incidence of chemically induced lung cancer is similar in

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immunocompetent and T cell deficient littermates while other investigators, using similar methodology, have demonstrated the importance of T cells in immunosurveillance for fibrosarcoma (15-17). Based on these and other data one must conclude that the existence of immunosurveillance for lung cancer is not rigorously supported by clinical or animal data. Thus, unlike clinical trials of immune based therapy for melanoma and renal cell carcinoma, the rationale for immunotherapy in the treatment of lung cancer deserves more background investigation. Since lung cancer, especially non small cell lung cancer (NSCLC), is the leading form of cancer related death (18,19), funding and execution of such trials is a critical priority to our field.

History of tumor immunotherapy

The first organized trials of immunotherapy can be rightfully credited to a New York Surgeon William Coley, whose line of clinical research began in the 1890's with an anecdotal observation that the facial sarcoma of a German immigrant, Fred Stein, miraculously regressed after a post-operative bacterial infection. This incident initiated Coley's clinical research as he started to infect cancer patients with various bacterial isolates (20). Coley's findings led to the creation of "Coley's toxin", a concoction derived from cultures of Streptoccocus pyogens and Serratia marcasens (21). Only in the 1990's was it defined that the anti-tumor effects of these bacterial toxins were the result of "tumor necrosis factor" produced by recipient macrophages rather then direct tumor toxicity (22). Multiple other strategies to stimulate an antitumor immune response have been similarly tried using bacterial products such as Corynebacterium parvum (23). The modern era of tumor immunotherapy can be traced to the use of adaptive cell therapy described in 1985 by Rosenberg and colleagues (24). In this classic translational trial, based directly on preclinical animal models, autologous lymphokine-activated killer cells were administered along with recombinant interleukin-2 (IL-2) to patients with advanced cancer. Despite failure of all other therapy prior to entry into this trial, cancer regression was documented in 11 out of 25 patients. While the response of malignant melanoma was most striking, including one complete and long-lasting responder, tumor regression or stability was noted in other cancers as well, including one patient with lung cancer. Based on the overwhelming response documented in patients with metastatic melanoma, significant effort has focused on immunotherapy for this specific malignancy. Furthermore, the majority of clinical efforts in other types of malignancies are based on data generated from the melanoma model.

Definition of tumor immunotherapy

Passive immunotherapy is defined as administration of an immunologically active agent to a patient that is made outside the body. In theory such a system does not rely on the function of the host's own immune system to have its effect. Currently used examples of such therapy include the administration of monoclonal antibodies (25) or adaptive cell therapy, which is described below (26). Active immunotherapy focuses on stimulating the hosts own immune system to eradicate cancer, whether by vaccination with tumor antigens and an adjuvant, non specific immunomodulation using bacterial products, or targeting negative regulatory receptors that prevent the development of the tumor immune response. As described above some of the earliest trials and successes of immunotherapy involved active immunotherapy in the form of non-specific immunomodulation using bacterial products to stimulate the immune response. Despite the early success reported by Coley, others were unable to obtain a similar degree of success using Coley's toxin (27). This resulted in the search for another form of immune stimulation. In 1908 Albert Calmette and Camille Guerin of the Pasteur Institute in Lille, France began work with the attenuated form of bovine tuberculosis, Mycobacterium bovis, or Bacille Calmette-Guérin (BCG), in an effort to develop a vaccine for human tuberculosis. Their work combined with the startling observation in 1929 by Raymond Pearl that patients with tuberculosis had a lower rate of cancer led to investigation into the use of live attenuated BCG as a form of tumor immunotherapy (28,29). To date BCG remains a clinically accepted and approved form of therapy for bladder cancer, with responses equal to if not superior to chemotherapy (30).

Advances in understanding immune regulation have resulted in the development of more defined methods of stimulating the immune system. Based on the central role that IL-2 plays in orchestrating inflammatory responses, and its success in mediating regression of some forms of human cancer (24,31,32), it was approved for human use in 1992 for renal cell carcinoma and in 1998 for malignant melanoma. Recent work in elucidating pathways used by malignancies to downregulate the immune response has led to experimental and clinical success in the palliation of metastatic malignant melanoma (33).

The administration of cancer vaccines, in the form of active immunotherapy, is based on the notion that malignancies express either mutated proteins that can be recognized as foreign antigens, overexpress normal proteins, or reexpress fetal antigens not present in the normal adult tissue. Such tumor associated antigens (TAA) form the basis for recognition of malignant tissue as a "foreign" entity (34,35). As a large number of such antigens can be presented to T lymphocytes by antigen-presenting cells, a major effort has focused on immunizing cancer patients using either whole cells, proteins, or synthetic peptides in order to stimulate a tumor immune response. This form of active immunotherapy is also known as antigen-specific immunotherapy. Similar to other forms of immune based

therapy, the greatest success has been demonstrated in clinical trials for malignant melanoma, especially when combined with efforts to ameliorate pathways that downregulate the immune response. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is receptor expressed on the surface of T cells that interacts with dendritic cell surface co-stimulatory molecules that then act to inhibit T cell activation (36). This interaction occurs in a competitive manner to the engagement of other T cell receptors, such as CD80 and CD86, which provide the signal necessary for the initiation of the T cell response. To this end the humanized form of anti-CTLA-4 IgG antibody (ipilimumab) was recently demonstrated to foster an anti-melanoma immune response leading to a significant survival advantage for patients with unresectable stage III or IV melanoma previously unresponsive to conventional therapy (33). Further encouraging results were reported in several large randomized clinical trials for prostate cancer, utilizing a vaccine targeting prostatic acid phosphatase (37,38), and follicular lymphoma using a vaccine unique for each individual patient (39).

Adoptive cell transfer is a form of passive immunotherapy that involves identification, isolation, expansion and subsequential re-infusion of autologous lymphocytes with anti-tumor activity into patients. This form of therapy has been used with or without administration of appropriate growth factors to enhance T cell survival and expansion in vivo. Such an approach also has the theoretic advantage that identification and isolation of only a few tumor reactive lymphocytes is sufficient for therapy as these cells can be expanded significantly in ex vivo prior to reinfusion (40). The genetic modification of isolated cells and the introduction of T cell receptors with high avidity for tumor specific antigens also creates exciting therapeutic possibilities (26).

Immunotherapy for lung cancer

Active immunotherapy for lung cancer can be traced back to observational studies suggesting that, similar to the sarcoma observations of Coley, local inflammatory changes may influence the prognosis of lung cancer (41,42). Enthusiasm for BCG therapy as adjuvant or neoadjuvant therapy was tempered early on by the lack of demonstrated efficacy in several clinical trials. While a 1981 series reported a decrease in local recurrence after adjuvant post-operative BCG in patients with early stage disease, these results may have been skewed by the high recurrence rate in the control group (43) and this data was not supported by further studies (44,45). Similarly disappointing results were noted for neoadjuvant therapy as Matthay and colleagues demonstrated that preoperative injection of BCG into NSCLC did not affect the disease-free interval or prolong survival (46).

The discovery that, similar to malignant melanoma, NSCLC can express tumor associated antigens led to an ever increasing interest in the development of active immunotherapy protocols utilizing antigen specific immunotherapy. A few investigators have attempted to modify whole tumor cell vaccines to render them more immunogenic prior to patient administration in order to stimulate an anti-tumor immune response. The GVAX vaccine consists of autologous tumor cells engineered to express granulocyte monocyte colony stimulating factor (GM-CSF), which functions to enhance the production and migration of granulocytes such as neutrophils and monocytes with subsequential initiation of a tumor-specific immune response. Such treatment has been shown in animal models to increase the immunogenicity of certain tumors, especially malignant melanoma (47). Based on this data several early phase trials have been conducted using variable immunization protocols consisting of irradiated autologous NSCLC cells transduced with replication incompetent adenovirus expressing GM-CSF. One of the trials, reported in 2004, demonstrated encouraging results as three of the 33 patients with advanced disease had durable and complete responses, one patient had a minor response, and nine demonstrated a mixed response or disease stability (48). Interestingly a survival advantage was noted in patients receiving tumor vaccine secreting high levels of GM-CSF compared to those secreting lower levels. Based on this preliminary data other therapeutic strategies utilizing GVAX have been described (49).

Mucin-1 (MUC-1) is a protein normally expressed on secretory epithelial cells and is present in multiple epithelial cancers, including NSCLC (50). This mutated protein might be an ideal target for active immunotherapy as it is antigenically distinct from that found in normal tissue and functions as an oncogene (51,52). Based on this preliminary data a phase IIb study of patients with IIIa/IV stage lung cancer was conducted randomizing patients to immunization with a synthetic peptide designed to mimic the most antigenic portion of MUC-1 (L-BLP-25), or best supportive care. Eight weekly injections were followed by maintenance immunization every 6 weeks after standard first line chemotherapy and a single dose of cyclophosphamide. Of the 171 patients in the trial those in the immunization arm demonstrated a trend towards longer median survival compared to the best supportive care group (17.4 vs. 13 months) but this trend did not reach statistical significance (53). Such data, while limited, were encouraging and served as the foundation for a multicenter randomized double blind placebo control phase III trial in patients with unresectable stage III disease (START trial).

MAGE-3 (melanoma associated antigen E-3) is a commonly expressed cancer testis antigen that is not present in normal adult tissue but is expressed on multiple malignancies including NSCLC (54). Like multiple other TAA it was initially identified in malignant melanoma based on its ability to stimulate autologous cytotoxic T lymphocytes (55). Aside from its restriction to extragonadal malignant tissue in the adult, MAGE-3 is unique as this protein contains epitopes that can be presented

by both MHC Class I and MHC Class II histocompatibility antigens. Thus the ability of this TAA to activate both CD8+ and CD4+ cytotoxic T lymphocytes may offer a unique approach to stimulate a broad tumor-immune response using whole protein vaccination as a form of antigen specific immunotherapy (56, 57). Based on these findings a phase II trial was performed randomizing patients with completely resected MAGE-3 positive stage IB and II NSCLC to either post-operative vaccination with recombinant MAGE-3 protein or placebo. When reported in 2007 a total of 30.6% patients in the vaccine arm and 43.3% in the placebo arm had recurred (58). While these differences did not reach statistical significance the data did suggest a trend of improved outcomes in the vaccination group and only few vaccine related side effects were recorded. Such reassuring data led to the implementation of a larger phase III trial adjuvant therapy trial, powered to detect differences in survival (5). The MAGRIT study (MAGE-A3 Adjuvant Non-Small Cell Lung Cancer Immunotherapy) is designed to enroll 2,270 MAGE-A3 positive patients after anatomic resection of stage IB, II, or IIIA NSCLC. All patients will be randomized in a 2:1 fashion to receive either the vaccine or placebo with a clinical end point of disease free survival (59).

Another very novel approach designed to harness the power of the immune system to treat lung cancer has been advanced by a group from Cuba. Clinical application of this approach is based on the finding that certain subsets of NSCLC express the epidermal growth factor (EGF) receptor and inhibition of this signaling pathway has been shown to have a therapeutic effect (60). The EGF vaccine is produced in the Center of Molecular Immunology in Havana, Cuba and is composed of recombinant EGF chemically conjugated to the P64 Neisseria meningitides recombinant protein adjuvant. The clinical efficacy of this vaccination strategy has been recently reported in a large randomized phase II trial (61). In this trial a total of eighty patients with stage IIIB/IV NSCLC were randomized to receive either vaccination or best supportive care after finishing first line chemotherapy. While few side effects were observed in the vaccinated group over half of the vaccinated patients demonstrated a good anti-EGF antibody response with a significant decrease in the serum EGF concentration. There was a trend for improved survival in the vaccination group that reached statistical significance in patients younger then 60 years of age. This very novel line of investigation offers the opportunity to target other cytokine or chemokine pathways responsible for growth and maintenance of lung cancer.

Multiple other trials targeting TAA in NSCLC, such as carcinoembryonic antigen (62) and HER-2/neu (63), have been described and are currently in clinical trials. While recent dramatic success with adoptive cell therapy has been demonstrated for malignant melanoma (26) such strategy has not yet been adapted to the field of lung cancer immunotherapy due to the lack of a well-defined and established immunodominant tumor antigen. Perhaps success in clinical trials using vaccination with defined peptides or whole proteins will pave the way for adoptive cell therapy in the future.

Unlike NSCLC, small-cell lung cancer (SCLC) represents only 15-20% of all primary malignancies the lung. SCLC is usually a systemic disease at the time of diagnosis and only anecdotal long-term survival has been reported. Even limited disease, although initially responsive to chemo and radiation therapy, often recurs (64,65). Based on these reasons biologic therapy, such as immunotherapy, that can offer a long-term tumor-specific response may be an ideal treatment modality for this malignancy. Gangliosides are glycolipid components of the plasma membrane that play an integral role in multiple biologic functions. Compared to the normal cellular components of the lung, neutral glycolipids are more prevalent in SCLC (66). While poorly immunogenic in and of themselves, these antigens can be successfully targeted by anti-idiotypic antibodies. This form of immunotherapy takes advantage of the fact that "secondary" antibodies raised against a primary antibody to a defined antigen, even one of low affinity, will mimic epitopes present in the original antigen in the hypervariable region of the immunoglobulin. Administration of such anti-idiotypic antibodies can then be used to generate both a humeral and cellular TAA-specific immune response as the antibody itself acts as an antigen (67,68). GD3 is a ganglioside present in tumors of neuroectodermal origin including small cell lung cancer. While itself poorly immunogenic (69), the anti-idiotype antibody to this antigen (Bec2) produces a detectable anti-GD3 immune response in as many as one-third of the treated patients (70,71). A small pilot study of Bec2 vaccination combined with BCG performed at Memorial Sloan-Kettering Cancer Center, revealed a surprisingly positive response in the 15 treated patients and acted as a nidus for a large randomized prospective trial (72). Such a study was performed between 1998 and 2002 as 515 patients from 120 institutions in 17 countries with limited SCLC were prospectively randomized to receive either vaccination with Bec2 and a BCG adjuvant or observation alone after completing induction chemoradiotherapy. When published in 2005 the investigators were unable to detect an improvement in overall survival, progression-free survival, or quality of life in those in the vaccination arm (4). While a trend towards prolonged survival was detected in the one third of patients who developed a detectable humoral immune response to the vaccination, this did not reach statistical significance. Toxicity was mostly related to local skin-site irritation with occasional systemic effects such as lethargy, arthralgia, and myalgia. Thus while this Bec2 vaccination trial presented an overall negative result the potential for vaccination and immune-based therapy as an adjuvant method for treating SCLC remains a future possibility if vaccines capable of producing a better immunologic response can be

developed.

Limitations and future directions

Immune based therapy for the treatment of solid cancer remains an exciting approach that is quickly making headway from the laboratory to the clinic. A cautionary and rational approach needs to be taken, however, in order to prevent negative results from halting progression of this field. First of all the potential for autoimmune disease resulting from successful immunotherapy of what is essentially altered self requires a cautious approach to clinical experimentation. Even with current therapy autoimmune vitiligo is often a complication of immunotherapy for malignant melanoma and the use of allogenic bone marrow transplantation as a mechanism of immunotherapy for leukemia is often complicated by graft vs. host disease (73-75). Lessons learned from notable examples such as the National Emphysema Treatment Trial of lung volume reduction surgery, or the recent human gene therapy trial for ornithine transcarbamylase deficiency complicated by a patient death, demonstrate that patient selection is critical for maintaining public enthusiasm and support for clinical trials (76-78). Second of all it is important to note that multiple current trials of immune based therapy enroll patients with residual bulky primary or metastatic disease. Such a strategy is counterintuitive to data demonstrating that tumor volume directly impacts the ability to mount a successful tumor-specific immune response (79) . It also goes against experimental data, from out laboratory as well as others, which demonstrate that tumor associated stromal cells, both radiosensitive hematopoietic myeloid cells and radioresistant endothelial cells, can influence the tumor immune response (80-82). Thus a potential therapeutic approach might combine immunotherapy with complete gross tumor resection or aggressive debulking in order to target minimal residual disease in the adjuvant setting. Patient enrollment in the MAGRIT trial described above, for example, is limited only to those with completely resected disease, thus avoiding the immunologic barriers imposed by a bulky tumor mass (58,59).

Summary

Unlike the case for malignant melanoma, immunotherapy for NSCLC lags behind in both experimental and clinical development. In fact most clinical trials are based on successful therapy defined in preclinical models for malignant melanoma. It is possible, however, that lung cancer may not respond to similar immunotherapy strategies. Since the lung is a naturally tolerogenic organ and intratracheal instillation of antigen is a successful and accepted method for induction of tolerance (83), it is possible that lung cancer directed immunotherapy needs to be routinely combined with strategies to downregulate mechanisms of tolerance induction (33). It is also possible that tumor immunoediting, or the elimination of immunodominant antigens by the immune system, is highly prevalent in lung cancer, resulting in resistance to immunotherapy (12). It is however, not inconceivable that lung cancer develops completely independent of immunologic influences and remains completely invisible to the immune system. If this were to be the case other strategies, such as targeted therapy of growth factor receptors, might be necessary for adjuvant treatment of lung cancer instead of immunotherapy (60).

Inbred mice offer an efficient and reproducible method to study carcinogen-induced lung cancer as a highly reliable model of lung adenocarcinoma exists. A single injection of urethane leads to the development of early adenomatous tumors in the lung within 10 weeks with progression to fully invasive adenocarcinoma after 32 weeks (84). The progression of such cancers from hyperplasia to benign adenoma to fully invasive adenocarcinoma with metastatic potential mimics progression of human disease. Interestingly multiple studies have defined different strains of mice as either tumor-susceptible "high-responders" (A/J for example) or tumor resistant "low responders" (C3H He/J for example) with 100% of susceptible animals and few resistant ones developing tumors after injection of carcinogen (85). This variability in strain susceptibility to induction of lung adenocarcinoma suggests that inherent genetic factors may alter the development and/or progression of NSCLC independent of carcinogen administration or the environment (85). The use of such models to develop a better understanding of lung cancer immunology and immune evasion, as has been done for malignant melanoma, might facilitate a more rational approach toward lung cancer immunotherapy.

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