Immune Modulation as a Therapeutic Strategy for Non–Small-Cell Lung Cancer

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ABSTRACT

Active tumor immunotherapy may provide hope for patients with non–small-cell lung cancer (NSCLC) because, in more than 20 years, current therapies have yet to change mortality statistics. Creating an efficacious vaccine involves selection of important tumor antigens and formulation of their immunogenic epitopes into a construct for delivery to antigen-presenting cells. The method of immunization will confer significant properties to the potency of the vaccine and might require augmentation with certain adjuvant agents like interleukin-12 and granulocyte-macrophage colony-stimulating factor. So far, clinical trials in NSCLC immunotherapy have shown promise with the induction of immune responses and the presence of clinical responses compared with historical controls treated with standard therapy. Immunotherapy could merge seamlessly into the current standard of care for NSCLC with the emergence of data supporting a beneficial role of chemotherapy and radiation in the production of antitumor immune responses. With continued work in this field, active immunotherapy may provide the necessary therapy for the successful treatment of this common disease.

Key words: Cytokines, Epitope, Immunogenic agents, MUC1, Tumor antigens, Vaccine therapy

INTRODUCTION

Lung cancer is the leading cause of cancer-related death in the United States, with estimates in 2007 attributing more deaths to lung cancer than breast, prostate, and colon cancer combined.1 Unfortunately for people with this disease, it is usually diagnosed at advanced stages, resulting in 5-year survival rates between 1% and 5%. For non–small-cell lung cancer (NSCLC), surgical resection represents the only truly curative therapy, but with relapse rates of > 40% after definitive resection, oncologists need better therapies to improve patient survival.2

Active immunotherapy provides a unique approach to potentially consolidate surgery and/or standard chemotherapy. Stimulating the immune system through the induction of a cellular immune response to harness CD4+ T-helper cells and CD8+ cytotoxic T lymphocytes (CTLs) capable of selectively destroying cancer cells by targeting the antigens they express could result in tumor eradication. Use of lymphocytes as a therapeutic intervention takes advantage of the immune system's specificity, surveillance properties, and memory induction. The remarkable specificity of the immune system would theoretically allow a more targeted therapy with fewer side effects than the generalized effect that chemotherapy and radiation have on all proliferating cells. The ability of the immune system to circulate throughout the body could allow the removal of micrometastases that remain cryptic until they grow to clinical significance.3 In addition, production of a long-lasting memory response would theoretically protect against new tumors that might arise through the effect of field cancerization.4 The growth kinetics of NSCLC makes the disease potentially amenable to immunomodulation. Given the usual late presentation of NSCLC, the predominant time course from diagnosis to disease progression is 1 year on average. This interval allows time for standard of care therapy followed by weeks of repetitive cycles of immunization and evaluation for clinical effect. Because it is estimated that there will be > 213,000 new cases of NSCLC this year, predominantly presenting in advanced stages without available curative therapy, there will be vast opportunities for clinical trials.1

More recent data suggest lung cancer, previously thought to be a relatively nonimmunogenic tumor, might stimulate the immune system. To date, there have been a multitude of antigens identified in lung cancer, many of which have been found to be immunogenic in other cancer models. Given the growing collection of antigens expressed in NSCLC, its
**Immune Modulation in NSCLC**

growth kinetics, and the large numbers of patients affected by the disease, this tumor represents an important model for clinical trials in immunotherapy.

**Antigen Selection**

The future success of any vaccine depends on the exact selection of antigens because their inclusion dictates the potential scope of the induced immunity. A NSCLC vaccine will require the inclusion of a diverse collection of antigens because there is variable expression of antigens in individual tumors. Several antigens, such as Wilm's Tumor Antigen 1 (WT1) and carcinoembryonic antigen (CEA), have been found to be expressed in 96% and 70% of NSCLCs, respectively.\(^5,6\) Other antigens such as MAGE-A4 and NY-ESO-1 have been found to be expressed in only 28% and 8% of NSCLCs, respectively, and their inclusion in a vaccine might have less of an impact.\(^7\) Ensuring that specific antigens are not mutually exclusive is also important. Thus, the choice of antigens will be partially dictated by the breadth and frequency to which they are expressed in individual NSCLCs.

In addition, tumors have been known to exhibit potent immune escape mechanisms, including loss of antigens,\(^8,9\) mutagenesis of targeted epitopes,\(^10\) and downregulation of major histocompatibility complex (MHC) molecules.\(^11,12\) Given the continuous state of mutagenesis in cancer cells, the predictable loss of the specific targeted antigen, epitope, or MHC haplotype by a NSCLC cell would effectively render it immune to the vaccine's effect, and the tumor would continue to proliferate. To counteract these pitfalls, it will be necessary to incorporate multiple epitopes of several antigens with varying MHC haplotype restrictions. Moreover, the vaccine must elicit potent tissue-destructive inflammation. Thus, antigen selection and vaccine delivery are keys to the success of lung cancer vaccines.

One caveat to the need for inclusion of multiple antigens in a vaccine formulation concerns the idea of epitope spreading. Theoretically, epitope spreading occurs after the initial immune response modulates the tumor microenvironment to enhance presentation of tumor cell antigens by antigen-presenting cells (APCs).\(^13\) This broadening of the immune response could strengthen immunity via the diversification of the immune responses to antigens not incorporated in the original vaccine. Generation of epitope spreading underscores the need for a powerful initial immune response necessary to cause the inflammation needed to activate APCs.

It will be critical to select antigens that have some biologic significance promoting the growth of the NSCLC cells. Targeting proteins necessary for cell proliferation or metastasis would remove the cells with the most aggressive phenotype or, theoretically, create a situation in which the loss of 1 of the antigens might prevent total tumor eradication but result in the outgrowth of tumor cells with a less aggressive phenotype. WT1 is a transcription factor found to have important tumorigenic effects in many tumors and has been found to be overexpressed in 96% of NSCLCs.\(^5\) Additionally, increases in matrix metalloproteinase 2 in NSCLC cells is seen in patients with advanced metastatic disease and might represent an ideal antigen to pursue.\(^14\)

One final consideration of antigen selection involves the changing scope of expressed antigens depending on tumor stage. Cho et al showed the differential protein expression of antigens in small-cell lung cancer cells depending on the stage of a patient's cancer.\(^15\) Most likely, a similar shifting antigenic repertoire with disease stage is operative in NSCLC as well. Additionally, the multiple subclasses of NSCLC will also dictate a change in the selected antigens as demonstrated by MAGE-A4, which is expressed more often in squamous cell carcinomas than in adenocarcinomas.\(^16\)

**Vaccine Formulation**

The final formulation of a vaccine can be divided into 2 separate components, each with significant variables that will influence its potency. First, vaccines typically include full-length copies of the antigen or the individual epitope, described as the minimal sequence necessary to fit into an MHC groove and induce an immune response. Second, the chosen form of the antigen must be delivered to APCs via an immunization strategy, usually in the form of free antigen emulsified in adjuvant, whole tumor cell, dendritic cell (DC), viral, or DNA-based methodology.

To minimize the need for antigen discovery and include all relevant antigens by default, whole-cell tumor vaccine trials have been conducted, and clinical trials have been performed in patients with NSCLC. Three phase I/II clinical trials have been conducted in which the patient's tumor was resected and transferred with the virus for production of granulocyte-macrophage colony-stimulating factor (GM-CSF) prior to immunization, creating a vaccine known as GVAX.\(^9,17,19\) Although in early phase, 2 of the studies demonstrated small but significant tumor responses in a minority of patients who received the autologous tumor cell vaccine. Specifically, 2 patients exhibited radiologic evidence of tumor regression with durable complete responses (CRs) lasting 6 months to > 22 months. In addition to the small clinical effect, one of the more pressing limitations of the above-mentioned studies lies in the difficulty of creating the vaccine for all enrolled. Although investigators were able to generate vaccine for 81%–97% of the enrolled patients, the reasons for failure were predominantly microbiologic contamination and insufficient tumor cell numbers. Expanding this vaccine method to the general population will be difficult because it demands collection of tumor tissue that might not be possible without increased risk in many patients as a result of their comorbidities.

To circumvent the problem of developing autologous cell-based vaccines, the identification of broadly expressed antigens that are present in NSCLC cells would allow their specific inclusion in an antigen-specific vaccine that could be uniformly manufactured. Modern techniques allow for the production of antigens in nucleic acid, viral, or protein forms capable for long-term storage, thus alleviating production problems.

Human telomerase reverse transcriptase (TERT) has been associated with NSCLC, and recently, 2 trials have evaluated the ability of subcutaneously injected TERT peptides to induce immune responses and clinical effects. Bolonaki et al vaccinated 22 patients with advanced NSCLC with a single peptide subcutaneously, with only minor skin reactions as adverse effects.\(^20\) Peptide-
specific CTLs were found in 91% of patients who received the full vaccination course (n = 11). Compared with nonresponders, patients with elicited CTLs had a statistically significant longer time to progression (4.2 months vs. 2.3 months; log-rank P = .046) and overall survival (OS; 30 months vs. 4.1 months; P = .012) at a median follow-up time of 10 months. In a previously published study also using a vaccine consisting of subcutaneously injected TeRT peptides, the only CR was seen in a patient who developed peptide-specific CTLs.21 In congruence with the postulated mechanism of action, the induction of peptide-specific effector cells is required for production of clinical effect. Improvement of the vaccine’s potency is thus critical for development of therapeutic interventions with better efficacy.

One of the more robust studies evaluated the immunization of patients with stage IIIb and IV NSCLC with a liposomal formulation of a MUC1 peptide in hopes of improving the strength of the induced immune response.22 A total of 171 patients were randomized to receive the vaccine with best supportive care (BSC) or BSC alone and were followed for clinical effects as well as immunologic parameters. Although not statistically significant, there was a 4.4-month median increase in OS for patients immunized with the vaccine versus those who received BSC (P = .112). In subgroup analysis, patients with stage IIIb locoregional disease demonstrated the best responses because their median survival time was unavailable at time of publication, compared with 13.3 months for BSC (P = .069). Such studies have been viewed as encouraging because improvements in antigen breadth and vaccination potency might achieve better results.

Kosmrl et al conducted experiments in which patients with NSCLC were vaccinated with DCs loaded with MUC1 peptides or tumor lysate and evaluated for clinical outcome.23 The investigators demonstrated a survival advantage in vaccinated patients whose tumors were found to express MUC1 compared with patients with MUC1-negative tumors (16.75 months vs. 3.8 months, respectively; P = .0101). Although the numbers of subjects are limited in this study, all of the patients who exhibited partial responses were vaccinated with peptides rather than tumor lysate.

The observed increased potency of an epitope-focused approach over a vaccine consisting of the full-length antigen, i.e., as part of the tumor lysate, is not a new concept and has been explored in murine models.24 This advantage might stem from the isolation of the pertinent epitopes of the antigen without diluting them with immunologically irrelevant sequences. Vaccines incorporating multiple epitopes from the same antigen produce strong immune responses to each peptide in a codominant manner instead of a single immunodominant peptide as can occur in vaccines using the full-length antigen. Because some of the targeted antigens represent oncoproteins, there is a select advantage of including only short segments of the gene rather than the full-length transcript with its associated biologic activity.

In the hopes of achieving better presentation of antigens, researchers have sought different mechanisms of delivering the vaccine to APCs and modulating the milieu toward one that is more favorable for inducing immune responses. Morse et al directly added antigen to ex vivo-generated DCs via an engineered fowlpox virus that expressed full-length CEA and TRICOM, an adjuvant agent consisting of 3 co-stimulatory molecules: B7.1, intracellular adhesion molecule (ICAM)-1, and lymphocyte function-associated antigen 3.25 Although only 3 of the 14 patients had NSCLC and the author did not differentiate the responders’ underlying disease, they were able to induce T-cell responses in 71% of patients, with 5 exhibiting stable disease (SD) for 3 months.

Taking advantage of the natural immunization via viral infection, modified vaccinia virus was engineered to express the entire MUC1 gene and subsequently used to vaccinate patients with advanced cancer expressing MUC1.26 Of the 14 patients, 4 were found to have SD for 6-9 months, and 5 had induced T-cell responses. One of the 3 patients with NSCLC was actually removed from the study because of rapid disease progression. Without further systemic therapy, his disease ultimately decreased in size after enlarging from what, in retrospect, might have been an inflammatory response to his tumor. His disease eventually progressed to metastasize to bone 14 months after vaccination, and he died nearly 2 years later. This clinical course is remarkable considering that his disease before vaccination was described as multiple pulmonary nodules with mediastinal lymphadenopathy, bilateral kidney metastases, presumed liver metastasis, and multiple abdominal lymph nodes.

The use of DNA-based vaccines has steadily increased after the initial finding of the ability to induce gene expression after injection of naked DNA into the muscle of a mouse.27 Because of the increased concentration of DCs in the skin,28 increased velocity of CTL induction with an intradermal route versus an intramuscular injection was demonstrated and argues for that route of immunization using DNA-based strategies.29 Thompson et al demonstrated the strength of a DNA approach through the injection of a DNA construct encoding 8 class I-restricted epitopes in mice, resulting in the induction of a strong immune response to each peptide with the ability to protect the mice from a challenge of tumor cells.30 In addition, the easily obtained consistency in creating multiple copies of a DNA vaccine, coupled with its relative ease and low production cost compared with other methods, makes it a highly attractive method of vaccination.

Although the injection of naked DNA into mouse muscle results in immune responses, several modifications were necessary before the approach could be considered immunogenic. Constructing a DNA-based vaccine of epitopes in a “beads on a string” method in which each peptide is included sequentially in the translated protein was made more potent when defined spacer sequences were installed between epitopes. This method was further augmented to the point where vaccination could result in rejection of established tumors in mice through the C terminus addition of an ubiquination signal.31

Multiple adjuvant agents have also been tested for their ability to augment the efficacy of a DNA-based vaccine.32 These additives tend to influence the cytokine milieu, target the DNA toward APCs, or protect the DNA from degradation. Some of the more successful methods include the addition of unmethylated oligonucleotides of alternating cytosine and guanine residues, which interact with toll-like receptors, fusion of heat shock protein–70 to the target protein, or addition of genes.
for cytokines like interleukin-123,5 and GM-CSF.3,4 Carionic or anionic liposomal additives seem to enhance humoral and cellular responses through easy fusion with cellular membranes with cytosolic deposition of the genetic material and increasing stability compared with naked DNA.

The route of vaccination has also been evaluated for its ability to induce a strong immunity and seems to point toward a parallel between immunogenicity and deposition of antigen in close proximity to DCs. The higher quantity of Langerhans cells in the dermis probably accounts for the seemingly greater immune response when a vaccine is given intradermally versus intramuscularly.28 In fact, a method that uses a "tattoo" of genetic material found gene expression within the dermis and resulted in the rejection of established tumors in mice.35

To date, there exists few clinical trials evaluating DNA-based vaccines, but studies of the approach are beginning to be reported. Early phase I studies have used the injection of naked DNA containing complete genetic sequences for gp100 or prostatic acid phosphatase for the evaluation of safety in patients with melanoma or prostate cancer, respectively.26,37 Although preliminary studies, both showed induction of specific immune responses without achieving clinical effects.

DNA vaccination is not without risk; however, the apparent toxicities remain hypothetical because they have yet to be documented in mouse or human experiments. There is a theoretical risk of the induction of anti-DNA antibodies akin to a lupus-like syndrome. In mice, it is possible to induce anti-DNA antibodies, but it requires mice with an unusual autoimmune phenotype, and the induced antibodies typically have different specificities than those found in patients with lupus.8 Moreover, the constitutively active viral promoter used to drive overexpression of the desired vaccine transcript has the potential to insert near a proto-oncogene with subsequent cellular transformation. Thankfully this phenomenon has not been observed clinically and remains a theoretical concern.

To date, immunotherapy trials in NSCLC have shown safety and immune responses but without significant therapeutic efficacy (Table 1).17-23,25,39-51 In part, this is secondary to the fact that the majority of these studies are early phase I/II clinical trials with small numbers of patients. Additionally, these trials are often uncontrolled experiments, and thus, all comparisons are made to historical controls with the limitations that lie therein. Most importantly, the majority of the studies are immunizing patients with significant tumor burden and evaluating for tumor regression. Active immunization might be more effective in tumor prevention or prevention of disease relapse rather than in actively vaccinating cancers in which standard chemotherapy has failed. Predominantly, these experiments have also typically targeted

### Table 1. Summary of Clinical Trials Involving Active Immunotherapy in Non-Small-Cell Lung Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Antigen</th>
<th>Method</th>
<th>N</th>
<th>Immune</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fong et al (2001)39</td>
<td>CEA</td>
<td>DC + peptide</td>
<td>2</td>
<td>+*</td>
<td>-</td>
</tr>
<tr>
<td>Ich et al (2002)40</td>
<td>CEA</td>
<td>DC + peptide</td>
<td>2</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Chan et al (2003)41</td>
<td>Autologous tumor cells</td>
<td>Adoptive T-cell transfer</td>
<td>21</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Kontani et al (2003)33</td>
<td>MUC1</td>
<td>DC + peptide or lysate</td>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mine et al (2003)42</td>
<td>SART, CYII, 1ck, ART</td>
<td>Peptide</td>
<td>10</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Raez et al (2003)43</td>
<td>Allogeneic tumor cells</td>
<td>Whole tumor cell</td>
<td>14</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Salgia et al (2003)37</td>
<td>Autologous tumor cells</td>
<td>Whole tumor cell</td>
<td>35</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Ueda et al (2004)46</td>
<td>CEA</td>
<td>DC + peptide</td>
<td>5</td>
<td>+</td>
<td>TM</td>
</tr>
<tr>
<td>Butts et al (2005)32</td>
<td>MUC1</td>
<td>Peptide</td>
<td>171</td>
<td>NP</td>
<td>+</td>
</tr>
<tr>
<td>Morse et al (2005)25</td>
<td>CEA</td>
<td>DC + peptide</td>
<td>3</td>
<td>+*</td>
<td>HC</td>
</tr>
<tr>
<td>Morse et al (2005)47</td>
<td>MAGE-A3, -A4, -A</td>
<td>DC + peptide</td>
<td>13</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Nemunaitis et al (2006)48</td>
<td>TGF-β</td>
<td>Antisense RNA</td>
<td>75</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Nemunaitis et al (2006)19</td>
<td>Autologous tumor cells</td>
<td>Whole tumor cell</td>
<td>86</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bolonaki et al (2007)50</td>
<td>TeRT</td>
<td>Peptide</td>
<td>22</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>González et al (2007)49</td>
<td>EGF</td>
<td>Protein</td>
<td>83</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mayordomo et al (2007)51</td>
<td>Tumor lysate</td>
<td>DC + lysate</td>
<td>2</td>
<td>+</td>
<td>HC</td>
</tr>
<tr>
<td>Meyer et al (2007)51</td>
<td>Ras</td>
<td>Peptide</td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Immune responses elicited; however, the authors do not specify whether they were from patients with NSCLC.
*Trend toward improved time to progression and overall survival.

Abbreviations: EGF = epidermal growth factor; HC = positive clinical results compared with historical controls; NP = not performed; TGF = transforming growth factor.

TM = positive clinical results when vaccination shows a decrease in serum tumor markers.
### Table 2: Immunogenic Antigens Found to be Expressed in Non-Small-Cell Lung Cancer

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Level of Expression</th>
<th>Immunogenicity</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-3-3 Theta</td>
<td>8/14 (57%)</td>
<td>Humoral</td>
<td>Cell signaling</td>
</tr>
<tr>
<td>α1 Anti-Trypsin</td>
<td>NA</td>
<td>Humoral</td>
<td>Protease inhibitor</td>
</tr>
<tr>
<td>Annexin 1</td>
<td>NA</td>
<td>Humoral</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>Annexin 2</td>
<td>NA</td>
<td>Humoral</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>CAYB R</td>
<td>13/36 (36%)</td>
<td>Humoral</td>
<td>Cancer-testes antigen</td>
</tr>
<tr>
<td>CAGE</td>
<td>7/7 (100%)</td>
<td>Humoral</td>
<td>Cancer-testes antigen</td>
</tr>
<tr>
<td>c-Myc</td>
<td>11/19 (58%)</td>
<td>Humoral</td>
<td>Transcription factor</td>
</tr>
<tr>
<td>Cyclin B1</td>
<td>17/77 (22%)</td>
<td>Humoral</td>
<td>Cell cycle regulation</td>
</tr>
<tr>
<td>GBU4-5</td>
<td>NA</td>
<td>Humoral</td>
<td>DEAD-box protein</td>
</tr>
<tr>
<td>KK-LC-1</td>
<td>40100 (40%)</td>
<td>CTL</td>
<td>Unknown function</td>
</tr>
<tr>
<td>L514S</td>
<td>~50%</td>
<td>Humoral</td>
<td>Unknown function</td>
</tr>
<tr>
<td>L552S</td>
<td>~50%</td>
<td>Humoral</td>
<td>Cancer-testes antigen</td>
</tr>
<tr>
<td>Livin/ML-IAP</td>
<td>13/17 (76%)</td>
<td>CTL</td>
<td>Inhibitor of apoptosis</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>13/157 (8%)</td>
<td>CTL</td>
<td>Cancer-testes antigen</td>
</tr>
<tr>
<td>Protein Gene Product 9.5</td>
<td>19/95 (19%)</td>
<td>Humoral</td>
<td>Protease</td>
</tr>
<tr>
<td>Retinol-Binding Protein</td>
<td>NA</td>
<td>Humoral</td>
<td>Transporter protein</td>
</tr>
<tr>
<td>Survivin 2B</td>
<td>17/42 (40%)</td>
<td>CTL</td>
<td>Inhibitor of apoptosis</td>
</tr>
<tr>
<td>Tara</td>
<td>NA</td>
<td>CTL</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>U7-A snRNP</td>
<td>NA</td>
<td>Humoral</td>
<td>Ribosomal nucleoprotein</td>
</tr>
<tr>
<td>Ubiquilin-1</td>
<td>NA</td>
<td>Humoral</td>
<td>Proteosome regulator</td>
</tr>
<tr>
<td>XAGE-1b</td>
<td>15/49 (31%)</td>
<td>T helper</td>
<td>Cancer-testes antigen</td>
</tr>
</tbody>
</table>

This table represents antigens found to be expressed on NSCLC tumors and have been shown to induce immune responses in patients with lung cancer. None of these antigens have been used in clinical trials of lung cancer immunotherapy and, thus, might represent novel targets for future study.

*Exact numbers not available in report.

Abbreviations: CTL = cytotoxic T-lymphocyte-specific immune responses; NA = not available

Single antigens or used a non-epitope-focused approach. Specific enhancements in antigen selection and optimization of vaccine strategy should produce better clinical responses.

### Chemotherapy and Radiation Treatment

Currently, chemotherapy and radiation play an important role in the current treatment strategies for most stages of NSCLC, with the sole exception in stage IA disease. It could be argued that systemic chemotherapy and radiation would have negative effects on an anticancer immune response through pervasive immunosuppressive effects. Indeed, it has been shown that the number of prevaccination chemotherapy regimens is negatively correlated with T-cell responses. Additionaly, T-cell responses from a vaccine improve with time after the last cycle of chemotherapy, further providing evidence as to the negative effect chemotherapy has on tumor vaccines. There exists equipoise on this matter, however, because the impact of chemotherapy was not seen in a mouse model, where peptide-vaccinated mice were protected from lethal tumor cell challenges despite concurrent administration of cisplatin.

Indeed, there is a growing body of evidence that chemotherapy and radiation might have significant benefits to immunotherapy. In colon cancer, the use of cisplatin has been associated with increases in ICAM-1 and fas expression with resultant increases in the level of fas-dependent and fas-independent cell killing. In a murine Lewis lung cancer model, the use of 1 dose of cisplatin augmented immunity, resulting in improved survival when the agent was given before vaccination. Radiation might also augment immune responses, as several patients with lung cancer have demonstrated upregulation of fas, ICAM-1, and MHC class I expression after treatment. The immune-enhancing effects of chemoradiation are particularly important when contemplating immunization in NSCLC as cisplatin and radiation are the standard of care for this disease.

To further elucidate the important role chemotherapy might play in enhancing immune responses, Nowak et al reported on a mouse model that used the ligation of CD40 to stimulate anticancer immunity. Mice given gemcitabine followed by the vaccine fared better than mice given the vaccine alone. However, administration of gemcitabine after the vaccine abrogated the immune response and tumor protection. This augmenting effect of gemcitabine was not a result of debulking the tumor burden, as cohorts of mice who underwent partial resection of the tumor mass to the same size as the gemcitabine-treated animals had lower survival rates when otherwise given identical therapy. Thus, timing of the administration of chemotherapy might have important effects on the development of an immune response after active immunization.
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Because gemcitabine is also a frequently used chemotherapeutic agent for the treatment of lung cancer, these observations might have clinical significance for treating humans. Although the mechanism is not completely elucidated, the improvement in immune responses with chemotherapy might result from the processing of necrotic or apoptotic tumor cells by the draining lymph nodes. The vaccine then boosts this initial immunity induced via chemotherapy. In terms of translating these observations into the human experience, it would appear to be advantageous to vaccinate patients after usual therapy involving surgical resection followed by chemotherapy with cisplatin/gemcitabine and possibly radiation as indicated.

Conclusion

In conclusion, the sheet number of patients, coupled with a lack of curative therapy for most patients with NSCLC, makes this cancer a necessary disease for the evaluation of new modalities of treatment. The theoretical and somewhat proven ability to treat tumors, at least in mice, makes vaccination a promising method that one day might provide new hope to those afflicted.

The relative newness of the therapy and current lack of clinical success indicates a continued need for experimentation. The exact selection of antigens needed is unknown but will most likely require multiple epitopes of a diverse set of genes restricted to multiple haplotypes to generate a vaccine that will combat the various tumor escape mechanisms. As new proteins are discovered in NSCLC and found to be immunogenic, researchers can begin to test their suitability for inclusion in vaccines (Table 2).

Use of an epitope-focused approach takes advantage of its ability to produce a strong, codominant immunity toward each immunogenic peptide involved without risking inclusion of the entire gene and its associated function.

The route and method of vaccination is also debatable, but a DNA-based vaccine has many advantages. Although DCs and viral-based vectors have also shown strength and superiority over peptide and whole-cell–based vaccines, DNA is not as labor intensive to use as DCs or as potently risky as viral vaccines. With DNA, there is neither a need for prolonged culturing of cells nor concerns over neutralizing antibody responses.

Finally, the timing of the immunization to augment current standard of care is an important issue. The risk of surveillance and, thus, select removal of micrometastatic disease will be tested in patients after resection. Application of vaccines after chemotherapy and radiation might be a more effective method to enhance tumor-specific immunity.

The promise of an immunotherapy in cancer is a daunting challenge and one that will hopefully live up to its potential. To create a vaccine that will alter current morality in NSCLC would be of great benefit to society as outcomes from this disease have not changed in more than 20 years.

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