Therapeutic cancer vaccines: Using unique antigens

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A decade ago, it seemed rational that our rapidly increasing knowledge of the molecular identities of tumor antigens and a deeper understanding of basic immunology would point the way to an effective therapeutic cancer vaccine. Significant progress has been made, but we do not yet have a cancer vaccine that can reliably and consistently induce tumor destruction or improve patient survival. Random mutations in cancer cells generate unique antigens in each individual, and this may be important in terms of generating a therapeutic immune response. Autologous heat shock protein–peptide complexes produced from each patient’s tumor is a logical personalized approach that may obviate the need to identify the unique antigens contained in the individual vaccine. Heat shock proteins elicit adaptive and innate immune responses and have been tested in a variety of animal models and different human cancers. Activity has been seen in several animal studies. Early-phase human studies have also suggested some activity in certain cancers. Large, randomized phase 3 studies are ongoing, and these will effectively answer the question of efficacy regarding this approach to therapeutic vaccination. There are sufficient data to support the notion that cancer vaccines can induce antitumor immune responses in humans with cancer. How best to translate this increase in immune responsiveness to consistently and reproducibly induce objective cancer regression or increased survival remains unclear at this time.

Lessons from Melanoma

Much experimental work with cancer vaccines is done in patients with metastatic melanoma. There are three valuable lessons in examples of treatments in melanoma that seemed to show clear clinical benefit in early-phase trials but were unable to be proven efficacious in large, phase 3 randomized studies. The first is the Dartmouth regimen (dacarbazine, cisplatin, carmustine, and tamoxifen) for stage 4 melanoma. Several single-institution phase 2 trials reported that the Dartmouth regimen can induce major tumor responses in 40–50% of stage 4 melanoma patients (8, 9). In a subsequent large phase 3 randomized study of 231 patients, there was no difference in survival time between patients treated with Dartmouth regimen vs. dacarbazine alone (10).

The second is the addition of immunotherapy to chemotherapy (biochemotherapy). Several phase 2 trials with biochemotherapy have shown encouraging response rates in metastatic melanoma, and metaanalyses and one small, single institution, phase 3 trial suggested survival benefit (10–13). Subsequently, a large, phase 3 study was conducted by the U.S. intergroup (Eastern Cooperative Oncology Group, Southwest Oncology Group, and Cancer and Leukemia Group B) to determine the relative efficacy of biochemotherapy (14). Patients were randomly assigned to receive cisplatin, vinblastine, and dacarbazine (CVD) either alone or concurrent with IL-2 and IFN (biochemotherapy). In that study 405 patients were evaluated. The overall response rate was 17.1% in the biochemotherapy group and 11.4% in the CVD alone group. This did not translate into a survival advantage, and the median survival for the biochemotherapy group was 8.4 months and in the CVD alone group it was 8.7 months. Thus, although biochemotherapy appears to produce a slightly higher response rate and progression free survival than CVD alone, this does not appear to be associated with either improved quality of response or overall survival.

The third example is the GM2 ganglioside vaccine. The ganglioside GM2 is a serologically well-defined melanoma antigen and the most immunogenic ganglioside expressed on
melanoma cells. Several studies conducted at the Memorial Sloan–Kettering Cancer Center have demonstrated that administration of GM2 in combination with Bacillus Calmette-Guérin induced IgM antibodies in the majority of patients and that these antibody responses were correlated with improved recurrence-free survival and overall survival in stage 3 melanoma patients. A variety of GM2 vaccine formulations were studied, and a commercial vaccine preparation was selected consisting of GM2 coupled to keyhole limpet hemocyanin and combined with the QS-21 adjuvant (GMK). Immunization of melanoma patients with the GMK vaccine has shown to induce high titers of IgM antibodies in >80% of patients as well as IgG antibodies that had not been previously observed with GM2 plus Bacillus Calmette–Guérin. These induced anti-GM2 antibodies have been reported to mediate complement-dependent cytotoxicity and antibody-dependent cellular cytoxicity of melanoma cell lines in vitro (15–19). A large randomized study was conducted by the Intergroup mechanism (Intergroup trial E1694) (14). In that study, 880 patients with stage 3 melanoma were randomized. The trial was closed after interim analysis indicated inferiority of GMK compared with high-dose IFN.

Thus, findings in early-stage clinical trials are often not borne out in later-phase studies. In the absence of highly significant numbers of patients who experience objective responses, claims for cancer vaccine efficacy are likely only to be reliable if demonstrated by large, randomized studies.

**Heat Shock Proteins (HSPs)**

HSPs were purified from tumor cells and shown to provide protective immunity in animals in 1984 (20). Autologous HSP-based immunotherapy studies in humans started in 1995 in Berlin and 1997 in New York (7, 21). As of 2003 >500 patients with eight different types of cancer have been treated (22). The gap between the first observation in laboratory animals and human clinical trials is a testament to the intensive efforts of Srivastava and his colleagues (23–31) to understand how HSPs elicit immunity to cancer. This body of work is a noteworthy example of translational biology where remarkable efforts to understand a powerful immunological phenomenon have led to advanced clinical testing in humans.

**HSPs as Chaperones**

Because HSPs were known to be chaperones, aiding in the transport of peptides throughout the cell, it was proposed by Srivastava (24) and Srivastava and Amato (32) that HSPs isolated from tumor cells contain low-molecular-weight antigenic peptides and that these HSP–peptide complexes conferred protective immunity to cancer. Several lines of evidence corroborated this hypothesis, the most important of which has been the isolation and identification of antigenic peptides stripped from purified HSP preparations of tumor cells. The peptides were shown to be of a large variety and included cytotoxic T cell epitopes (25–31, 33–41). It is perhaps apt therefore to call HSPs the “Swiss army knife” of the immune system (42). These activities are likely important in immune rejection of cancer.

The early discovery that animals could be specifically vaccinated against autologous cancers led several investigators to search for the molecules within tumor cells that might be responsible for conferring immunity. Tumor cell lysates were fractionated biochemically, and individual fractions were tested for activity in tumor protection experiments in vivo. The protective fractions were observed to contain HSPs (24, 32, 33). Consistent with the early demonstration that immunity generated with whole tumor cells is tumor specific, it was observed that HSPs elicited immunity only to the tumor from which they were purified.

Tumor rejection activity has been seen with several different HSPs in multiple cancer models (24). When mice with bulky metastatic lesions are treated with HSPs, what is typically observed is a slowing of the rate of tumor growth or stabilization of disease. Generally, only a small proportion of the animals achieve complete tumor regression. In contrast, HSP treatment of mice with resected primary tumors or minimal residual disease confers long-lasting protection from recurrence of tumors and the vast majority of animals live their normal life span (24).

The finding that HSPs elicit specific cancer immunity is ironic because HSPs are among the most highly conserved proteins in invertebrate and vertebrate biology. How were these molecules, which are ubiquitous and so similar in structure and sequence among species, able to elicit specific cancer immunity? Adding to the mystery was the finding that no differences in gene sequences were observed between HSPs isolated from normal tissues and tumor tissues (29, 30).

Immunity elicited by HSP vaccination was shown to require professional antigen-presenting cells (APCs) and CD8+ T cells because depletion of either cell type abrogates protection. The efficiency with which HSPs elicit immune response suggested a receptor-mediated mechanism of HSP uptake by APCs, and one such receptor was recently identified as CD91 (23).

Despite their exogenous route of administration, HSP–peptide complexes prime CD8+ T cell responses, suggesting that HSP preparations treated to unbind peptides were not immunogenic. Thus, in cancer the specific immunogenicity of the HSP preparations can be attributed to the unique repertoire of antigenic peptides that exists in different cancers. The unique peptide repertoire is a product of mutations in cancers, and as mutations arise randomly, this repertoire is highly likely unique to each cancer. The HSP–peptide complexes thus confer specific immunity only to the cancer from which they are isolated.

### Table 1. Translational biology: Characteristics of HSPs in immune response observed in animals have been observed in humans

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Can induce objective tumor response</th>
<th>Binds peptides</th>
<th>Primes T cells</th>
<th>Activates innate immunity</th>
<th>HSP receptors identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Rat</td>
<td>Yes</td>
<td>Yes</td>
<td>NT</td>
<td>NT</td>
<td>Yes</td>
</tr>
<tr>
<td>Mouse</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Human</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NT, Not tested.
HSP-chaperoned peptides gain access to the MHC class I antigen processing pathway in the immunized host’s dendritic cells and macrophages.

In studying the interaction of purified HSP preparations with various immune cells in both murine and human systems, it became clear that HSPs elicit a variety of innate immune responses that are independent of chaperoned peptides. These responses include cytokine and chemokine release by antigen-presenting cells and T cells, maturation of dendritic cells, and induction of migration of dendritic cells to draining lymph nodes. Several studies suggest that this innate immune response elicited by HSPs contributes to cancer immunity; however, the strongest anti-tumor effects are observed upon concurrent priming of tumor-specific T cells by HSP-chaperoned tumor-specific peptides.

Translation: Human Clinical Trials

Autologous HSP vaccines deliver a personalized vaccine targeting the individually unique antigenic repertoire of each cancer. The unique antigens used for vaccination, the HSP-bound peptides, do not need to be identified to create the vaccine. The translation of autologous HSP vaccination with unique antigens is logical. These molecules constitute one of the most highly conserved structures and systems in biology, and one could argue would be more likely to translate from laboratory to clinic (see Table 1). In addition, the use of antigens unique to an individual cancer may reverse the inefficiency of translating from homogeneous laboratory models into heterogeneous human tumors. The first autologous HSP vaccine introduced in clinical trials was HSPPC-96 (Oncophage), gp96 HSP peptide complex, purified from resected tumor tissue and then formulated for intradermal or s.c. injection. Since 1995, 10 phase 1 or 2 clinical studies have been performed to define the safety profile of the vaccine, explore and characterize immune responses, identify the most practical and effective dose and route, and test for clinical activity (see Table 2) (22).

It is important to note that for phase 1 or 2 clinical trials, interpretations of data are often confounded by variables inherent in any early, nonrandomized clinical trial. Thus, in the absence of a clinical response rate that is significantly above the background variability that is observed in any diverse patient cohort, it is often difficult to accurately interpret data. There is fundamental artificiality when reducing any diverse set of clinical data to a single outcome. We are not yet sure of the correct or most efficient outcome measurement when assessing vaccination effects in early-stage human studies (2).

Like most vaccines, the safety profile of HSPPC-96 is characterized by mild and transient side effects, including injection site inflammation or pain and low-grade fever. Not surprisingly, when compared to conventional chemotherapy or high-dose cytokine-immunotherapy, HSPPC-96 results in a substantially improved quality of life during treatment (43). In addition, no measured laboratory nor clinical signs of autoimmunity have been observed in >500 patients treated to date.

T cell responses in cancer patients have been detected after vaccination with HSPPC-96, as measured by ELISPOT and Tetramer assays. It is difficult to know with any certainty as to whether there is any causality between these responses and any clinical responses. In a pilot clinical trial in patients with advanced cancers a CD8+–restricted response against autologous tumor was observed in 6 of 12 patients after vaccination with HSPPC-96 (21). In another phase 1 trial of patients with resected pancreatic cancer there was a postvaccination increase in CD8+ ELISPOTS in two of five patients tested (7). In a phase 2 study in patients with metastatic melanoma a postvaccination increase in IFN-γ release from peripheral blood mononuclear cells cultured with HSPPC-96-pulsed autologous monocytes was noted (6). In about half of the patients (11/23) tested in this study, an increase in frequency of melanoma-specific T cells was detected after vaccination. A similar level of detectable immune response was also demonstrated in a phase 2 trial in patients with colon cancer who were vaccinated with autologous HSPPC-96 (44).

Measurable immune responses were seemingly correlated with clinical outcome in both the melanoma and colorectal studies (6, 44). In patients with metastatic melanoma, most clinical responders also showed measurable immune responses (6/7), whereas patients without clinical responses were less likely to have a measurable immune response (5/16) (6). In the colorectal cancer study, patients clinically free of disease after surgery had a significantly better overall and disease-free survival if they developed measurable T cell responses after vaccination than if they did not. The 2-year survival in these patients was 100% in immunological responders versus 50% in nonresponders. Although there seems to be a correlation between detectable immune response and survival, it is important to note that healthier patients (who survive longer) may have the best immune responses.

In the study of patients with metastatic melanoma, 64 patients underwent surgical resection of metastatic tissue required for vaccine production, 42 patients were vaccinated without toxicity, and 39 were evaluable (6). Of 28 patients with measurable disease, two had objective responses (complete responses 559+ and 703+ days). An additional three had stable disease (153, 191, and 272 days) at the end of follow-up. In a separate study, patients clinically free of disease after complete resection of liver metastases from colorectal cancer were treated with HSPPC-96 produced from liver metastases. Two-year survival in this patient population was 79%. This overall survival is similar to that seen in large series of patients treated with surgical resection of liver metastases (45). All patients mounting a measurable immune response against their tumors were alive after 2 years (44). It is again important to note that this does not imply causality.

The effects of autologous HSP vaccination have also been studied in 61 patients with stage 4 renal cell carcinoma (36). In

Table 2. Summary of clinical trials testing autologous HSPs in immunotherapy of cancer

<table>
<thead>
<tr>
<th>Clinical phase</th>
<th>Cancer type</th>
<th>Clinical stage</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>Pancreatic cancer</td>
<td>Stage 1–3</td>
<td>10</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Gastric cancer</td>
<td>Resectable</td>
<td>15</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Melanoma</td>
<td>Stage 3/4</td>
<td>36</td>
</tr>
<tr>
<td>Phase 1/2</td>
<td>Renal cell carcinoma</td>
<td>Stage 4</td>
<td>38</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Colorectal cancer</td>
<td>Stage 4</td>
<td>29</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>Low grade</td>
<td>10 ongoing</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Melanoma</td>
<td>Stage 4</td>
<td>42</td>
</tr>
<tr>
<td>Phase 2</td>
<td>Renal cell carcinoma</td>
<td>Stage 4</td>
<td>71</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Melanoma</td>
<td>Stage 4</td>
<td>350 ongoing</td>
</tr>
<tr>
<td>Phase 3</td>
<td>Renal cell carcinoma</td>
<td>Stages 2 and 3</td>
<td>650 ongoing</td>
</tr>
</tbody>
</table>

Like most vaccines, the safety profile of HSPPC-96 is characterized by mild and transient side effects, including injection site inflammation or pain and low-grade fever. Not surprisingly, when compared to conventional chemotherapy or high-dose cytokine-immunotherapy, HSPPC-96 results in a substantially improved quality of life during treatment (43). In addition, no measured laboratory nor clinical signs of autoimmunity have been observed in >500 patients treated to date.
addition to the HSPPC-96, s.c. IL-2 was given to those patients who progressed while on HSPP vaccine. Two patients had partial responses and one had a complete response. It is difficult to determine whether the objective response rate of ~5% is different to that seen as part of the background response variability in this disease.

Multicenter, large, randomized phase 3 trials are now underway in both adjuvant (renal cell carcinoma at high risk for recurrence after nephrectomy) and metastatic disease settings (metastatic melanoma) to ultimately define the magnitude of clinical efficacy of this autologous HSP vaccine used as a monotherapy.

**Conclusions**

There are sufficient data to support the notion that therapeutic cancer vaccines can induce anti-tumor immune responses in humans with cancer. How best to translate this increase in immune responsiveness to consistently and reproducibly induce objective cancer regression or increased survival remains unclear at this time. Despite monumental advances in our understanding of molecular and cellular immunology, we have thus far been unable to translate this into a proven and measurable clinical benefit.

The translation of autologous HSP vaccination, with unique antigens is logical. These molecules constitute one of the most highly conserved structures and systems in biology, and one could argue would be more likely to translate from laboratory to clinic. In addition, the use of antigens unique to that cancer may reverse the inefficiency of translating from homogeneous laboratory models into human tumor heterogeneity. The results of the ongoing, randomized, phase 3 studies will answer the question of how efficacious this method of therapeutic vaccination is in humans.

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