Vaccination of High-Risk Breast Cancer Patients with Mucin-1 (MUC1) Keyhole Limpet Hemocyanin Conjugate plus QS-21


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ABSTRACT

Our objective was to determine whether an immune response can be generated against MUC1 peptide and against tumor cell MUC1 after vaccination with MUC1-keyhole limpet hemocyanin (KLH) conjugate plus QS-21 in breast cancer patients.

Nine patients with a history of breast cancer but without evidence of disease were treated with MUC1-KLH conjugate plus QS-21, containing 100 μg of MUC1 and 100 μg of QS-21. S.c. vaccinations were administered at weeks 1, 2, 3, 7, and 19. Peripheral blood was drawn at frequent intervals to assess antibody titers. Skin tests were placed at weeks 1, 3, 9, and 21 to determine delayed type hypersensitivity reactions.

Common toxicities included a local skin reaction at the site of the vaccine, usually of 4–5 days’ duration, and mild flu-like symptoms usually of 1–2 days’ duration. High IgM and IgG antibody titers against synthetic MUC1 were detected. IgG antibody titers remain elevated from a minimum of 106–137 weeks after the first vaccination. Binding of IgM antibody to MCF-7 tumor cells was observed in seven patients, although there was minimal binding of IgG antibody. Two patients developed significant antibody titers post-high-dose chemotherapy and stem cell reinfusion. There was no evidence of T cell activation.

This MUC1-KLH conjugate plus QS-21 was immunogenic and well tolerated in breast cancer patients. Additional trials are ongoing to determine the optimal MUC1 peptide for use in larger clinical trials. Further investigation of vaccine therapy in high-risk breast cancer is warranted.

INTRODUCTION

Despite recent advances, current therapeutic regimens for both metastatic and early stage breast cancer remain suboptimal. Standard therapy for metastatic breast cancer results in minimal impact on survival (1, 2), whereas adjuvant therapy reduces the annual risk of recurrence by approximately one-third (3, 4). Patients at a particularly high risk for relapse include those with a history of metastatic (5–7) or locally advanced breast cancer (8) and those with increasing levels of tumor markers, including CEA, CA15-3 or BR2729 (9–11). In an attempt to provide greater clinical benefit, evaluation of an immunotherapeutic approach has generated considerable enthusiasm (12).

Investigation of vaccines for potential therapeutic purposes has been accelerated by technological advances and the identification of various differentiation antigens found on breast cancer cells. For example, carbohydrates (i.e., gangliosides and blood-group related antigens), and peptides are possible antigenic targets (13–16). Of particular interest is the mucin MUC1, which is a transmembrane glycoprotein composed of a polypeptide core containing multiple tandem repeats of a 20-amino acid sequence with numerous carbohydrate side chains (17). MUC1 was initially characterized by the development of murine monoclonal antibodies reactive with mucins from human milk or malignant cells derived from the breast or pancreas (17, 18). It is commonly found on a variety of normal epithelial cells, including lung, breast, pancreas, stomach, colon, salivary gland, kidney, endometrium, and prostate (16, 18, 19), as well as malignant cells of breast, ovary, pancreas, endometrium, colon, lung, and prostate origin (16, 18–20).

However, there are differences between MUC1 expression on normal cells and tumors. For example, the monoclonal antibody SM3 demonstrates significant reactivity with MUC1 on paraffin embedded breast cancer but minimal reactivity with normal breast cells or benign breast lesions (18, 21). On normal cells, mucins are located on the apical surface and are extensively glycosylated. However, in tumors, the usual structure of the tissue is disrupted so that mucin may be found on multiple cell surfaces (22). In addition, abnormal glycosylation in the cancer results in less complex and fewer carbohydrate side chains (23). Therefore, in tumors, there is greater exposure of MUC1 epitopes to the immune system, compared to normal cells (24). Although the function of MUC1 is not clearly estab-

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The abbreviations used are: CEA, carcinoembryonic antigen; AJCC, American Joint Committee on Cancer; BCG, Bacillus Calmette-Guerin; DNCB, dinitrochlorobenzene; KLH, keyhole limpet hemocyanin; MBS, m-maleimidobenzoyl-N-hydroxysuccinimide ester; NED, no evidence of disease.
lished, it may allow for tumor growth through its interactions with adhesion molecules and lymphocytes (22). The cloning and sequencing of MUC1 led to the production of synthetic peptides of various lengths for use in vaccines (25).

There are preclinical data in rodents demonstrating that MUC1 based vaccines are immunogenic in both a humoral and cellular fashion. For example, inhibition of tumor growth has been associated with antibody production (26-28) and T cell activation (29-31). Vaccination of chimpanzees with MUC1 vaccine constructs has also resulted in an immunogenic response (32, 33).

In addition, there is evidence that MUC1 is occasionally immunogenic in un-vaccinated breast cancer patients. First, MHC unrestricted cytotoxic T cells that lyse breast tumor cells but not normal breast cells have been derived from axillary lymph nodes of patients with breast cancer (34). Second, a humoral response directed against MUC1 has also been noted. In patient sera, IgM antibodies reactive with MUC1 peptides and circulating immune complexes containing MUC1 have been detected (35, 36). Despite these findings, the development of an immune response against MUC1 on tumor cells may be limited because of low tumor immunogenicity.

An effective method of inducing antibodies against weak immunogens is conjugation with a protein carrier, such as KLH, and the use of an immune adjuvant, such as QS-21. KLH is a large immunogenic protein obtained from the blood of the keyhole limpet. QS-21 is a saponin fraction derived from the bark of the South American tree *Quillaja saponaria* Molina (37). This combination of KLH and QS-21 is an effective regimen for inducing antibody responses against a variety of antigens.

For example, in preclinical analyses, high antibody titers reactive with MUC1 peptide and tumor cells expressing MUC1 were observed after vaccination of mice with synthetic MUC1 peptides conjugated to KLH and mixed with QS-21 (28). In addition, inhibition of tumor growth was noted in these mice after injection with E4 cells (MUC1-expressing mammary cell line), despite the lack of a detectable T cell response. In contrast, vaccination with unconjugated MUC1 peptides mixed with either BCG or QS-21 yielded minimal antibody responses. In clinical trials, vaccination of melanoma patients with a variety of ganglioside KLH conjugates plus QS-21 has also yielded higher antibody titers than vaccines containing the same gangliosides with other immune carriers or adjuvants (38). A dose of 100 μg of QS-21 has been established as effective and well tolerated (39).

Based on these studies, we initiated a trial in high-risk breast cancer patients with no clinical evidence of disease. The primary objective was to determine whether an immune response could be elicited against MUC1 peptide and against tumor cell MUC1 after vaccination with the MUC1-KLH conjugate plus QS-21. Based on statistical models, a relatively small number of patients were enrolled to answer this question (40).

**PATIENTS AND METHODS**

Breast cancer patients without evidence of disease and with one of the following characteristics were potentially eligible for the study: (a) AJCC stage IV disease after eradication of all detectable disease by surgery, radiation, or chemotherapy; (b) AJCC stage I, II, or III disease with rising tumor markers (CA15-3, BR2729, or CEA); or (c) AJCC stage III disease with an initially unresectable primary tumor after completion of adjuvant therapy within 12 months. A rising CA15-3 or CEA level was defined as a previously normal value with a subsequent elevation above the normal range documented on two consecutive occasions at least two weeks apart. For patients with a significant smoking history and a chronically elevated CEA level < 15 ng/ml, a rising CEA level was defined as one that increased at least 1.5× the uppermost chronic value on two consecutive occasions at least 2 weeks apart.

Chemotherapy, radiation therapy, or surgery must have been completed at least 4 weeks prior to treatment and immunotherapy within 6 weeks. Hormonal therapy was allowed during the study. Other requirements included: Karnofsky performance status > 80%, lymphocyte count ≥ 0.5 x 10⁹/ml, no other active cancers, and no history of a seafood allergy. Premenopausal patients were required to have a normal β-human chorionic gonadotropin level.

A history and physical examination, rectal examination with Hemoccult, chest X-ray, complete blood count with differential, CEA, CA15-3 (or BR2729), creatinine, chemistry profile, and gamma glutamyltranspeptidase level were required within three weeks of treatment. A CT scan of the chest/abdomen/pelvis and bone scan were obtained within four weeks of treatment. A colonoscopy within at least 5 years was required for patients with an isolated elevation of the CEA level. Patients were required to sign an informed consent form that had been approved by the Institutional Review Board and the Food and Drug Administration.

**Treatment Plan and On-study Evaluation.** All patients received MUC1-KLH conjugate plus QS-21, containing 100 μg of MUC1 and 100 μg of QS-21 via s.c. vaccination. A total of
five s.c. vaccinations were administered at weeks 1, 2, 3, 7, and 19 as an outpatient in the Immunology Unit at rotating sites, including the arm, thigh, or buttocks (Table 1).

A history and physical examination, complete blood count with differential, chemistry profile, CEA, and CA15-3 (or BR2729) levels were obtained intermittently as outlined in Table 1. A bone scan and CT scans of the chest/abdomen/pelvis, CEA, and CA15-3 (or BR2729) levels were obtained at weeks 21–24 for patients with elevated tumor markers and for other patients at the discretion of their primary physician. All patients were asked to complete a diary after each vaccination to document any symptoms.

Peripheral blood to assess immune response was drawn at week 1 (pretreatment) and at time intervals as noted in Table 1. Sera was analyzed by ELISAs for antibody titers against synthetic MUC1 and against a cell line expressing MUC1 (MCF-7). Additional blood was drawn at weeks 1, 5, 9, and 21 to assess for T cell response. Skin tests with topical DNCB were performed in the laboratory of Dr. Olivera Finn Joyce Taylor Papadimitrou, was the positive control against MUC1.

**Vaccine Preparation.** The primary antigenic component of this vaccine contains 15 repeats of the 20-amino acid sequence of the MUC1 peptide. This 30-amino acid sequence is C-VTSAPDTRPA-PGSTAPPAHGVTSAPDTRPA. Attached to the N-terminal carboxyl end is cysteine (Fig. 1). This MUC1 peptide was synthesized in an automated peptide synthesizer at the NCI. The other vaccine components were obtained from the following sites: MBS from Pierce (Rockford, IL); KLH from PerImmune Inc. (Rockville, MD); and QS-21 from Aquilla Biopharmaceutical, Inc. (Worcester, MA).

Production of the vaccine began with a mixture of MBS/dimethylformamide and KLH as described previously (28). The MBS is a bifunctional linker that facilitates covalent binding of the KLH to the terminal cysteine on the MUC1 peptide. This mixture was passed through a Sephadex G25 column to remove unconjugated MBS. The remaining MBS-KLH was then combined with MUC1 peptide and incubated for 3 h. A molecular weight 30,000 Centriprep filter was then used to eliminate free MUC1 peptide (Amicon Inc., Beverly, MA). The conjugate MUC1-KLH epitope ratio was 560:1. The MUC1-KLH conjugate was mixed with QS-21, placed in a vial, and tested for sterility, purity, immunogenicity, and safety.

**Dose Modifications and Off-study Criteria.** Toxicity was graded according to the Common Toxicity Criteria. A 50% reduction in doses of all vaccine components was planned for individual patients with grade III or greater local or systemic toxicity. Discontinuation of treatment was planned for recurrence of disease requiring systemic therapy or radiation.

**Serological Analysis.** ELISAs were performed to detect IgG and IgM antibody production against MUC1. ELISA plates were coated with the MUC1 peptide (30- or 32-amino acid sequence) at 0.1 μg/well, in carbonate buffer, and incubated at 4°C overnight. To block unreacted sites, 3% human serum albumin was added for 2 h. Serial dilutions of patient sera were then added to the ELISA plates. After a 2-h incubation, the plates were washed, and alkaline phosphatase labeled goat anti-human IgM antibody (Kierkegaard and Perry Labs, Gaithersburg, MD) was added. For IgG detection, unlabeled mouse anti-human IgG (Southern Biotechnology, Birmingham, AL) was used instead of IgM antibody, followed by alkaline-phosphatase labeled goat anti-mouse IgG (Southern Biotechnology). After a 45-min incubation, the plates were washed and developed. The change in color was measured at 414 nm on the ELISA reader. The antibody titer was defined as the highest serum dilution with an absorbance of ≥0.100 (28). Measurement of IgG subclasses was determined by ELISA assays using goat anti-human IgG1, IgG2, IgG3, or IgG4 labeled with alkaline phosphatase (Zymed, San Francisco, CA).

Flow cytometric assays were performed to determine whether the IgG or IgM antibodies were binding to tumor cells. Tumor cells (1 × 10^5) from the MCF-7 human mammary carcinoma cell line (provided by Dr. Neil Rosen, Memorial Sloan-Kettering Cancer Center), which expresses MUC1, or from the SKMEL 28 melanoma cell line, which does not, were incubated with individual patient sera (1:5 dilution) from pre-treatment samples (in all patients except week 2 for patients 2 and 4) and from posttreatment samples with elevated antibody titers. After washing, 20 μl of 1:25 dilution of FITC-labeled goat anti-human IgM or IgG antibody (Southern Biotechnology) was added, as described previously (28). After a 30-min incubation on ice, the percentage of positive cells was determined by flow cytometry (EPICS Profile II, Coulter Co., Hialeah, FL). The monoclonal antibody HMF52, generously provided by Dr. Joyce Taylor Papadimitrou, was the positive control against MUC1.

**Assays for T-lymphocyte Immunity.** Cell mediated immunity was determined using limiting dilution chromium release assays performed in the laboratory of Dr. Olivera Finn (University of Pittsburgh School of Medicine, Pittsburgh, PA) with cryopreserved peripheral blood mononuclearocytes obtained pre- and postvaccination for the initial six patients treated. After a 10-day in vitro cultivation period with the MUC1 positive cell line CAM-1, MHC unrestricted cytotoxicity against a second MUC1 positive but MHC distinct cancer cell line (BT-20) was tested as described previously (32). K562 cells were used to block nonspecific cytotoxicity.

**RESULTS**

Between June 1995 and June 1996, nine patients were enrolled in this study. All patients had a history of breast cancer...
Vaccination of High-Risk Breast Cancer Patients

Flu-like symptoms, including low-grade fever, headaches, chills, and myalgias, were self-limiting, usually of 1–2 days’ duration. Approximately 3 days after the third vaccine, one patient reported grade 2 nausea and grade 3 emesis, in association with a headache. This was felt to be consistent with her history of migraine headaches, and no dose reduction was made. Two patients noted a transient mild increase in inguinal adenopathy near the vaccine site, and three patients noted a skin recall reaction at earlier vaccine sites after later vaccinations.

No hepatic or renal toxicity was observed. No significant hematological toxicity was noted during the study. Two patients developed transient leukopenia (one with a pretreatment grade 1 value), and one patient developed a transient grade 2 neutropenia (pretreatment grade 1 value). The lowest hematological values throughout the entire study for each patient are noted in Table 4.

There was no clinical evidence of an autoimmune reaction as determined by symptoms, physical examination, or laboratory findings.

**Immunological Response.** IgM and IgG antibody titers against MUC1 were evaluated by ELISA for each patient at the time points mentioned previously. If feasible, blood samples were also obtained approximately every 3 months after completion of the study. The values of reciprocal titers are outlined for each patient in Fig. 2. All patients developed a significant increase in both IgM and IgG antibody titers after vaccination. The IgG titers remain elevated in all patients at a minimum of 106 weeks from the first vaccination. Analysis of IgG subclass titers for anti-MUC1 antibodies were performed for each patient using samples with high IgG titers in comparison to the pretreatment value (data not shown). IgG1 and IgG3 were detected in all patients postvaccination. Three patients had a minimal increase in IgG2. IgG4 was not detected in any patient.

To determine whether these antibodies were able to bind to MUC1 naturally expressed on tumor cells, flow cytometric analysis was performed to test IgM and IgG reactivity of patient sera against MCF-7 cells. Pre- and posttreatment values for all patients are noted in Tables 5 and 6. Seven patients (all but patients 2 and 4) demonstrated a clear increase in IgM reactivity against MCF-7 cells after vaccination. However, there was minimally significant increase in IgG reactivity against MCF-7 cells, with only three patients (patients 3, 4, and 9) demonstrating a tripling of the percentage of positive cells.

BR2729 levels were obtained prevaccine therapy and during treatment. These values were within the normal range for all

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**Table 2** Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9</td>
</tr>
<tr>
<td>Stage 4 NED</td>
<td>8</td>
</tr>
<tr>
<td>Increased CEA level</td>
<td>1</td>
</tr>
<tr>
<td>Patients with prior chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Adjuvant only</td>
<td>4</td>
</tr>
<tr>
<td>Metastatic only</td>
<td>4</td>
</tr>
<tr>
<td>Patients with prior hormone therapy</td>
<td></td>
</tr>
<tr>
<td>Adjuvant only</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic only</td>
<td>5</td>
</tr>
<tr>
<td>Adjuvant + metastatic</td>
<td>2</td>
</tr>
<tr>
<td>Patients with prior radiotherapy</td>
<td></td>
</tr>
<tr>
<td>Adjuvant only</td>
<td>3</td>
</tr>
<tr>
<td>Metastatic only</td>
<td>3</td>
</tr>
<tr>
<td>Adjuvant + metastatic</td>
<td>0</td>
</tr>
<tr>
<td>Patients on hormone therapy during the study</td>
<td>8</td>
</tr>
<tr>
<td>Age Range</td>
<td>38–61 years</td>
</tr>
<tr>
<td>Median</td>
<td>49 years</td>
</tr>
</tbody>
</table>

**Table 3** Common toxicities: number of episodes that occurred following all vaccinations

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Skin Reaction</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Myalgias</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>9</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 4** Numbers of patients with hematologic toxicity

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia</td>
<td>7</td>
<td>1</td>
<td>1a</td>
<td>0</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>8</td>
<td>0</td>
<td>1b</td>
<td>0</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>2</td>
<td>1</td>
<td>5a</td>
<td>1c</td>
</tr>
<tr>
<td>Anemia</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>7</td>
<td>2a</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a These patients had pretreatment values of grade 1.

b Three of these patients had pretreatment values of grade 2; two patients had pretreatment values of grade 1.

c Pretreatment value.
patients at all of these time points. Therefore, no clear correlation between antibody titers and BR2729 levels could be made.

No delayed type hypersensitivity skin test reactivity against MUC1 was detected. All patients had reactivity to the DNCB skin tests. There was no evidence of increased precursor frequency of CTLs in the six patients evaluated. Precursor frequencies ranged between 1 in 664,000 lymphocytes and 1 in 1,759,000 lymphocytes prior to vaccination, and these values did not change significantly after vaccination.

Clinical Response. Because all patients were without evidence of clinical disease at the start of this study, tumor response was not an end point. The range of follow-up from administration of the first vaccine was 106–162 weeks (median, 135 weeks). However, two patients have developed a recurrence during this time. Patient 5 underwent resection of a chest wall recurrence in week 47 and was placed on hormonal therapy. She remains without active disease. Patient 6 developed a chest wall recurrence in week 80 and remains on hormone therapy with stable disease. All other patients are without evidence of recurrence. The patient with the mild CEA elevation continues to have a CEA level in the 6–7 ng/ml range.

Fig. 2 IgM and IgG antibody titers after vaccination with MUC1-KLH conjugate + QS-21.

DISCUSSION

Thus far, there has been minimal evaluation of vaccine therapy in breast cancer patients. The majority of previous studies noted a lack of significant clinical benefit after vaccine administration with either tumor cells or general immune stimulants in advanced disease (41, 42) or in the adjuvant setting (3, 43, 44). Overall, these unfavorable results were likely caused by several factors, including small numbers of patients, poorly defined eligibility criteria, and most importantly, limitations of the agents used. However, recent clinical investigation of a sialyl Tn glycoconjugate documented an immunogenic response with little clinical toxicity (45). These results are encouraging and support further exploration of vaccine therapy in breast cancer.

Several conclusions can be made from our study. First, vaccination of breast cancer patients with the MUC1-KLH conjugate plus QS-21 was well tolerated. The most common toxicities included transient local skin reactions and mild flu-like symptoms. The changes in hematological parameters are unlikely to be caused by the vaccine, as most patients had low pretreatment values. The transient variation in values could be
attributable to normal biological fluctuations. Although mucin is present on a variety of normal cells, we did not observe any evidence of an autoimmune reaction. Several mechanisms may decrease the potential for an autoimmune reaction or an endogenous anti-mucin response. As noted previously, the glycosylation of mucins from normal and malignant breast cells is different (34, 46, 47). In addition, the secretion of mucin primarily on the luminal surface of many normal epithelial cells limits its exposure to the immune system (22, 34). These data support the suggestion that aberrant glycosylation and cellular architecture may allow for greater exposure of mucin epitopes to antibodies and T cells.

Second, vaccination of breast cancer patients with this vaccine results in significant production of both IgM and IgG antibodies against synthetic MUC1, but no evidence of T lymphocyte activation. For most patients, the IgM and IgG antibody titers were elevated (22, 34). These data suggest that a larger number of patients would be necessary to evaluate this point. As all patients were without clinical evidence of disease, it is not possible from this small trial to conclude whether an association exists between antibody titers and risk of relapse. Longer follow up with a larger number of patients would be necessary to evaluate this point.

Although many vaccine studies have focused on T cell activation to elicit an antitumor response, there are significant data to indicate that antibodies may also result in antitumor activity (48). For example, increased disease-free and overall survival associated with antibody production has been observed in melanoma patients after vaccination with ganglioside vaccines (49). It is too early to determine whether antibodies against MUC1 or activation of T cells will correlate with improved clinical outcome in breast cancer patients. It is not possible from this small trial to conclude whether an association exists between antibody titers and risk of relapse. Longer follow up with a larger number of patients would be necessary to evaluate this point.

Only a few clinical trials exploring various MUC1 based vaccines have been conducted thus far. A Phase I trial at the University of Pittsburgh treated breast cancer and gastrointestinal cancer patients with a synthetic MUC1 peptide (105-amino acid sequence) plus BCG. Toxicities were limited except for skin breakdown probably caused by BCG. An increase in CTLs after vaccination was found in several patients (50, 51). Another Phase I trial in Australia of a mannan-MUC1 fusion protein in advanced cancer patients resulted in minimal toxicity. High titers of anti-MUC1 IgG antibodies were found in 13 of 25 patients. Whether these antibodies will eventually become undetectable remains undetermined. Although some patients had proliferation of T cells (52).

However, results of T cell assays can be difficult to reproduce and clearly require further evaluation. It is unclear whether the type of protein carrier (KLH) or immune adjuvant (QS21) in our vaccine results in significant production of both IgM and IgG antibodies (50, 51). Another Phase I trial in Australia of a mannan-MUC1 fusion protein in advanced cancer patients resulted in minimal toxicity. High titers of anti-MUC1 IgG antibodies were found in 13 of 25 patients, and 4 of 15 patients had proliferation of T cells (52).

Whether these antibodies will eventually become undetectable requires continued follow up of these patients. The optimal number of doses and duration of vaccine administration has not been established, and it is possible that patients may require subsequent “booster” vaccinations.

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However, results of T cell assays can be difficult to reproduce and clearly require further evaluation. It is unclear whether the type of protein carrier (KLH) or immune adjuvant (QS21) in our trial affected T cell activation.

Third, significant antibody titers were produced in both patient 3 and patient 8, who had been treated with high-dose chemotherapy and stem cell reinfusion approximately 44 and 48 months, respectively, prior to the vaccine. Immunosuppression occurs after high-dose therapy, and it may be prudent to allow...
a reasonable time period to elapse prior to antitumor vaccinations. All other patients except patient 9 (no prior chemotherapy) had completed chemotherapy 3–70 months prior to the first vaccination. The median time from completion of chemotherapy to the first vaccine for these eight patients was 45.5 months. Prior chemotherapy does not preclude a strong serological response to the MUC1 vaccine; however, the optimal time between completion of chemotherapy and initiation of vaccine therapy is not known.

Fourth, we observed binding of IgM antibody to MCF-7 tumors cells in seven of nine patients but minimal cell surface binding of IgG antibody. Based on inhibition assays of sera from six patients, we found that these antibodies bind primarily to the APDTRPA epitope of the MUC1 peptide (53). Similar immunodominant epitopes have been confirmed by others (24, 35). In our 30-amino acid MUC1 immunogen, this epitope was located in both a medial and a COOH-terminal position, yet the antibody bound primarily to the COOH-terminal epitope. However in tumor cells, most of this epitope is not expressed in the COOH-terminal position (54). This explains our finding of modest cell surface reactivity in the face of very high antibody titers against MUC1 peptide. It is probable that other MUC1 peptides may generate antibodies against different epitopes. For example, a clinical trial of a MUC1 peptide mannan conjugate resulted in antibody production against other epitopes (52). Therefore, further evaluation of different MUC1 peptides is warranted, with the goal of finding a synthetic peptide that will more closely resemble the epitope exposure in patients.

The availability of immunological assays for precisely defining the specificity and cell surface reactivity of vaccine induced serological responses allows for analysis of a series of vaccines with relatively small numbers of patients. Based on our results, we have designed additional clinical trials aimed at improving the immunogenicity of the current vaccine. First, we have recently completed a clinical trial to assess a 32-amino acid MUC1 peptide containing two APDTRPA epitopes, neither of which is at the terminal position. Second, an ongoing trial will determine the immunogenicity of a 106-amino acid MUC1 peptide that should naturally assume a β-helix configuration similar to the proposed configuration on tumor cells. Third, another ongoing trial will evaluate glycosylated MUC1 peptides because they may more closely resemble the secondary structure of MUC1 on tumor cells.

Development of breast cancer vaccines involves determination of an immunological response as well as clinical benefit. Therefore, the results from these studies will allow for the selection of the optimal MUC1 peptides for use in larger Phase II or III clinical trials. Because of the heterogeneity of breast cancers, it is also possible that an effective antitumor vaccine will require multiple antigenic components, in addition to MUC1. These antigens are undergoing evaluation in separate clinical trials. Although the optimal role of vaccines for breast cancer therapy has not yet been identified, it is reasonable to propose that clinical benefit be evaluated after adjuvant chemotherapy, particularly in patients at high risk of recurrence, in whom micrometastatic disease is likely.

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Clinical Cancer Research

Vaccination of High-Risk Breast Cancer Patients with Mucin-1 (MUC1) Keyhole Limpet Hemocyanin Conjugate plus QS-21

Teresa Gilewski, Sucharita Adluri, Govindaswami Ragupathi, et al.


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