

Pilot Study of Immunization of High Risk Breast Cancer Patients with a Sialyl Lewis^a-Keyhole Limpet Hemocyanin Conjugate plus the Immunological Adjuvant QS-21

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a pilot study of injection of sialyl lewis^a (sLe^a) conjugated to keyhole limpet hemocyanin (KLH) plus the immunological adjuvant QS-21. Eligible patients include those with metastatic

breast cancer who have no evidence of disease or stable disease on hormone therapy. Initially 3 patients will be entered at a lower dose (10 mcg) of sLe^a. If safe, 10 patients will be accrued at the full dose of 30 mcg. If 6 or more of these 10 patients have an immune response at any time point then an additional 10 patients will be accrued and injected with a higher dose of QS-21.

Injections will be administered subcutaneously during weeks 1, 2, 3, 7 and 19. Prior to and during therapy, blood samples will be obtained to assess immune response.

The primary endpoints of this trial are safety and immune response. Clinical response will be monitored but will not be a primary endpoint. A secondary endpoint is the evaluation of blood samples for circulating tumor cells.

2.1 OBJECTIVES AND SCIENTIFIC AIMS

The primary objectives of this trial are to determine the following endpoints after injection with sLe^a-KLH plus QS-21:

- Safety
- The IgG and IgM antibody response to sLe^a and breast cancer cells expressing this antigen

The secondary objective of this trial is to monitor the presence of circulating tumor cells (CTC) prior to, during and after injection with sLe^a-KLH plus QS-21.

Clinical response will be monitored but will not be an endpoint. As part of standard care, patients on this trial will be followed for progression of disease. It is unknown how many patients will enroll with evidence of disease. Therefore, we will report any responses that are observed for these patients in a purely descriptive manner (see section12). This is consistent with the pilot vaccine trials we have previously conducted.

3.0 BACKGROUND AND RATIONALE

3.1 Breast Cancer

Invasive breast cancer is now predicted to affect one in seven American women at some point in their lifetime.¹ A significant number of patient will develop metastatic breast cancer, which in contrast to early stage breast cancer is generally considered incurable.² While patients with AJCC Stage IV breast cancer who have no evidence of disease or stable disease on hormone therapy can live for many years, ultimately their disease will likely become resistant to current treatment options. Additional therapeutic strategies for these patients are needed.

3.2 Treatment Background

Dr. Livingston and colleagues have shown that some patients have antibodies in their serum which react with highly restricted ganglioside antigens present on melanomas and a variety of other cancers. In some cases, they and others^{3,4} noted that the presence of these antibodies has been associated with an unexpectedly favorable course. As only few patients

have these antibodies in their serum, we are attempting to induce antibody formation by immunizing the patients with tumor vaccines containing the relevant antigens.

Adjuvant immunotherapy of breast cancer with immunizations must be based on the identification of antigens expressed by breast cancers, which are immunogenic. While many antigens have been identified on breast cancers using murine monoclonal antibodies, very few of these are known to be recognized by the human immune system. Several of the antigens commonly identified on breast cancer cells include the gangliosides GM2 and Globo H⁵, blood group-related antigens TF, sTn, Lewis y (Le^y)⁶ and the protein MUC1⁷.

3.2.1 Keyhole Limpet Hemocyanin (KLH) Various methods have been used to increase the immunogenicity of antigens, in particular to induce an IgG response. The approach that Dr. Livingston and colleagues have found to be most successful at inducing an IgG response against the closely related T antigen in the mouse has been covalent attachment to keyhole limpet hemocyanin (KLH). KLH is an immunogenic protein which is derived from the blood of the keyhole limpet. This approach also resulted in anti-T antigen delayed type hypersensitivity reactions and partial protection against cancer cells expressing T antigen.⁸ Studies of immunological tolerance and of ways to overcome it have shown that in a variety of experimental systems, T-cell (not related to T antigen) unresponsiveness is more rapidly induced and more easily maintained than B cell unresponsiveness.⁹ Levels of circulating antigens suitable for maintaining T-cell tolerance frequently fail to maintain B cell tolerance. Consequently if T-cell help is provided (as by potent irrelevant antigens such as KLH covalently attached to the desired immunogen), antibodies can be induced to tolerated T-cell dependent antigens. This approach has been successfully used to induce IgG antibodies against a variety of carbohydrate antigens, and the titer of antibody induced is greatly augmented with the use of immunological adjuvants.¹⁰

3.2.1.1 Conjugation of antigens to KLH can be accomplished using a direct method or with a bifunctional linker MMCCH (M2C2H): (4-(4-N-maleimidomethyl) cyclohexane-1-carboxyl hydrazide. This latter method of conjugation was developed to improve the antigen epitope:KLH ratio.¹¹ In mice, this method of conjugation resulted in higher antibody titers following immunization with an sTn based vaccine. This method of conjugation has also been evaluated at MSKCC with the Globo H-KLH plus QS-21 construct in breast cancer patients (protocol 97-86).

3.2.2 Immune Adjuvants

QS-21 In addition to KLH, the immunological adjuvant QS-21 has also been used to increase immunogenicity of various antigens. QS-21 is a saponin fraction from the bark of the South American tree Quillaja saponaria Molina.¹² Immunization of mice with synthetic MUC1 peptides conjugated to KLH and mixed with QS-21 resulted in significant antibody titers against MUC1 peptide and MUC1 positive tumor cells in comparison with minimal immune response to unconjugated MUC1 peptides mixed with BCG or QS-21.¹³ Groups of melanoma patients have been immunized with various constructs containing the adjuvants DETOX, BCG and QS21 or no adjuvant at all. QS-21 was a significantly more effective adjuvant than the others, producing significantly higher titer IgM and IgG antibodies.¹⁴ A dose of 100 mcg of QS-21 was found to be minimally toxic and immunogenic.¹⁵ Therefore, QS-21 was selected for

future trials due to its adjuvanticity and lack of toxicity. It has also proven nontoxic and highly effective at augmenting the immunogenicity of an FeLV subunit vaccine in cats¹⁶ and an HIV-1 recombinant vaccine in Rhesus monkeys.

3.3 MSKCC Experience with Immunization of Breast Cancer Patients

Immunization against antigens found on tumor cells represents a potential therapeutic strategy for breast cancer patients. We have conducted several clinical trials at MSKCC in high risk breast cancer patients to assess the safety and immunogenicity of various single antigens conjugated to keyhole limpet hemocyanin (KLH) and mixed with the immune adjuvant QS21. Antigens included the peptide MUC-1 and the carbohydrates Globo-H, GM2 and STn[clustered] (protocol numbers 94-130, 95-67, 97-123, 99-023). These immunizations were well-tolerated with the most common toxicities including transient local skin reactions at the injection site and mild flu-like symptoms. Although, there were no definite autoimmune reactions attributable to the injection, several patients developed hypothyroidism or hyperthyroidism. The majority of patients developed IgM and IgG antibody titers reactive with the immunizing antigen. There were no significant differences in the immune response observed among patients with no evidence of disease or those with stable disease. No definite T cell responses were elicited in the MUC-1 trials.¹⁷⁻²⁰

We subsequently evaluated patients on protocol 01-019 with a polyvalent construct containing 7 antigens conjugated to KLH and mixed with either QS21 or GPI-0100 (a semi-synthetic saponin).²¹⁻²³ The injections were well tolerated with common local skin reactions and mild flu-like symptoms. Several patients receiving GPI-0100 developed a transient Grade 1-3 elevation in the hepatic enzymes (AST, ALT). Following an extensive hepatic evaluation, it was felt that the GPI-0100 was the likely source of the hepatotoxicity. Therefore, the adjuvant GPI-0100 will not be incorporated into the current breast cancer trial. The majority of patients also developed antibody titers reactive with multiple immunizing antigens.

3.4 Rationale for Evaluation of sialyl Lewis^a (sLe^a)-KLH plus QS-21

The carbohydrate antigen sLe^a is the antigen recognized by monoclonal antibody 19.9 and is commonly expressed on breast cancer cells. In a study of 43 breast cancer patients with infiltrating ductal carcinoma it was found in about 79% of samples.²⁴ There was high expression in patients with greater node involvement. sLe^a may be a ligand for endothelial leukocyte adhesion molecule and therefore have an impact on metastatic potential.

sLe^a is expressed primarily as a glycolipid on the surface of cancer cells. We are preparing a polyvalent vaccine for evaluation in patients with breast cancer. It contains four antigens expressed on mucins (MUC1, Tn, sTn and TF) and three antigens expressed as glycolipids (GM2, globo H and Lewis Y). There are advantages to glycolipid antigens including greater susceptibility to activation of complement in the presence of antibodies and greater induction of complement mediated cytotoxicity, as recently described.^{24a} We have recently learned that Lewis Y is poorly immunogenic, inducing antibodies in fewer than 30% of vaccinated patients. If sLe^a proves immunogenic in this trial, we plan to replace Lewis Y in the polyvalent construct with sLe^a.

In this protocol, we propose to evaluate a construct containing the antigen sLe^a for toxicity and immunogenicity. If the injections are well tolerated and immunogenic, then sLe^a will be incorporated into a future polyvalent construct. This is the first time that sLe^a is being evaluated in humans.

Our prior breast cancer immunization protocols used 100 mcg of QS-21. The greatest toxicity primarily occurred during weeks 2 and 3. In an attempt to increase the immunogenicity of the construct, if six or more of the initial ten patients demonstrate an immune response against sLe^a and the injection is well tolerated, the dose of QS-21 will be increased to 150 mcg during weeks 1, 7 and 19 in a second group of 10 patients. We do not anticipate significant increases in toxicity as prior evaluations of 200 mcg of QS-21 were tolerated.

The potential benefits of this study include generation of an anti-tumor response by stimulation of the immune system. While it is possible that this treatment will result in shrinkage of breast cancer tumors, delay a recurrence or decrease the risk of progression, it is not known whether these effects will occur. If this study demonstrates that the sLe^a-KLH conjugate is safe and immunogenic, it will be incorporated into a polyvalent vaccine that will be evaluated in high risk breast cancer patients for clinical benefit.

3.5 Evaluation of Circulating Tumor Cells (CTC)

Circulating tumor cells (CTC) can be detected in the blood from patients with various primary or metastatic carcinomas.²⁵⁻²⁹ Recently the development of immunomagnetic separation strategies has increased the sensitivity of these assays.^{28,29} Several studies have reported that the presence of CTC predicted shortened disease free and overall survival times. These studies demonstrate that detection of CTC correlates with prognosis at baseline and that changes in CTC levels during and after treatment may be more predictive than other measures.³⁰⁻³⁴ Monoclonal antibody based therapy (trastuzumab) with or without additional chemotherapy was particularly effective at eliminating CTC. This was associated with improved disease free and overall survival.²⁸

Use of peripheral blood immunomagnetically enriched for tumor cells using labeled antibodies targeting epithelial cell surface markers on breast cancer cells and quantitated using a CellSpotterAnalyzer is now FDA approved for this purpose in breast cancer patients. Veridex, LLC. has provided MSKCC with a CellSpotterAnalyzer for this purpose in patients with prostate and breast cancer. To date, Dr. Martin Fleisher, Chief of the MSKCC Department of Clinical Laboratories, has validated the assay in prostate cancer patient using the same labeled antibodies which are FDA approved for use in breast cancer patients.

Two studies have identified CTC in approximately 95% of breast cancer patients with Stage I/II disease prior to surgery.^{35,26} After resection of all known disease, 30% of patients continued to have CTC that persisted for at least 2 weeks. The patients that we propose to evaluate are patients at a high risk for recurrence. We estimate that at least 30% of these patients will have demonstrable CTC.

In this study, CTC will be evaluated in an exploratory manner to determine whether they can be detected in this patient population at baseline and if so whether there is evidence

of a decline in those patients who have an immune response. CTC will be monitored prior to, during and after injection with sLe^a-KLH plus QS-21. The patient and their primary oncologist will be notified of all CTC results. These results will be used for research purposes and will not be used during the study to alter clinical care. Although some physicians at other centers may use CTC results to evaluate response to therapy, this is not considered standard care at MSKCC.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a pilot study designed to assess the safety and immunogenicity of a sLe^a-KLH plus QS-21 construct in patients with metastatic breast cancer. Initially 3 patients will be accrued to the study at a 10 mcg dose of sLe^a. If safe, then 10 patients will be accrued at the 30 mcg dose of sLe^a. If 6 or more of these 10 patients have an immune response at any time point then an additional 10 patients will be accrued and injected with a higher dose of QS-21. Toxicities will be monitored during the study. Patients will be instructed to complete a patient diary and document any side effects.

Patient blood samples will be evaluated for an IgG and IgM antibody response to sLe^a and breast cancer cells expressing this antigen. Immunological response for a patient is defined as an antibody response of greater than 1:80 if baseline value was 0 and at least an increase of 8-fold if baseline value is greater than 0. Patient blood samples will also be monitored for the presence of circulating tumor cells pre study and then during weeks 5, 13, and 23.

4.3 Intervention

Patients will receive sLe^a-KLH plus QS-21 subcutaneously during weeks 1,2,3,7 and 19. The first 3 patients will receive a 10 mcg dose of sLe^a and subsequently all patients will receive a 30 mcg dose of sLe^a. The dose of QS-21 is 100 mcg for the initial 13 patients. If immune responses are observed at the 100 mcg level of QS-21 then the dose of QS-21 will be escalated to 150 mcg, in a subsequent group of patients during weeks 1, 7 and 19. Blood samples will be obtained pre study and during weeks 1,2,3,5,7,9,13, 19 and 23 to assess for immune response.

sLe^a is one of multiple antigens found on breast cancer cells. Data from this trial will be used to determine whether the sLe^a antigen will be included in a future polyvalent construct. Ultimately we plan a multicenter randomized clinical trial to assess potential clinical benefit of immunization with a polyvalent construct in high risk breast cancer patients.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Clinical Material

5.1.1 Sialyl lewis^a (sLe^a)-KLH conjugate The conjugate is prepared at Optimus Pharmaceutical, Inc. sLe^a pentenyl glycoside was synthesized using a proprietary synthetic method by Optimus Pharmaceuticals, Inc. under GMP. Its identity was confirmed by MS and NMR. The glycoside was purified by reverse phase

chromatography and the purity established to be greater than 99% by NMR and HPLC analysis.

The pentenyl glycoside group on sLe^a was bound to M2C2H heterobifunctional linker following ozone treatment. Keyhole limpet hemocyanin (KLH) was activated by introduction of thiol groups on the KLH amino groups with 2-Iminothiolane. Activated KLH was mixed with sLe^a-M2C2H and the resulting sLe^a-M2C2H-KLH purified and washed by diafiltration. The carbohydrate and protein components were quantitated and the conjugate was tested for toxicity and immunogenicity in mice. The sLe^a/KLH ratio was determined to be greater than 600/1. The dose of sLe^a-KLH to be studied is 30 mcg. However, due to concerns regarding potential toxicity 3 patients will first be entered at a dose of 10mcg.

5.1.2 QS-21 QS-21 is a saponin prepared from the bark of Quillaja saponaria Molina. It will be supplied by Antigenics, Inc.. All injections will be vialed with a dose of 100 mcg of QS-21. If there is an immune response in at least six of the ten patients, then in the second group of patients, an additional 50 mcg of QS-21 will be added to the preparation during weeks 1, 7 and 19.

5.1.3 The injection will contain sLe^a-KLH conjugate 30 mcg (10 mcg for the first 3 patients) and QS-21 100 mcg per 1 ml 0.9% saline. It will be stored at -80°. The total volume per vial is 1.1 ml.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

Breast cancer patients with a history of metastatic disease will potentially be eligible.

6.2 Subject Inclusion Criteria

- **AJCC Stage IV breast cancer who have no evidence of disease**
OR
AJCC Stage IV breast cancer who have stable disease on hormonal therapy. Stable disease must be present for at least 2 months prior to enrollment in this study. Stable disease includes less than a 30% decrease or less than a 20% increase in the sum of the longest diameters of target lesions, taking as a reference the baseline sum of the longest diameters recorded at the last set of scans. Stable disease also includes no new target lesions and no unequivocal progression of non-target lesions.
(These patients may or may not have an elevation in the CA153 (BR2729) or CEA values).
- Performance status ≥ 80 (Karnofsky).
- Patients must have completed radiation, chemotherapy or surgery at least 4 weeks prior to treatment. Patients must have completed prior immunotherapy at least 6 weeks prior to vaccination. **Ongoing hormone therapy is permitted.**
- Pathology slides from the metastatic site must be reviewed by the Department of Pathology at MSKCC to confirm diagnosis.

6.3 Subject Exclusion Criteria

- Pregnant women are ineligible. Premenopausal patients must have a pregnancy test to exclude pregnancy within 2 weeks of starting treatment. Women who may yet bear children and sexually active men must use appropriate contraception during the course of this study. Patients must be counseled not to become pregnant during the study. Patients must also be counseled that injection of sLe^a may have unknown affects on the viability of a future fetus.
- Patients who are nursing
- Patients with other active cancers are ineligible, (excluding basal cell or squamous carcinomas of the skin).
- Patients with:
 - total lymphocyte count of $< 0.5 \times 10^6/\text{ml}$.
 - total white blood cell count of $< 3,000 \text{ cells}/\text{cmm}$
 - serum creatinine $> 1.5 \times \text{upper limit of normal}$
 - serum aspartate aminotransferase $> 1.5 \times \text{upper limit of normal}$
 - serum alkaline phosphatase $> 1.5 \times \text{upper limit of normal}$.
- Patients with a history of a seafood allergy.
- Patients with clinically significant NY Heart Association Class 3 or 4 cardiac disease.
- Patients who have previously been immunized with the sLe^a antigen.
- Patients with a known history of immunodeficiency or autoimmune disease. Current use of immunosuppressive medications (ie corticosteroids) is prohibited.
- Active infection requiring antibiotic treatment.

7.0 RECRUITMENT PLAN

Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or research team at Memorial Sloan-Kettering Cancer Center (MSKCC). If the investigator is a member of the treatment team, s/he will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator/research staff of the study.

The principal investigator may also screen the medical records of patients with whom they do not have a treatment relationship for the limited purpose of identifying patients who would be eligible to enroll in the study and to record appropriate contact information in order to approach these patients regarding the possibility of enrolling in the study.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and

enrollment process. The investigator/research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes.

In most cases, the initial contact with the prospective subject will be conducted either by the treatment team, investigator or the research staff working in consultation with the treatment team. The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened and minimal PHI will be maintained as part of a screening log. For these reasons, we seek a (partial) limited waiver of authorization for the purposes of (1) reviewing medical records to identify potential research subjects and obtain information relevant to the enrollment process; (2) conversing with patients regarding possible enrollment; (3) handling of PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).

Patients will be recruited for this protocol primarily by their doctor at MSKCC. In addition, patients outside of MSKCC may be aware of this trial from other patients, their local doctors or the National Cancer Information Center.

Once a patient has indicated an interest in participation, he/she will be referred to one of the consenting physicians for further evaluation and discussion of informed consent. If the patient is determined to be eligible, he/she will be registered according to standard MSKCC policy.

Most patients with breast cancer are female, but male patients will not be excluded. Children are excluded from participation in this study since breast cancer is rare in this patient population and therefore toxicity analysis would be of questionable significance.

8.1 PRETREATMENT EVALUATION

Within 2 weeks of the first injection:

Pregnancy test for premenopausal patients

Within 3 weeks of the first injection:

- Complete history and physical examination
- Complete blood cell count with differential and platelet count (CBC)
- Comprehensive panel (COMP): aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, alkaline phosphatase, bilirubin, calcium, creatinine, glucose, total protein, BUN, sodium, potassium, chloride, C02
- CEA, CA153(or BR27-29 or CA27-29)

- Thyroid stimulating hormone (TSH) level
- Urinalysis
- Rectal examination with Hemoccult
- Blood (3 red-top tubes) for immune response studies will be sent to Dr. Philip Livingston's laboratory in 723 Kettering building. An additional 7.5 ml of blood will be drawn in special tubes to go to Dr. Fleisher's lab in Clinical Chemistry for assessment of circulating tumor cells. These blood samples may be drawn on the day of the first injection, but prior to the injection.

Within 4 weeks of the first injection:

Imaging and Diagnostic Studies

- CT scan of the chest/abdomen/pelvis
- Radionuclide bone scan

9.0 TREATMENT/INTERVENTION PLAN

The initial 3 patients will be accrued at 1 or more week intervals and will receive 10mcg of sLe^a in the sLe^a-KLH + 100mcg QS-21 vaccine. Four weeks or more after accrual of the third patient, 3 additional patients may be accrued at 1 week intervals at the full 30mcg sLe^a dose. Four weeks after accrual of the sixth patient, 7 additional patients may be accrued at a rate no greater than two patients per week.

The sLe^a-KLH plus QS-21 vaccines will be injected during weeks 1, 2, 3, 7 and 19 for a total of five injections. They will be stored in a freezer at -80 degrees Centigrade. At the time of administration the vaccine will be thawed and 1ml drawn up into a syringe. Each 1.1 ml vial will contain sLe^a - KLH conjugate 30 mcg (except for the 10 mcg dose) and QS-21 100 mcg per 1 ml of 0.9% saline.

If a second group of 10 patients is evaluated, then the dose of QS-21 in that group will be increased to 150 mcg during weeks 1, 7 and 19 with the dose remaining the same for the other injections. The same precautions will apply to the rate of patient accrual. The initial 3 patients in this group will be accrued at 1 or more week intervals. Four weeks or more after accrual of the third patient, 7 additional patients may be accrued.

All injections will be administered by a trained nurse on an outpatient basis in the Experimental Therapeutics Center at 53rd Street outpatient building. All patients will be observed for 30 minutes after each injection to monitor for acute allergic reactions.

The sites of immunization (arm or thigh usually- buttocks may be used if necessary) will be rotated for each of the immunizations. No injection will be given at a location in which the draining lymph nodes have been removed.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

The study evaluation will include parameters to assess the safety of the injections as well as assessments of immunity to sLe^a (Appendix A). Interval safety assessments will include the „Patient Diary“ (Appendix B). Patients will be provided with Appendix A and B. Long term followup will also be maintained to assess toxicity. Although not a primary endpoint, patients will be monitored for any evidence of an anti-tumor effect. Imaging to assess extent of disease will be performed at baseline, then as appropriate for clinical circumstances. Patients with metastatic disease are often evaluated with imaging every 3 months as standard of care. Clinical benefit will also be assessed by physical examination and biochemical studies as outlined below.

SUMMARY TABLE OF EVALUATION

STUDY WEEK VACCINATION	Pre	1	2	3	5	7	9	13	19	23	q3 months ^g
Clinical:											
History	X	X				X		X	X	X	X
Physical Exam	X	X				X		X	X	X	X
CBC w/ Diff	X					X		X	X		
COMP ^b	X				X	X		X	X	X	
CEA, CA153	X										X ^f
TSH	X						X				X
Urinalysis	X										
Pathology Review	X										
Pregnancy Test	X ^a										
Rectal/Hemoccult	X										
Imaging:											
Bone scan	X									X ^f	
CT Scans	X									X ^f	
Response Assessments ^d									X ^f	X ^f	
Anti-sLe ^a											
Immunity:											
Serology (3 RTT)	X	X ^e	X ^e	X ^e	X	X ^e	X	X	X ^e	X	X
CTC Assay	X				X		X		X		X

^a Within 2 weeks of the first injection for a premenopausal patient..

^b Includes total bilirubin, AST, ALT, Sodium, Potassium, Chloride, CO₂, Calcium, Glucose, Total Protein, Albumin, Alkaline Phosphatase, Creatinine, BUN, Bilirubin.

^c Patients may be asked to obtain tissue blocks from previous diagnostic/therapeutic procedures.

^d Overall response assessment includes the measurement of tumor by non-imaging studies such as physical exam.

^e Blood must be drawn before administration of vaccine at week 1,2, 3, 7 and 19. An extra 100ml of heparinized blood may be drawn once (during weeks 9-23) during the course of immunizations depending on the serologic response in individual patients, at the principal investigators discretion, for evaluation of the immune response at the clonal level by hybridoma production.

^f These studies will be performed as standard of care if deemed appropriate by the patient's physician.

^g If feasible, a history and physical examination and serology will be obtained every 3 months until evidence of disease progression.

10.1 Clinical Course

Patients will be examined by oncologists at Memorial Hospital and have laboratory and radiographic studies performed as outlined in the above table. As this is a pilot study primarily concerned with effect of injection of sLe^a on immune response, intensive clinical follow-up at Memorial Hospital will not be required. However, if feasible, patients who remain clinically stable will be asked to return to Memorial Hospital every 3 months for a history and physical examination and laboratory studies.

The patient and their primary oncologist will be notified of all CTC results. These results are for research purposes and will not be used during the study to alter clinical care.

10.2 Immunologic Response

Serological response: Three 10 ml red top tubes will be drawn before the first injection, before each subsequent injection and also during weeks 5, 9, 13 and 23. Sera will be evaluated for reactivity against sLe^a in ELISA and/or immunoprecipitation-western blot assays. These assays detect antibodies against sLe^a and confirm identity of the reaction. If serologic reactivity is detected, then additional blood (~10ml) may be drawn at later times after the injections are completed to determine the duration of this response (every 3 months if feasible). An extra 100ml of heparinized blood may be obtained once during the course of immunizations depending on the serologic response in individual patients at the principal investigators discretion, for hybridoma production. This will be used for evaluation of the B-cell response to vaccination at the clonal level by establishment of monoclonal antibodies. This will be performed in collaboration with MabVax Therapeutics, Inc., (San Diego, CA). It will make possible more detailed analysis of the most immunogenic epitopes on sLe^a and more detailed comparison of the avidity of the induced IgG and IgM antibodies than is possible with sera.

T cell response will not be evaluated since this is a carbohydrate antigen and significant T cell response is not anticipated. The immune response analyses will be conducted in the laboratory of Dr. Livingston under Dr. Ragupathi's supervision.

10.3 Circulating Tumor Cell Methodology

Epithelial cell adhesion molecule (EpCAM) is used to concentrate circulating epithelial tumor cells from a 7.5 ml peripheral blood specimen. A semi-automated system called CellSearch, uses EpCAM antibody-based immunomagnetic capture of epithelial cells to identify and isolate circulating tumor cells (CTC).

Immunomagnetically captured CTCs are stained and enumerated using specially prepared buffers and three immunofluorescent markers: DAPI to detect cells with intact nuclei; Cytokeratin-PE to detect epithelial cells; and CD-45-APC to detect leukocytes. CTCs are defined as DAPI(+), CK-PE(+) and CD-45(-) cells. Digitally archived images of candidate CTCs meeting these criteria also must meet standardized morphologic inspection.

11.0 TOXICITIES/SIDE EFFECTS

In our earlier breast cancer immunization trials, common toxicities included local skin reactions at the injection sites, flu-like symptoms (ie, fever, fatigue, myalgias, arthralgias), and less frequent symptoms such as pruritis, rashes, diarrhea, nausea and vomiting. These symptoms were transient and usually of less than 5 days duration. In addition, minimal transient decreases in hematologic values, primarily white blood cell counts and lymphocyte counts were noted. It is unlikely that these fluctuations were due to the vaccine. Transient elevation in various laboratory values (ie glucose, liver enzymes) were observed and also thought unrelated to the vaccine.

Since the antigen in this injection is present on the surface of normal tissues, the theoretical possibility of a serious autoimmune reaction exists. In our previous breast cancer trials, there was no consistent evidence of an autoimmune reaction due to the immunization. Several episodes of hyperthyroidism or hypothyroidism have occurred on these trials. Patients were subsequently treated with radioactive I-131 or thyroid supplementation. It is unclear whether these events were related to the immunization. Therefore, the thyroid stimulating hormone level will be monitored pre therapy, during therapy and post therapy.

In addition, no definite evidence of autoimmunity has been observed in immunization trials in prostate or ovarian cancer at MSKCC (personal communication with Dr. Slovin and Dr. Sabbatini). Autoimmune reactions may be controlled by cessation of the immunization and anti-inflammatory medications. The symptoms would be dependent on the organs involved. These may include but are not limited to diarrhea, dyspnea, numbness, weakness, vaginal bleeding, inflammation of the breast or pancreas and blood in the stool. This autoimmunity is considered unlikely however, since cytotoxic T-cells and antibodies against various antigens found on breast cancer cells have been recognized in the peripheral blood of patients with breast cancer in the absence of any evidence for autoimmunity. Some patients have also demonstrated baseline antibody titers against various antigens used in our prior trials.

In MSKCC protocol 01-019 several episodes of transient asymptomatic elevation of the AST and ALT levels (grade 1-3) were observed. The finding was thought to be due to the GPI-0100 and we do not anticipate this toxicity on this trial using QS-21.

The rare possibility of allergic reaction exists (ie wheezing, hives, fevers, chills, dyspnea).

In prior immunization trials, moderate local discomfort at injection sites and flu-like symptoms were observed with QS-21 at the 200 mcg dose. The 100 mcg dose was found to be effective with minimal toxicity. We do anticipate significant toxicity with the higher dose of QS-21 (150 mcg). The only expected toxicity of KLH is local injection site reaction.

Grade III or greater local or systemic toxicity will result in removal of the patient from the study. Grade II or greater toxicity of autoimmune etiology will result in removal from the study. Grade II or greater allergic toxicity, excluding a fever or flushing, will result in removal from the study. However, patients who develop a fever \geq 38 degrees Centigrade, thought to be due to the vaccine, will be monitored for 60 minutes in the Immunology Unit following the next vaccination.

Any patient removed from the study due to toxicity will result in suspension of the study pending further investigation and discussion with the CBER IND review team. In that case, if mutually agreed on, the trial may resume at the 10mcg sLe^a dose, if that was well tolerated. Patients will be counseled to avoid pregnancy.

Note: Grade 3 lymphopenia is acceptable since the eligibility criteria allows for a grade 3 baseline value.

Adverse events will be reported to the Institutional Review Board and to the Food and Drug Administration. Patients will be asked to complete a diary after each vaccine. (Appendix B)

Criteria for Toxicity: Toxicity will be graded in accordance with the Common Toxicity Criteria Version 3.0 developed by the National Cancer Institute (NCI). (Refer to the Internet web site <http://www.nci.nih.gov>)

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary endpoints of this trial are toxicity and immunologic response. Patients will have baseline imaging studies to assess the extent of the disease. However, patients will not routinely undergo followup imaging studies unless felt to be appropriate by their physician as standard of care.

12.1 Long Term Followup

Patients will be followed every 3 months if feasible following completion of the vaccine trial, until evidence of disease progression. Shorter time intervals between visits may be required, according to the standard of care for the patient's state of disease.

12.2 Evaluability

Efforts shall be made to account for all patients entered into the study during the evaluation of results. All patients will be considered evaluable for safety and immune response.

12.3 Clinical Response

Patients will be monitored for anti-tumor effect, although this is not a primary endpoint. Patients who have evidence of disease will be evaluated at week 19 or at the discretion of their physician. Responses will be categorized using RECIST response criteria as follows:

Complete Response (CR):

Target Response Criteria: The disappearance of all known disease and no new sites or disease related symptoms confirmed at least 4 weeks after initial documentation. All sites must be assessed, including non-measurable sites, such as effusions, or markers

Non-Target Response Criteria: Disappearance of all non-target lesions and normalization of tumor markers level confirmed at least 4 weeks after initial documentation.

Partial Response (PR):

Target Response Criteria: At least a 30% decrease in the sum of the longest diameters of target lesions, taking as a reference the baseline sum of the longest diameters confirmed at least 4 weeks after initial documentation. PR is also recorded when all measurable disease has completely disappeared, but a non-measurable component (i.e., ascites) is still present but not progressing.

Stable Disease (SD):

Target Response Criteria: Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease.

Non-Target Response Criteria: Persistence of one or more non-target lesions and/or the maintenance of tumor marker level above the normal limits.

Progressive Disease (PD):

Target Response Criteria: At least a 20% increase in the sum of the longest diameters of target lesions, taking as reference the smallest sum of the longest diameters recorded since the treatment started; or the appearance of one or more new lesions; or the unequivocal progression of a non-target lesion.

Non-Target Response Criteria: Appearance of one or more non-target lesions and/or unequivocal progression of existing non-target lesions.

13.1 CRITERIA FOR REMOVAL FROM STUDY

Cessation of treatment

Therapy may be discontinued at any time due to severe unacceptable side effects, dose-limiting toxicities as defined, life-threatening events, patient's non-compliance with the defined treatment plan, the patient becomes pregnant or the patient's request to withdraw consent.

With evidence of recurrent disease or progression of disease, the patient's primary physician will be notified immediately by the protocol investigators. The decision regarding optimal therapy will be made by the patient and the primary physician. The patient may continue on study if the patient's primary physician feels it is acceptable and the only change in therapy is hormone therapy.

The following dose limiting toxicities as evaluated during weeks 1-21 will result in cessation of treatment:

- Grade 2 or greater autoimmune reactions
- Grade 2 or greater allergic reactions (excluding isolated fever and/or flushing)
- Grade 3 or greater toxicity of any kind (excluding grade 3 lymphopenia)

If any of these toxicities occur, no additional patients will be treated except following mutual agreement after discussion with the CBER IND review team.

14.0 BIOSTATISTICS

This is a pilot study designed to assess the safety and immunogenicity of immunization with sLe^a-KLH plus QS-21 in patients with high risk breast cancer. If immune responses are observed at the 100 mcg level of QS-21 then QS-21 will be escalated to the 150mcg level.

The immunization schedule comprises five injections administered during weeks 1,2,3,7 and 19. The primary endpoint of this trial is to evaluate immunological response to this single antigen and evaluate toxicity. Immunological response for a patient is defined as an antibody response of greater than 1:80 if baseline value was 0 and at least an increase of 8-fold if baseline value is greater than 0. Initially, three patients will be accrued to the study at a dose of 10 mcg of sLe^a. If safe, then ten patients will be accrued to the study at the full 30mcg sLe^a dose. If 6 or more of these 10 patients have an immune response at any time point then an additional 10 patients may be accrued and receive sLe^a-KLH plus QS-21 (at 150mcg) during weeks 1, 7 and 19. This sample size is chosen to minimize the total number of patients who need to be immunized to identify a promising agent with a probability of an immunological response greater than 50% in a series of immunization trials. The error rates of missing a promising agent or selecting a nonpromising agent are set to be at most 10%.³⁶

At the end of the trial a qualitative assessment of the number of responses and frequency of toxicities will be made to determine which dose of QS-21 will be carried forward in future studies.

Severe toxicities are not anticipated. Toxicities will be tabulated according to the NCI CTC version 3.0. Any grade 3 or greater toxicity (excluding grade 3 lymphopenia) or grade 2 or greater allergic/immunologic toxicity (except isolated fever and/or flushing) will result in removal of the patient from the study. Since the above listed toxicities are unexpected, any event of this sort will result in suspension of the study pending further investigation. Descriptive statistics summarizing the antibody responses will be provided for each patient. The length of the study will be approximately 1-2 years.

Circulating tumor cells (CTC) will be evaluated in an exploratory manner to determine whether CTC can be detected in this patient population at baseline and if so whether there is evidence of a decline in those patients who have an immune response.

Severe toxicity is not anticipated. Frequencies of all toxicities will be recorded. We plan to accrue approximately 1-2 patients per month and therefore accrual should be completed in 10 months.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

The following persons can obtain informed consent:

Teresa Gilewski M.D.
Maura Dickler M.D.
Clifford Hudis M.D.
Larry Norton M.D.

Confirm in the electronic medical record that the patient has received the Notice of Privacy Practice. This must be obtained before the eligibility confirmation and obtaining of the research informed consent.

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain written informed consent, by following procedures defined in section entitled Informed Consent Procedures.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am - 5:30pm at (646) 735-8000. The PPR fax numbers are (646) 735-0008 and (646) 735-0003. Registrations can be phoned in or faxed. The completed signature page of the informed consent form, the completed signature page of the Research Authorization and a completed Eligibility Checklist must be faxed to PPR.

During the registration process registering individuals will be required to answer specific eligibility questions and provide the following information:

Registering Individual	[Last, First Name]
Notice of Privacy Status	[Yes, No, N/A]
Research Authorization	[Date]
MSKCC IRB Protocol#	
Attending of Record (if applicable)	[Last, First Name]
Consenting Professional	[Last, First Name]
Informed Consent Date	
Participant's Full Name	[Last, First Name]
Participant MRN	

16.1 DATA MANAGEMENT ISSUES

A data profile in Appendix A will be completed for all patients.

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization and coordination of activities of the protocol study team.

The data collected for this study will be entered into a secure database at MKSCC. Data from this trial will be entered in the Clinical Research Data Base (CRDB). Source documentation will be available to support the computerized patient record.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at:

<http://cancertrials.nci.nih.gov/researchers/dsm/index.html>. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at:

<http://mskweb2.mskcc.org/irb/index.htm>

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center’s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g. NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.1 PROTECTION OF HUMAN SUBJECTS

The study will maintain the privacy of the participant as noted in Section 7.1. The patient will be informed that the study is voluntary and the participant may withdraw at any time. Alternative options for the patient are not to participate in this study and receive no therapy or to receive other treatments that may include hormone therapy, chemotherapy, radiation therapy, targeted therapy, surgery or other investigational therapy. The patient will be informed that there is no known clinical benefit to the immunization under evaluation in this study. Potential benefits include generation of an anti-tumor response by stimulation of the immune system.

The patient is responsible for all costs excluding the sLe^a-KLH plus QS-21 immunization, the evaluation of immune response and the assessment of circulating tumor cells. Potential toxicities are outlined in Sections 11 and 13.

17.2 Privacy

It is the responsibility of the Research Staff to ensure that protocol subjects received the Center's Notice of Privacy Practices. If the subject has not received one, MSK personnel must provide a Notice of Privacy Practices and obtain acknowledgment before the subject participates in the study.

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board.

17.3 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB as soon as possible but no later than 5 calendar days. The IRB requires a Clinical Research Database (CRDB) AE report to be delivered to the Institutional SAE Manager (307 East 63rd Street, 1st Floor) containing the following information:

Fields populated from the CRDB:

- Subject's name
- Medical record number
- Disease/histology (if applicable)
- Protocol number

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
 - A explanation of how the AE was handled
 - A description of the subject's condition
 - Indication if the subject remains on the study

- If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:

The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. The report will be forwarded to the FDA by the Institutional SAE Manager through the IND Office.

17.2.1 IND Safety Reports

This protocol is sponsored under an IND. Therefore, any serious adverse events will be reported to the Food and Drug Administration through the IND office of Memorial Sloan-Kettering Cancer Center, as set forth in FDA regulation 312.32.

17.3 Reporting of Other Adverse Events

Unexpected Toxicities- an event that is not identified in terms of its nature, severity or frequency of occurrence in the expected toxicities.

The MSKCC IRB must be notified in writing within 5 working days of the event.

Non-treatment Related Toxicities

If a toxicity is not a serious adverse event and unrelated to the protocol treatment, this must clearly be documented on the flow sheets which are submitted to the MSKCC clinical trials office. This does not in any way obviate the need for reporting the toxicities described above.

18.1 INFORMED CONSENT PROCEDURES

A consenting professional will explain the protocol to the patient. In addition to the verbal explanation, patients will receive the informed consent document to read. If the patient agrees to participate in the trial, the patient will sign three copies of the informed consent. One copy will be placed in the patient's medical record, one copy will be returned to the patient. One copy will be placed in the research file.

18.2 Research Authorization

Procedures for obtaining Research Authorization: Before any protocol-specific procedures are carried out, investigators and/or designated staff will fully explain the details of the protocol, study procedures, and the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must sign the Research Authorization component of the informed consent form. The Research Authorization requires a separate set of

signatures from the patient. The original signed documents will become part of the patient's medical record, and each patient will receive a copy of the signed documents.

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20.0 APPENDICES

APPENDIX A: PATIENT CALENDAR

APPENDIX B: PATIENT DIARY