

## **Pilot Study of a Heptavalent Vaccine-Keyhole Limpet Hemocyanin Conjugate plus QS21 in Patients with Epithelial Ovarian, Fallopian Tube, or Peritoneal Cancer**

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**Abstract Purpose:** To characterize the safety and immunogenicity of a heptavalent antigen-keyhole limpet hemocyanin (KLH) plus QS21 vaccine construct in patients with epithelial ovarian, fallopian tube, or peritoneal cancer in second or greater complete clinical remission.

**Experimental Design:** Eleven patients in this pilot trial received a heptavalent vaccine s.c. containing GM2 (10 µg), Globo-H (10 µg), Lewis Y (10 µg), Tn(c) (3 µg), STn(c) (3 µg), TF(c) (3 µg), and Tn-MUC1 (3 µg) individually conjugated to KLH and mixed with adjuvant QS21 (100 µg). Vaccinations were administered at weeks 1, 2, 3, 7, and 15. Periodic blood and urine samples were obtained to monitor safety (complete blood count, comprehensive panel, amylase, thyroid-stimulating hormone, and urinalysis) and antibody production (ELISA, fluorescence-activated cell sorting, and complement-dependent cytotoxicity).

**Results:** Eleven patients were included in the safety analysis; 9 of 11 patients remained on study for at least 2 weeks past fourth vaccination and were included in the immunologic analysis (two withdrew, disease progression). The vaccine was well tolerated. Self-limited and mild fatigue (maximum grade 2 in two patients), fever, myalgia, and localized injection site reactions were most frequent. No clinically relevant hematologic abnormalities were noted. No clinical or laboratory evidence of autoimmunity was seen. Serologic responses by ELISA were largely IgM against each antigen with the exception of Tn-MUC1 where both IgM and IgG responses were induced. Antibody responses were generally undetectable before immunization. After immunization, median IgM titers were as follows: Tn-MUC1, 1:640 (IgG 1:80); Tn, 1:160; TF, 1:640; Globo-H, 1:40; and STn, 1:80. Only one response was seen against Lewis Y; two were against GM2. Eight of nine patients developed responses against at least three antigens. Antibody titers peaked at weeks 4 to 8 in all patients. Fluorescence-activated cell sorting and complement-dependent cytotoxicity analysis showed substantially increased reactivity against MCF7 cells in seven of nine patients, with some increase seen in all patients.

**Conclusions:** This heptavalent-KLH conjugate plus QS21 vaccine safely induced antibody responses against five of seven antigens. Investigation in an adequately powered efficacy trial is warranted.

The current standard treatment for patients with advanced ovarian cancer consists of aggressive surgical cytoreduction followed by taxane- and platinum-based chemotherapy. Although the median overall survival for optimally debulked

patients has increased to 65.6 months, <30% of patients will remain free of disease (1). Many patients will have initially chemotherapy-sensitive disease at recurrence and can reenter successive remissions with additional treatment. Subsequent remissions are of progressively shorter duration until chemotherapy resistance uniformly develops (2). Immune directed therapy is ideally suited for evaluation in first and subsequent clinical remission populations when the disease burden is lowest, and evaluating treatments designed to prolong the duration of such remissions remains the highest priority (3). Antibodies are well suited for eradicating tumor cells from the bloodstream and eliminating early tissue invasion (4). Preclinical models have shown the clearance of circulating tumor cells and the elimination of systemic micrometastasis through the use of both passively administered and vaccine-induced antibodies (5).

Ovarian cancers express a rich array of cell surface antigens. These include carbohydrate epitopes, such as GM2, Globo-H, Lewis Y, sialyl Tn (STn), Tn, Thompson Friedreich antigen (TF),

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and mucin 1 (MUC1; refs. 6–9). For the production of antibodies against defined cell surface antigens such as these, the best approach has been described to include chemical conjugation of the antigen to a highly immunogenic carrier protein plus the use of a potent immunologic adjuvant. The best carrier protein in our experience has been keyhole limpet hemocyanin (KLH) and the best immunologic adjuvant has been a saponin such as QS21 (10). Advances in the chemical and enzymatic synthesis of carbohydrate and glycopeptide antigens and optimization of adjuvant use have permitted the exploration of a variety of synthetic antigen vaccines with antibody production as the end point (11–13). Polyvalent vaccines will likely be required due to tumor cell heterogeneity, heterogeneity of the human immune response, and the correlation between overall antibody titer against tumor cells and antibody effector mechanisms (5). We showed in preclinical models that a heptavalent-KLH conjugate plus QS21 vaccine induced antibody titers comparable with those induced by monovalent vaccines containing Tn, STn, TF, MUC1, and Globo-H. Lower titers were produced against Lewis Y and GM2 (14). A series of monovalent antigen vaccine trials at Memorial Sloan-Kettering Cancer Center evaluating single antigen-KLH constructs with QS21 as the adjuvant have shown antibody responses against the glycolipids GM2 and Globo-H, and the mucin backbone MUC1, as well as cancer cells expressing these antigens (15–17). Other antigens have required modification for optimal immunogenicity resulting in trimers or clusters (c) of Tn, STn, and TF rather than unmodified monomers (18, 19). We have not identified a good approach for augmenting the immunogenicity of Lewis Y in patients despite several attempts (11, 20).

In this pilot study, we sought to test a heptavalent vaccine in ovarian cancer patients in second or greater complete clinical remission. The objectives were to characterize safety and immunogenicity in preparation for an adequately powered randomized efficacy trial.

## Materials and Methods

**Eligibility criteria.** Eligible patients had histologically documented epithelial ovarian carcinoma arising in the ovary, fallopian tube, or peritoneum from stages II to IV at diagnosis. Primary treatment must have included cytoreductive surgery and a platinum-based chemotherapy regimen, and patients must have been in complete clinical remission for study entry. Alternatively, patients may have relapsed following primary treatment and returned to complete clinical remission after additional chemotherapy. Complete clinical remission was defined as serum CA125  $\leq 35$  IU/mL, computed tomography scan without evidence of disease, and normal physical examination. Other requirements included Karnofsky performance status  $\geq 60\%$ ; adequate organ function defined as absolute neutrophil count  $\geq 1.0 \times 10^3$   $\mu\text{L}$ ; platelets  $\geq 100,000$  cells/ $\text{mm}^3$ ; serum creatinine  $\leq 1.5 \times$  institutional upper limits of normal; and total bilirubin, aspartate aminotransferase, and alkaline phosphatase  $\leq 2 \times$  institutional upper limits of normal. Patients were ineligible if they had a known autoimmune disease or immune deficiency, a known allergy to seafood, child-bearing potential, or an expected survival  $< 3$  months.

All patients provided written informed consent. The protocol was approved at the Memorial Sloan-Kettering Institutional Review Boards and reviewed annually. A Food and Drug Administration Investigational New Drug was obtained.

**Treatment plan.** The administered heptavalent vaccine contained GM2 (10  $\mu\text{g}$ ), Globo-H (10  $\mu\text{g}$ ), Lewis Y (10  $\mu\text{g}$ ), Tn-MUC1 (3  $\mu\text{g}$ ),

Tn(c) (3  $\mu\text{g}$ ), STn(c) (3  $\mu\text{g}$ ), and TF(c) (3  $\mu\text{g}$ ) individually conjugated to KLH and mixed with adjuvant QS21 (100  $\mu\text{g}$ ). Vaccinations were administered at weeks 1, 2, 3, 7, and 15. The vaccine was administered in 1 cc total volume using a 25-gauge needle s.c. into the buttocks, thighs, abdomen, or arms.

**Vaccine preparation.** Globo-H, Lewis Y, Tn(c), STn(c), and TF(c) were synthesized in the laboratory of Bio-Organic Chemistry at the Sloan-Kettering Institute as described previously (18, 20–25). MUC1-32mer(-CHGVTSAPDTRPAPGSTAPPAGVTSAPDTRPA) was synthesized in an automated peptide synthesizer in the Memorial Sloan-Kettering Cancer Center Core Peptide Synthesis Facility, and the Tn-MUC1-32 GalNAc-glycopeptide with 8 mol GalNAc incorporated was produced by *in vitro* glycosylation (see Fig. 1) with recombinant human polypeptide GalNAc-transferases T2 and T4 as described previously (26, 27). GM2 was extracted from rabbit brains as GM1, treated with  $\beta$ -galactosidase to yield GM2, and provided by Progenics, Inc. (28).

With regards to conjugation to KLH, Globo-H, MUC1-32mer, GM2, Lewis Y, Tn(c), STn(c), and TF(c) were covalently attached to KLH directly by reductive amination (GM2), using the 4-(4-maleimido-methyl) cyclohexane-1-carboxyl hydrazide heterobifunctional linker group (Globo-H and Lewis Y) and using the m-maleimidobenzoyl-N-hydroxysuccinimide ester heterobifunctional linker group [MUC1, Tn(c), STn(c), and TF(c)] as described previously (18, 20–25, 29). The structures of these seven conjugates are summarized in Fig. 1. Antigen and adjuvant doses were selected based on previous phase 1 monovalent vaccine trials as follows: 3  $\mu\text{g}$  Tn-MUC1-32mer (24), 10  $\mu\text{g}$  Globo-H (15, 22), 10  $\mu\text{g}$  GM2 (30), 10  $\mu\text{g}$  Lewis Y (20), 3  $\mu\text{g}$  Tn(c) (23), 3  $\mu\text{g}$  STn(c), 3  $\mu\text{g}$  TF(c) (18), and 100  $\mu\text{g}$  QS21 (15, 30). These seven conjugates and QS21 were vialled together in 1 mL PBS. Vialled samples underwent testing for toxicity and immunogenicity in mice, as well as for sterility and endotoxin. Vaccines were given s.c. on weeks 1, 2, 3, 7, and 15 to rotating sites on arms and legs.

**Dose adjustment.** Dose modification or delay was not permitted. Patients were to be removed from study for a dose-limiting toxicity defined using National Cancer Institute toxicity criteria as (a)  $\geq$  grade 2 allergic reaction, (b)  $\geq$  grade 2 autoimmune reaction, and (c)  $\geq$  grade 3 hematologic or nonhematologic toxicity, including fever, or grade 3 injection site reaction. Patients were removed from study for disease progression.

**Evaluation during study.** Pretreatment evaluation included a complete medical history, physical and radiologic examination (computed tomography), vital signs, Karnofsky performance status assessment, and clinical laboratory tests, including hematologic, biochemistry, CA125, and immunologic testing. Baseline stool guaiac had to be negative for occult blood, and it was repeated at weeks 7, 15, and 27. Patients had repeat complete blood cell counts, comprehensive biochemistry panel, and serum amylase at regular intervals, and at off-study visit. Computed tomography imaging was done every 3 months while on study, or sooner to restage patients if signs or symptoms, blood tests, or physical examination suggested disease progression. Serum samples for immune testing were obtained at weeks 1, 5, 7, 9, 13, 15, and 17.

All patients were in complete clinical remission at the time of study enrollment. Although not the study end point, time to treatment failure was defined based on data from Rustin et al. (31). Treatment failure was characterized by (a) physical examination evidence of disease recurrence or (b) radiographic evidence of disease recurrence (biopsy will be done at the discretion of the principal investigator but is not required).

ELISA assays were tested against the following target antigens: Tn-MUC1-32mer, GM2, Globo-H ceramide, Lewis Y ceramide, desialylated ovine submaxillary mucin expressing Tn, ovine submaxillary mucin expressing STn, and desialylated porcine submaxillary mucin expressing TF. IgM and IgG antibody titers were measured by ELISA as described previously (15, 22, 24). Nunc 96-well ELISA plates were plated with 0.1  $\mu\text{g}$  of target antigen per well, in 60  $\mu\text{L}$  carbonate buffer, and incubated overnight at 4°C (for MUC1, Tn, STn, and TF) or plated with 60  $\mu\text{L}$  of 100% ethanol and left overnight to evaporate (GM2, Lewis Y,

and Globo-H). Following a PBS wash, unreactive sites were then blocked by incubation with 3% human serum albumin for 2 h at 37°C. Serial dilutions of the patient's sera were then added to the wells, left at room temperature for 1 h, and then washed. Secondary antibodies, either alkaline phosphatase labeled goat anti-human IgM or IgG, were added. Following a 10- to 20-min incubation, the plates were washed, developed, and read at 405 nm on the ELISA reader (Bio-Rad model 550 microplate reader). The titer was defined as the highest dilution yielding an absorbance of  $\geq 0.1$ . Patients going from undetectable titers pretreatment to 1:40 or greater posttreatment or those with an 8-fold or greater increase in titer over detectable retreatment levels were considered responders.

Fluorescence-activated cell sorting was done as described previously to show antibody binding to the cell surface of the cell line MCF7. MCF7 was chosen as it is known to express each of the seven antigens (15, 22, 24). The colon cancer cell line LSC and the ovarian cancer cell lines OVCAR3 were also used as targets. Preimmunization and postimmunization sera were diluted 2- to 4-fold from their maximum antibody titer determined by ELISA and incubated with cells for 30 min

on ice. The cells were washed and treated with goat anti-human IgM or IgG labeled with FITC and analyzed by flow cytometry. Pretreatment and experimental sera were read together and the pretreatment percentage positive cells gated at 10%. Sera with a 2-fold increase in percentage positive cells and 1.5-fold increase in mean fluorescence intensity over the pretreatment level are considered positive.

Complement-dependent cytotoxicity (CDC) was assayed on MCF7 cell lines using a 2-h 51-chromium release assay as described previously. CDC (15, 22, 24) was assessed before and at the time of peak ELISA reactivity (week 6 or 8). Ten to 20 million MCF7 cells were washed in FCS-free medium twice, resuspended in 500  $\mu$ L medium, and incubated with 100  $\mu$ Ci Cr for 2 h at 37°C, during which the cells were shaken every 15 min. The cells were washed thrice in medium and suspended to achieve a concentration of  $\sim 20,000$  cells per well and plated in round-bottomed plates. The plates contained either 50  $\mu$ L cells plus 50  $\mu$ L monoclonal antibody as positive control or 50  $\mu$ L cells plus serum (pre and post) diluted 1:4 as experimental. The plates were incubated at 4°C on a shaker for 45 min. Human complement (Sigma-Aldrich Co.) at a 1:5 dilution, (resuspended in 1 mL of ice-cold water

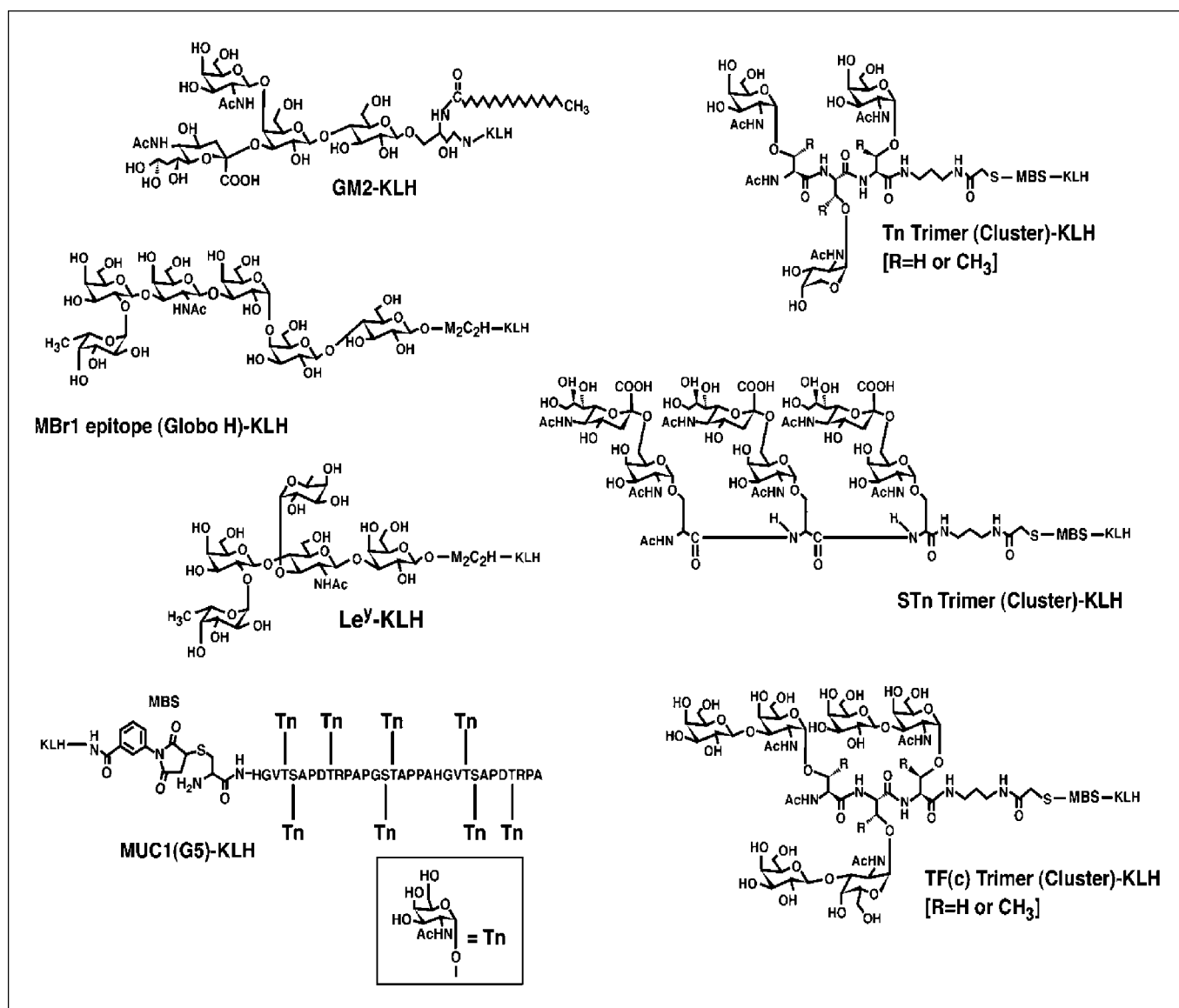


Fig. 1. Components of the heptavalent-KLH conjugate vaccine against ovarian cancer. The two heterobifunctional linker groups used are m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) and 4-(4-maleimidomethyl) cyclohexane-1-carboxyl hydrazide (MMCCCH). For Tn(c)-KLH and TF(c)-KLH, R is equal to H.

**Table 1.** Patient characteristics (N = 11)

Characteristic	n (%)
Median age (range), y	50 (43-64)
FIGO stage at diagnosis	
III	11 (100)
IV	0 (0)
Histology	
Papillary serous	9 (82)
Endometrioid	2 (18)
Primary surgery	
Optimal	7 (64)
Suboptimal	4 (36)
First remission at start of study	4 (36)
Second remission at start of study	3 (28)
Third or greater remission at start of study	4 (36)

Abbreviation: FIGO, International Federation of Gynecology and Obstetrics.

with 3% human serum albumin) was added to each well at a volume of 100  $\mu$ L. The 1:5 complement dilution was selected as a compromise between higher background in preimmune sera with lower dilutions, and lower CDC with immune sera with higher dilutions. In the six control wells, Triton (10%, 20  $\mu$ L) was added with medium to a total volume of 200  $\mu$ L. The plates were incubated for 2 h at 37°C and then centrifuged for 3 to 5 min. Then, 30  $\mu$ L of supernatant were removed for radioactivity counting. Positive results were those wells in which post-vaccination cytotoxicity was  $\geq$ 15% above the pretreatment level.

**Statistical considerations.** The primary end points of this pilot trial were safety and confirmation of immunogenicity in the heptavalent setting. Because one of the concerns was to evaluate safety in patients having an immune response, all patients (intention to treat) are recorded for safety analysis, but 11 patients were accrued to reach a target of patients who remained on study at least 2 weeks past the fourth vaccination for evaluation of immunogenicity. The same criteria for immunogenicity were used as were used in the preceding individual pilot trials: patients must have peak IgM titer  $\geq$ 1:40 or a peak 8-fold increase in prevailing antibody titer if present at baseline. Assuming a probability of response equal to 50%, nine patients were accrued and more than five of these patients should meet these criteria for three or more antigens to proceed with this construct in additional studies (32).

## Results

**Patient characteristics.** The patient characteristics are described in Table 1. The median age was 50 (range, 43-64 years). All patients were stage III at diagnosis and most of papillary serous histology (82%) with optimal primary debulking (64%). Patients entered this pilot study in first (36%) or second or greater complete clinical remission (64%). Seven patients received all 5 vaccinations, 3 received 4 vaccinations (one patient was withdrawn for progression of disease immediately following the fourth immunization), and 1 patient was removed after 3 vaccinations (progression of disease). Thus, nine patients remained on study for at least 2 weeks following the fourth vaccination and were included in the immunologic evaluation.

**Adverse events.** The heptavalent vaccine administration was well tolerated. The most common drug-related adverse events are listed in Table 2. Self-limited and mild fatigue (maximum

grade 2 in two patients), fever, myalgia, and localized injections site reactions were most frequent. Leukopenia and anemia (maximum grade 1) were seen in two patients, but no clinically relevant hematologic abnormalities were noted. No clinical or laboratory evidence of autoimmunity was seen. There were no serious adverse events reported. No hypersensitivity reactions were seen.

**Immune response.** Serologic responses as determined by ELISA were largely limited to IgM responses against each of the antigens except for Tn-MUC1 where both IgM and IgG antibody responses resulted. The serologic responses of all patients over time against the five most immunogenic antigens are shown in Fig. 2. Antibody titers peaked between weeks 4 and 8 in all cases and then generally declined to titers of 1:40 or less by 6 months, with the booster immunization at week 15 having limited effect. Tn-MUC1 and TF were the most potent immunogens with eight of nine patients showing serologic responses, whereas only one and two patients showed serologic reactivity against Lewis Y and GM2, respectively. Eight of nine patients showed at least an 8-fold increase in antibody titers against three of the seven antigens after immunization.

The median pretreatment and posttreatment ELISA titers against each of the seven antigens for the patients treated with this heptavalent vaccine were considered in the context of the titers against the individual antigens induced in patients treated previously with the individual monovalent vaccines. These results are summarized in Table 3. Although the antibody titers induced by the heptavalent vaccine were slightly lower than those induced by the monovalent vaccines, the difference in titers would be considered to be possibly clinically relevant only in the case of GM2. Each of the other antigens met our previously determined definition of "positive."

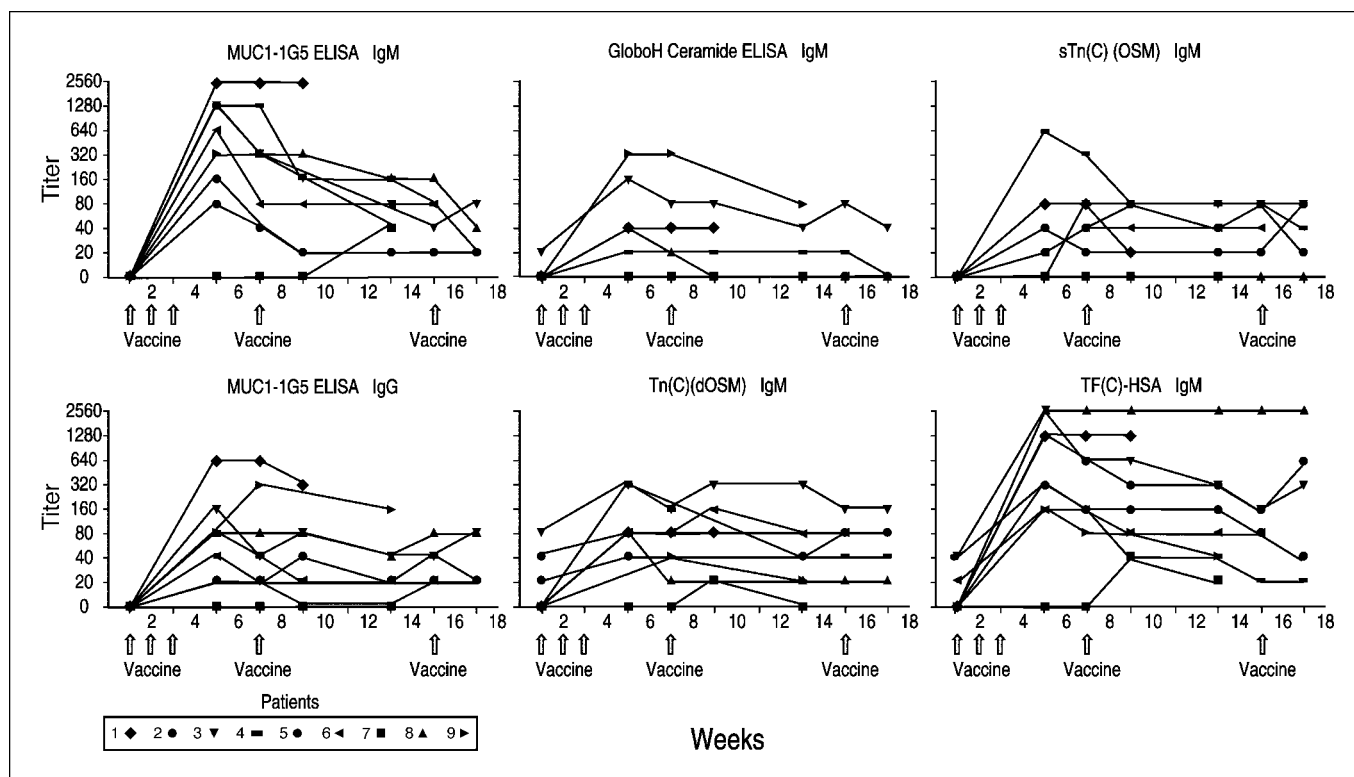
Fluorescence-activated cell sorting analysis showed increased reactivity against the MCF7 cells in six of the nine patients (patients 1, 3, 4, 5, 8, and 9), although some increase was detected in all nine patients. These results are shown in Fig. 3, where percentage positive cells and mean fluorescent intensity pre-vaccination and post-vaccination for each of the nine patients are indicated. Increases in cell surface reactivity after vaccination were also shown against LSC and OVCAR3 with

**Table 2.** Vaccine-related events in patients receiving heptavalent vaccine (N = 11)

Drug-related adverse event	Patients per maximum toxicity grade, n (%)			
	1	2	3	4
Laboratory based				
Leukopenia	0	0	0	0
Anemia	0	0	0	0
Platelets	1 (9)	0	0	0
Amylase	1 (9)	1 (9)	0	0
AST or ALT	2 (18)	0	0	0
Nonlaboratory based				
Fever	2 (18)	2 (18)	0	0
Fatigue	6 (54)	0	0	0
Myalgia	6 (54)	1 (9)	0	0
Injection site reaction	10 (90)	1 (9)	0	0

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.





**Fig. 2.** Results of ELISA assays against five individual antigens (excluding GM2 and Lewis Y) at all time points pre-vaccination and post-vaccination for patients receiving the heptavalent vaccine. Reciprocal antibody titers at each time point. Vaccines were administered on weeks 1, 2, 3, 7 and 15.

post-vaccination sera from three patients each (data not shown).

Sera from five of the nine immunized patients showed increases in complement-mediated cytotoxicity against MCF7 cells as shown by the solid lines in Fig. 4 (patients 1, 4, 7, 8, and 9). Levels of cytotoxicity using a 1:4 serum dilution and human complement at a 1:5 dilution in a 2-h assay increased in these five patients by 15% to 44%. Whereas sera from four of the five patients with CDC developed titers of reactivity against Globo-H and/or Lewis Y between 1:40 and 1:320, the fifth patient (patient 4) had no detectable antibody reactivity against the three glycolipids but developed reactivity against all four of the mucin antigens.

Clinical outcome was not the study end point in this phase I trial. The median time to treatment failure from date of first vaccination was 4.2 months (95% confidence interval, 2.7-8.5). Because the number of patients in this pilot study is small and all patients had some immune response, it is not possible to separate differences in time to treatment failure by the differences in immune titers. This study succeeded in confirming safety and immunogenicity. An adequately powered trial with efficacy as the end point is warranted.

## Discussion

Low immunogenicity of cancer antigens is the major obstacle in the development of cancer vaccines. This is in part because many cancer antigens are autoantigens or only slightly modified autoantigens. Each of the seven antigens in the vaccine described here are autoantigens expressed on a variety

of normal tissues (6–8). The relative cancer specificity is a consequence of (a) overexpression on ovarian cancer cells and (b) expression on normal tissues at secretory borders and other luminal sites that are largely inaccessible to the immune system. The expression of these antigens on normal tissues is of concern with regard to both the safety and the potential immunogenicity of this vaccine. Our results show that it is possible to safely induce antibody responses against these antigens using a heptavalent-KLH antigen construct with QS21 as the immune adjuvant.

We show here that all nine vaccinated patients had antibody responses against at least one of these antigens, and eight patients responded against at least three antigens after vaccination. Sera from seven of nine patients had levels of cell surface reactivity shown by fluorescence-activated cell sorting or CDC. These responses were slightly lower but comparable with responses seen previously when six of these antigens were administered as monovalent vaccines, with the exception being GM2. Antibody titers against GM2 after vaccination were lower than those generated in patients treated previously with the monovalent GM2-KLH vaccine. It is possible that one or more components of the vaccine specifically inhibited the antibody response against GM2 but more likely is that the lower dose of GM2 (10  $\mu$ g as opposed to 30  $\mu$ g) or decreased immunogenicity of the particular lot of GM2-KLH used in this trial is the cause. The antibody response against Lewis Y-KLH was also low, with only one of nine patients generating antibody titers against Lewis Y, but this was consistent with the low immunogenicity seen in previous trials with monovalent vaccines against Lewis Y (20).

**Table 3.** Median pre-vaccination and post-vaccination antibody titers by ELISA in patients treated with this heptavalent-KLH conjugate vaccine or previously treated with monovalent vaccines

Antigen	Median IgM ELISA titers after vaccination (based on previous studies)*			Median IgM ELISA titers after vaccination*	
	Monovalent vaccines			Heptavalent vaccine	
	Pre	Post	Reference	Pre	Post
Tn-MUC1	20	1,280	(24)	0	640
Tn (DOSM)	0	640	(23)	0	160
TF (DPSM)	0	1,280	(18)	0	640
Globo-H	20	80	(22)	0	40
GM2	0	160	(25, 40)	0	0
Lewis Y	0	0	(20)	0	0
STn (OSM)	10	640		0	80

Abbreviations: DOSM, desialylated ovine submaxillary mucin; DPSM, desialylated porcine submaxillary mucin; OSM, ovine submaxillary mucin.

\*Patients with IgM titer  $\geq 1:40$  or an 8-fold increase in prevailing antibody titer if present at baseline are considered "responders."

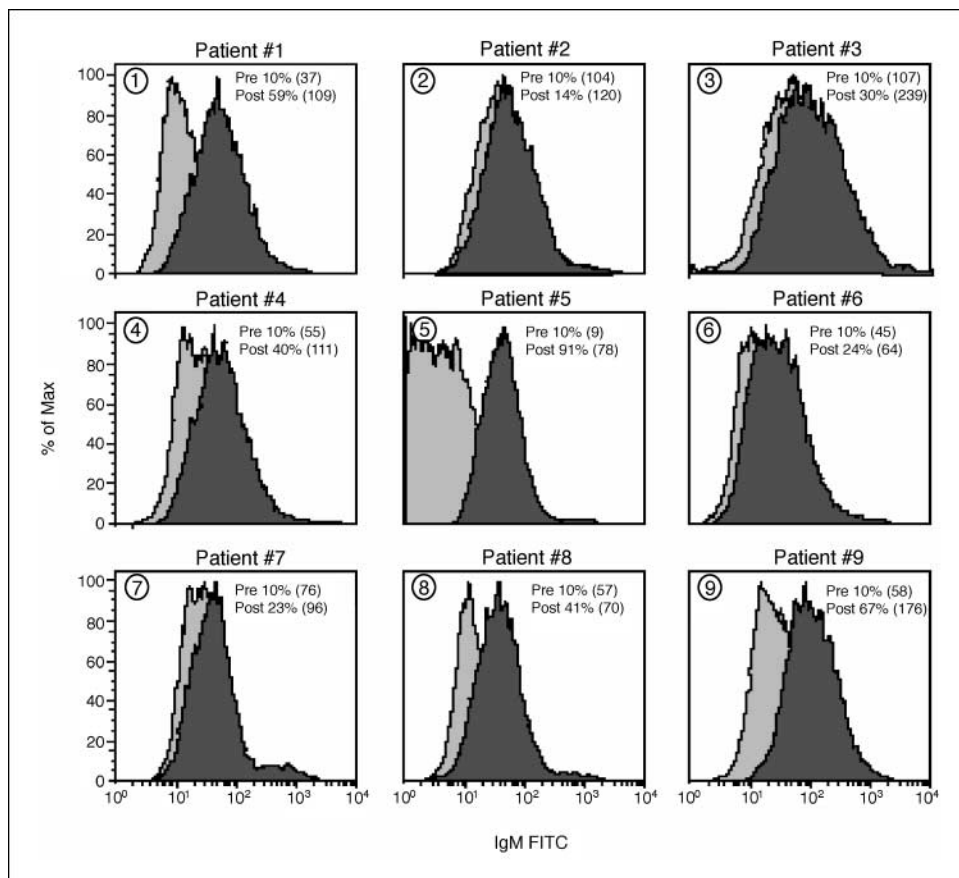
concern that antibody responses against the four mucin antigens as well as Globo-H, GM2, and Lewis Y might induce individual subclinical toxicities, which would prove additive or enhancing after a heptavalent vaccine. No such systemic adverse events were seen. Toxicity was restricted to local erythema and induration at vaccination sites as well as flu-like symptoms in occasional patients, all known to be consequences of the 100  $\mu$ g dose of QS21. No lines of evidence of elevated liver functions or amylase, occult blood in the stool, or other abnormalities were detected.

CDC using a 1:4 serum dilution and 1:5 human complement dilution in a 2-h chromium release assay was positive in five patients with 15% to 4% CDC. However, three additional patients showed complement activation at the cell surface as shown by the immune adherence assay, which was indicative of complement activation at the level of at least complement factor 3. We had shown previously that our monovalent vaccines induced antibodies against both glycolipids and mucins but that only the antibodies against glycolipids were able to induce potent complement-mediated cytotoxicity of antigen-positive cell lines (33). The basis for this is assumed to be the great distance from the cell surface that complement is activated following antibody recognition of antigens on mucins. The three patients with positive immune adherent assays but negative CDC all had higher levels of antibodies against mucins than glycolipids, consistent with complement activation primarily on mucins far from the cell surface.

The lack of CDC does not necessarily mean that these mucin antibodies have no therapeutic potential. Administered or

Documenting the safety of this heptavalent-KLH conjugate plus QS21 vaccine was a priority in this trial. The seven individual components had been tested separately in previous trials and their safety had been confirmed, but there was

**Fig. 3.** Percentage positive MCF7 cells (and mean fluorescent intensity) in fluorescence-activated cell sorting analysis of pre- and post- fourth vaccination sera from nine patients treated with the heptavalent vaccine.



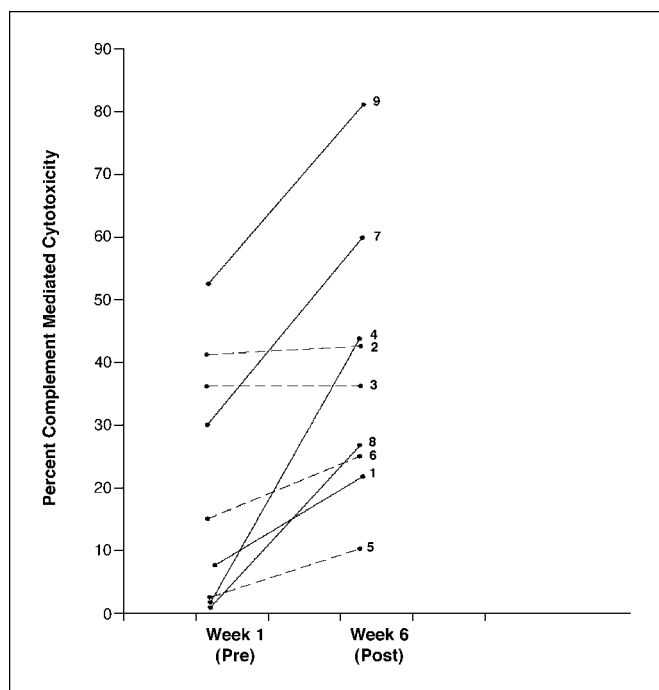


Fig. 4. Comparison of CDC with pre-vaccination and post-vaccination sera tested on MCF7 cells. Five of the nine patients showed CDC (solid lines).

induced antibodies against either glycolipid or mucin antigens protect from tumor recurrence in several syngeneic tumor models (4). In addition, antibodies against either glycolipid or mucin antigens correlate with a more favorable prognosis in cancer patients after resection of all known disease. Consequently, with regard to complement-mediated effector mechanisms, inflammation and opsonization are likely mechanisms contributing to prolonged survival seen in preclinical experiments and suggested in clinical trials with passively administered and actively induced antibodies against mucin antigens. With regard to bacterial infections, this is supported by the severe consequences of hereditary deficiency states involving either the classic or alternative complement pathway or the

comparable trivial consequences of deficiencies in the membrane attack complex. Evaluating the role of complement-mediated mechanisms here is further complicated by the series of complement-inhibitory proteins that have been described in ovarian cancers, ovarian cancer ascites, or ovarian cancer cell lines (34–36). These include membrane complement regulators, such as complement receptor 1, CD46, CD59, and CD55 (decay accelerating factor), and soluble inhibitors, such as factor H and H-like protein.

There are additional antibody-mediated effector mechanisms likely to be relevant here that are unrelated to complement. In addition to antibody-dependent cellular cytotoxicity, these include interference with cell surface adhesion receptor-ligand interactions from binding to Globo-H, which is a selectin ligand (37), and TF, which is a galectin 1 and 3 ligand (38). The relative roles of these complement-mediated effector mechanisms, complement-inhibitory proteins, antibody-dependent cellular cytotoxicity, and receptor-ligand interactions *in vivo* in the adjuvant setting in ovarian cancer patients with minimal disease remain to be determined.

Patients in second remission after a complete response to optimal chemotherapy have a 90% risk of developing additional recurrences over the next 3 years and eventually dying of their metastatic ovarian cancer. This is an ideal population of patients for testing potential adjuvant immunotherapy regimens. The trial described here was done in preparation for a randomized trial designed with time-to-treatment failure as the end point.

In addition to the antigens in our heptavalent vaccine as described, the cell surface antigens characteristic of ovarian cancers includes KSA (NCAM) and CA125 (MUC16). After several attempts, however, we have been unable to induce antibodies against KSA (27), consistent with the very broad expression of KSA on most epithelial tissues. CA125 is a very large complex mucin that will require further investigation to determine optimal epitopes for inclusion in pilot vaccine trials (39). Consequently, the five antigens shown to be immunogenic here (Globo-H, Tn-Muc1, Tn, STn, and TF) and in our previous studies (GM2; ref. 25) each conjugated to KLH and mixed with a purified saponin fraction adjuvant such as QS21 will be the vaccine to be used as we plan a randomized study.

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## Pilot Study of a Heptavalent Vaccine-Keyhole Limpet Hemocyanin Conjugate plus QS21 in Patients with Epithelial Ovarian, Fallopian Tube, or Peritoneal Cancer

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