



Use of the anti-idiotypic antibody vaccine TriAb after autologous stem cell transplantation in patients with metastatic breast cancer

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Summary:

Between April 1997 and March 1998 we evaluated the immune response and outcome in 11 chemosensitive patients who were treated with the anti-idiotypic antibody vaccine TriAb after recovery from intensive therapy and autologous stem cell transplant (ASCT). TriAb was commenced after recovery from the acute effects of ASCT; a minimum interval of 1 month was required from completion of consolidation radiotherapy, if given. Nine patients (82%) manifest anti-anti-idiotypic antibody (Ab3) responses post ASCT. The maximal Ab3 response was seen after a median of 10 doses (range 5–20), which corresponded to a median of 14 months (range 5–19) post ASCT. Evidence of a T cell proliferative response was seen in eight patients; the response was modest in most of these. At a median follow-up of 24 months (range 22–33) after ASCT, four patients are alive without evidence of disease progression. All four of these patients were in the subgroup with more vigorous immune responses. Subsequent efforts have been directed toward the achievement of higher levels of immune responses more rapidly post ASCT. *Bone Marrow Transplantation* (2000) 26, 729–735.

Keywords: anti-idiotypic breast cancer vaccine; metastatic breast cancer; autologous stem cell transplantation

Although intensive therapy and autologous stem cell transplantation (ASCT) produces durable progression-free survival in a small proportion of women with metastatic breast cancer, the majority of patients relapse despite the procedure.¹ A number of different intensive chemotherapeutic agents and dose schedules have been evaluated, but none has clearly produced superior results.^{1,2} One approach to try to improve the outcome involves the use of immunotherapy in conjunction with ASCT. The use of immune-based therapy has particular appeal after ASCT, at which time the tumor burden may be small. Several strategies have been proposed in breast cancer patients undergoing ASCT, including the use of cyclosporine to evoke an auto-

logous 'graft-versus-tumor effect'³ and the use of anti-tumor vaccines.⁴

Several vaccines which have utilized whole tumor cells or tumor cell lysates, or specific tumor antigens, have been designed for use in breast cancer.^{4,5} For instance, vaccines against the specific breast cancer antigens MUC-1 mucin,^{6–9} carcinoembryonic antigen (CEA),¹⁰ HER-2/neu⁴ and sialyl-Tn (STn)¹¹ have all been studied.

An alternative vaccine strategy exploits the immune network hypothesis of Lindenmann¹² and Jerne¹³ to transform epitope structures into idiotypic determinants expressed on the surface of antibodies. According to this concept, immunization with a tumor-associated antigen elicits production of antibodies against this specific antigen, which are called Ab1. In turn, Ab1 can be used to generate a series of anti-idiotypic antibodies directed against the Ab1, termed Ab2. Some of these Ab2 can effectively mimic the three-dimensional structure of the original tumor-associated antigen identified by the Ab1. Immunization with such Ab2 can lead to the production of anti-anti-idiotypic antibodies (Ab3) that recognize the initial tumor-associated antigen identified by Ab1.

Active immunization with tumor-specific anti-idiotypic vaccines can inhibit the growth of a variety of tumors in animal models.^{14–22} In humans, idiotypic vaccines have been used to elicit IgG humoral and cellular responses against tumor-associated antigens such as CEA on colo-rectal cancer cells,^{23,24} HMW-MAA²⁵ and disialoganglioside GD2²⁶ on melanoma cells, patient-specific immunoglobulin derived from B cell lymphomas²⁷ and gp37, a highly restricted T cell antigen on T cell lymphoma cells.²⁸ Moreover, clinical improvement has been observed in several settings.^{25–28}

Recently, the anti-idiotypic breast cancer vaccine TriAb (Titan Pharmaceuticals, South San Francisco, CA, USA) has been evaluated. TriAb is the aluminum hydroxide precipitate of 11D10, a murine monoclonal anti-idiotypic antibody that mimics an epitope of the human milk fat globule (HMFG) protein.^{29–32} HMFG is a tumor-associated antigen expressed on the surface of over 90% of human breast cancers; this antigen shares a similar amino acid sequence with MUC-1 mucin. HMFG is not expressed in normal breast tissue, but is also found on lung, ovarian, endometrial, and pancreatic cancer cells. 11D10 has only minimal reactivity with normal tissues, namely the alveolar lining of the lungs and the brush border of the renal tubules.^{32,33} HMFG itself is a poor immunogen, and purified antigen is not available

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for human vaccination.^{32,33} However, a murine monoclonal antibody, MC-10, was developed that identifies the epitope of HMFG. MC-10 was then used as an immunizing antibody (Ab1) in mice to produce 11D10 (Ab2).^{32,33} 11D10, or TriAb, can thus serve as a surrogate for the HMFG antigen for clinical vaccination. Initial studies in breast cancer patients not undergoing ASCT demonstrated that intracutaneous TriAb can result in the generation of Ab3 antibody responses that bind to purified HMFG and react by immunoperoxidase staining to HMFG-positive tumor specimens. Using models of breast cancer tumor cell lines, Ab3 from patients immunized with TriAb can initiate antibody-dependent cell-mediated cytotoxicity. As well, immunization with TriAb has been shown to elicit idiotype-specific T cell proliferative responses as well as responses to the HMFG antigen.³²⁻³⁴ In this pilot study, TriAb was utilized after recovery from ASCT to try to induce anti-tumor immunity of potential therapeutic benefit.

Patients and methods

Patient selection

Between April 1997 and March 1998, 11 patients with metastatic breast cancer underwent intensive therapy and ASCT, followed by vaccination with TriAb. Eligibility criteria included: documentation of metastatic breast cancer, age ≤ 70 , satisfactory organ function, ECOG performance status < 2 , absence of significant co-morbid conditions and informed consent. Patients were also required to have chemosensitive disease with $\geq 50\%$ reduction in the size of tumor masses, and/or bone scan findings that were stable or improved before ASCT. Patients with breast cancer involving the central nervous system or bone marrow were excluded. This protocol was approved by the institutional review board of the University of Kentucky.

Transplant protocol

Priming therapy for peripheral blood stem cell collection included cyclophosphamide (4 g/m² i.v), prednisone (100 mg p.o. daily \times 4 days) and G-CSF (10 mg/kg/day s.c.) in 10 patients and G-CSF alone in one patient. One patient required a supplemental bone marrow harvest to obtain sufficient cell numbers. After harvest, the stem cells of 10 patients were positively selected for CD34⁺ cells using the Ceparate SC stem cell separator. The median number of CD34⁺ cells reinfused was 3.26×10^6 /kg (range 1.78–15.01).

Patients were conditioned with one of two high-dose regimens. The first four patients received paclitaxel, cyclophosphamide, cisplatin and amifostine, as previously described,^{35,36} while the remaining seven were conditioned with cyclophosphamide, thiotepa and carboplatin (STAMP V).³⁷ All patients received G-CSF post ASCT. After blood count recovery, nine patients with ≤ 3 sites of prior metastatic disease underwent radiation therapy as consolidation. One patient who had presented with stage IV disease underwent mastectomy post ASCT. Nine patients whose tumors were estrogen and/or progesterone receptor-positive

received post-transplant hormone therapy, preferentially with tamoxifen, for 5 years or until disease progression occurred. Patients underwent re-staging at 100 days post transplant. Repeat staging studies were performed every 6 months or as clinically indicated.

Vaccination schedule

TriAb was commenced after blood count recovery and when the acute effects of the transplant had resolved. In patients receiving consolidation radiation therapy, a minimum interval of 1 month was required from completion of radiation therapy until the time of vaccine initiation. TriAb was given at a dose of 2 mg intracutaneously every 2 weeks for a total of four injections, followed by the same dose every 4 weeks for a maximum of 2 years. TriAb was stopped if disease recurrence was documented.

Toxicity of the vaccine was recorded at each visit and was graded according to the NCI criteria. Laboratory tests to assess the production of humoral and cellular immunity were performed before priming therapy, prior to the fourth dose and monthly thereafter.

Measurement of anti-idiotype immune responses

Humoral responses: The development of humoral immunity induced by TriAb was assessed by measuring the level of anti-anti-idiotypic antibodies (Ab3) in sera obtained from the patients at different time points. Patient sera samples were treated with unrelated murine immunoglobulins to remove anti-idiotypic and allotypic reactivity. Serial dilutions of sera were then tested for ability to inhibit Ab1-Ab2 binding. All assays were performed in triplicate. For the first direct binding inhibition assay between Ab1 and Ab2, microtiter plates were coated with MC-10 (Ab1) and incubated overnight at room temperature. The plates were then blocked with 1% BSA in PBS. Different dilutions of patient sera along with ¹²⁵I-11D10 (Ab2) (50 μ l/well, ~ 90000 c.p.m.) were added and incubated for 2 h at room temperature. After washing, the cells were counted in a gamma counter, and percentage inhibition calculated. In the second inhibition assay, the procedure was repeated using plates coated with Ab2 (11D10) (5 mg/ μ l, 100 μ l/well), and different dilutions of patient sera along with ¹²⁵I-MC-10 (Ab1) (50 μ l/well, ~ 90000 c.p.m.) were added. The percent inhibition was calculated by the formula:

$$\% \text{ Inhibition} = 1 - \left\{ \frac{R_T - R_C}{R_{\max} - R_C} \right\} \times 100$$

in which R_T was the average c.p.m. of the experimental well with inhibitors, R_C was the average background c.p.m. and R_{\max} was the average maximum binding without inhibitors. Inhibition greater than 25% by Ab3 sera at a 1:10 dilution was considered a positive response.^{24,38}

Proliferative T cell responses: Peripheral blood mononuclear cells were isolated by the standard Ficoll-Hypaque density gradient centrifugation method and 5×10^5 cells/well were incubated with different concentrations of 11D10 Alu gel and 11D10 IgG, and control 4DC6 Alu gel

and 1A7 (2 mg, 1 mg, 10 mg, 100 mg per well). After the cells had been incubated for 5 days at 37°C in an atmosphere containing 5% carbon dioxide, they were pulsed with ³H thymidine (1 μCi per well) for 20 h. Data were expressed as mean counts (triplicate wells) per minute of ³H thymidine incorporation and reported as the stimulation index (SI) compared with the control. The standard deviation of the data was <10% for each determination.³⁹

Results

Patient characteristics

Characteristics of the 11 patients entered on to this protocol are shown in Table 1. Eight had received prior adjuvant chemotherapy, and all had received an anthracycline before ASCT. Three had liver involvement with or without involvement of other organs.

Post-ASCT responses

At day +100 post-ASCT, four patients, including three already in CR pre-ASCT, met the criteria for CR. Five achieved a PR; two of these had only residual bone scan changes of uncertain significance. The bone scan of one of these patients continued to improve with time and was normal 1 year post ASCT. Two patients had no response (Table 2).

Table 1 Characteristics of 11 patients with metastatic breast cancer treated with ASCT followed by TriAb vaccine

Pre-ASCT features	
No.	47 (34–57)
Stage at initial diagnosis	
I	1
II	6
III	1
IV	3
Prior therapy	
Adjuvant therapy	8
Anthracyclines	11
Taxanes	5
Radiotherapy	5
Median No. prior regimens (range)	2 (1–2)
ER and/or PR+	9
Sites of metastasis	
Bone only	1
Nodal only	3
Liver +/- other	3
Disease status	
CR	3
PR ^a	8

^aFour with residual bone scan abnormalities.
ER = estrogen receptor; PR = progesterone receptor.

Initiation of vaccine therapy

TriAb therapy was started a median of 4.5 months (range 1–9.5) after ASCT in the 11 patients. The median time to begin the vaccine was 5 months (range 1–9) in the eight patients receiving consolidation radiotherapy immediately after recovery from ASCT. One other was treated with consolidation radiotherapy at the discretion of her referring physician 6 months post transplant. Two patients were not given radiotherapy; the vaccine was commenced 0.75 and 7 months after ASCT. The median number of doses administered per patient was 15 (range 5–26) at the time of this analysis. A total of 168 doses of vaccine have been given (Table 2).

Humoral responses

The median baseline Ab3 level was 6% (range 3–16%) in the inhibition 1 assay and 0% (range 0–17%) in the inhibition 2 assay. Nine patients (82%) showed evidence of a positive Ab3 response at a median of 9 months (range 4–16) post-ASCT and 4 months (range 1–8.5) after starting the vaccine. The median number of doses to achieve evidence of a positive response was six (2–10). Positive Ab3 responses were sustained throughout the period of vaccine administration in the responding patients, with the exception of UPN 695, whose antibody levels transiently decreased during delayed consolidation radiotherapy. The two non-responders received four and 16 doses, respectively, before progression was documented.

The median maximal Ab3 response in the inhibition 1 assay was 51% (range 39–96%), and in the inhibition 2 assay 60% (range 29–94%); these levels were achieved after a median of nine doses (range 4–20) (Table 2). The median time to achieve the maximal Ab3 response was 14 months (range 4.5–19) post ASCT and 9 months (range 7.2–16) after starting the vaccine (Table 2).

T cell proliferative responses

One patient did not have an evaluation of T cell response; this individual had received radiotherapy post transplant and had progressed 1.5 months after starting the vaccine. The median maximal T cell SI in the remaining patients was 3.5 (1.5–16.7) and was observed after a median of 10 doses (range 3–20); four patients had a maximal SI ≥7.5. Attainment of maximal response occurred a median of 13 months (range 4.5–19) post ASCT and 8 months (range 3.5–15) after commencement of the vaccine (Table 2).

All five patients with a T cell SI ≥3.5 also had Ab3 responses above the median maximal level, and four are alive and well; one progressed 20.5 months post ASCT. All five patients with lower SI levels have progressed post ASCT. Only one of the five patients with a SI <3.5 had an Ab3 response greater than the median maximal response.

Vaccine toxicity

Grade 2 local skin reactions were seen in all patients. One patient experienced grade 3 skin reactions with each dose and another grade 2 myalgias after one dose. One noted

Table 2 Results after post-ASCT vaccination with TriAb in 11 patients with metastatic breast cancer

Patient UPN	Sites of metastasis	Post ASCT		Total no. doses Triab	No. doses Triab to maximal response	Time to maximal Ab3 response post ASCT (months)	Maximal Ab3 response (% inhibition)		Maximal T cell SI	Response		Progression (months)	Current status (months)
		RT	HT				Inhibition 1	Inhibition 2		Pre-ASCT	Post-ASCT		
618	B	+	+	26	20	19	96	95	3.6	PR ^b	CR	—	A&W (33)
624	B, L, N	-	+	8 ^a	7	12	18	35	2.3	PR ^b	PR ^b	13	D, prog (10)
628	B, Br, N	+	+	12 ^a	10	16	38	28	1.8	PR	CR	19	D, prog (23.5)
632	Br, N	+	+	5 ^a	4	7	58	80	NA	PR	PR	7	D, prog (10)
649	N	+	-	20 ^a	16	17	18	22	1.5	CR	CR	21	A, prog (26)
677	B, N	+	+	18	15	19	55	77	7.5	CR	CR	—	A&W (25.5)
678	B, N	+	-	7 ^a	4	6	9	9	3.1	PR	NR	8.5	D, prog (11)
683	N	+	+	18 ^a	9	14	93	92	16.7	CR	CR	20.5	A, prog (24)
684	N	+	+	21	14	18	51	60	15.8	PR ^b	PR ^b	—	A&W (22)
694	B, L	-	+	7 ^a	5	4.5	35	56	3.1	PR	PR	6	D, prog (19)
695	B, L	+	+	26	13	5	93	83	9.7	PR	PR	—	A&W (22)

^aVaccine discounted due to disease progression. ^bResidual bone scan abnormalities.

N = lymph nodes; L = liver; B = bone; Br = breast; Ab3 = anti-anti-idiotype antibody; SI = stimulation index; prog = progression; A&W = alive and well; NA = not available; RT = consolidation therapy; HT = hormonal therapy.

grade 3 flu-like symptoms after the fifth vaccine dose; further vaccine was not administered due to the development of recurrent breast cancer shortly thereafter.

Current status

At a median follow-up of 24 months (range 22–33), four patients are alive and well. These include two in CR and two in PR. Two patients continue on vaccine, while two have completed the planned 2-year course. Seven patients have experienced progressive disease at a median of 10 months post transplant (range 6–20.5) and four of these have died a median of 11 months (range 10–23.5) post ASCT. The actuarial progression-free survival is 36% (95% CI 8–65%) (Figure 1).

Outcome by immune response

Four of the six patients with a maximal Ab3 response ≥ the median of 51% in the inhibition 1 assay and 60% in

the inhibition 2 assay are alive and well, compared with none of the five patients with lesser responses ($P = 0.06$, Fisher’s exact test, two-tail). The findings were identical for patients with a T cell SI above or below the median of 3.5.

Discussion

This study demonstrates that the majority of patients given an anti-idiotypic breast cancer vaccine after ASCT will mount an Ab3 response, and that at least 50% will manifest evidence of T cell proliferative response. However, both T cell and Ab3 responses were relatively delayed after ASCT. Several factors contributed to the length of time required to achieve an immune response. First, there were logistic delays in starting vaccine, largely due to the routine use of consolidation radiation therapy and, in one patient surgical therapy, post ASCT. Also, a several-month period of decreased immunocompetence is known to occur post

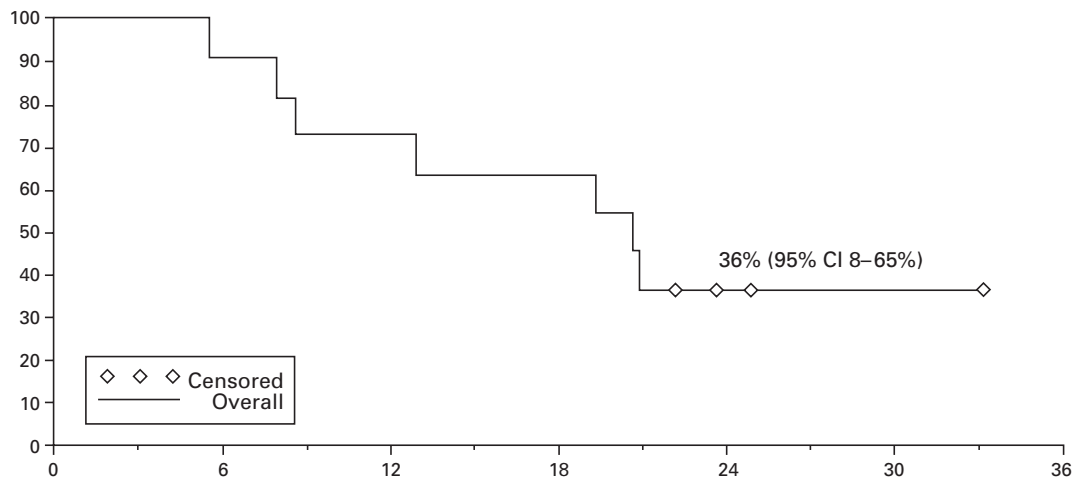


Figure 1 The progression-free survival of patients with metastatic breast cancer treated with ASCT followed by TriAb vaccine.

ASCT⁴⁰ and may have been accentuated by our use of CD34-positively selected stem cells.^{41–43}

In this study, patients with higher levels of humoral and T cell proliferative responsiveness appeared to have a better outcome than those with less brisk immune responses. However, only one of the four progression-free survivors had visceral metastases and all four had ER- and/or PR-positive disease. These features have previously been associated with a more favorable prognosis in other series of ASCT in metastatic breast cancer.⁴⁴

Currently, it is uncertain whether the immune response to TriAb represents a true anti-tumor effect, or rather an epiphenomenon which indicates that patients capable of mounting an immune response are simply destined to have a better outcome. The potential benefit of anti-idiotype vaccine therapy is felt to predominantly involve use of T cell helper mechanisms, which may lead to antibody-dependent cell-mediated cytotoxicity, as well as to other indirect effects to enhance cytotoxic T cell function. The *in vitro* demonstration that the various effectors mediating this immune response could destroy autologous breast cancer cells would argue strongly that the responses provide a meaningful anti-tumor benefit. However, given the proposed mechanism of anti-idiotype antibody vaccines, such studies would be difficult to perform and quantitate accurately. Alternatively, randomized clinical trials of ASCT with or without TriAb could help address this question.

The Seattle group has evaluated the use of the anti-tumor vaccine Theratope STn-KLH after ASCT in 40 patients with either high-risk stage II/III and stage IV breast cancer, or stage IV ovarian cancer.⁴⁵ STn is a carbohydrate antigen expressed by most human malignancies; it is associated with the MUC-1 mucin core peptide. This vaccine was started at a median of 4.5 months, an interval similar to that in the current study, and was given every 2–3 months for up to five doses. The majority of patients manifest an IgG anti-STn response, which peaked after the fourth or fifth dose. Eleven of 26 patients demonstrated antigen-specific T cell responses with gamma-interferon production. Peripheral blood lymphocytes after vaccination demonstrated increased lytic activity in LAK- and NK-sensitive lines, as well as in an STn-expressing cancer cell line.⁴⁵ Again, as in our study, maximal responses were observed relatively late post ASCT.

The relative merits of TriAb over other breast cancer vaccines, such as Theratope STn-KLH or those directed against MUC-1, are unknown at present. One theoretical advantage relates to the observation that the immune response to T cell-dependent antigens matures earlier than the T cell-independent response to carbohydrate antigen in animal models, which may lead to improved responses to an anti-idiotype vaccine in an abnormal immune system,⁴⁶ such as seen post ASCT.⁴⁷ Also, data indicate that an acquired state of tolerance to one antigen form can be broken with using a different molecular form of the same antigenic moiety, a finding which suggests an advantage for an anti-idiotype antibody.⁴⁶ Of note, Adluri *et al*⁴⁸ recently reported that breast cancer patients treated with vaccines consisting of MUC-1 peptides of different sizes in combination with potent adjuvants generated mostly anti-MUC-1 immunity which recognized the peptides but not

MUC-1-positive tumor cells. This phenomenon was likely due to the presumed conformation dependency of MUC-1 epitopes. On the other hand, MUC-1 and HMFG share amino acid sequences, and TriAb is capable of eliciting anti-HMFG antibodies that react with tumor cells. However, it is not yet known whether TriAb will result in the production of cytotoxic T cells.

Assuming that vaccine therapy can potentially improve the results of ASCT in metastatic breast cancer, strategies to achieve vigorous immune responses rapidly after the procedure are desirable. To this end, we are currently evaluating the use of TriAb both pre- and post ASCT in patients with metastatic breast cancer.⁴⁹

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