Up to 15-year clinical follow-up of a pilot Phase III immunotherapy study in stage II breast cancer patients using oxidized mannan–MUC1

Background: Targeting antigens to dendritic cell receptors has recently become a popular approach to inducing effective immune responses against cancer antigens. Almost 20 years ago, however, we demonstrated that targeting the mannose receptor on macrophages and dendritic cells leads to strong cellular immune responses. We conducted numerous human clinical trials demonstrating the effectiveness of oxidized mannan–MUC1 (M-FP) in MUC1+ adenocarcinoma patients. In one trial, the 5–8-year follow-up of breast cancer patients vaccinated with M-FP was published previously; we now report here the 12–15-year follow-up. Details regarding the preparation of the vaccine, inclusion and exclusion criteria, immunotherapy and follow-up schedule, were published previously. **Results:** The follow-up at 12–15 years showed that the recurrence rate in patients receiving placebo was 60% (nine of 15). In those receiving immunotherapy (M-FP), the rate was 12.5% (two of 16). The time of recurrence in the placebo group ranged from 7 to 180 months (mean: 65.8 months) and in the two patients of the vaccine group, the recurrence appeared at 95 and 141 months (mean: 118 months) after surgery. These findings are statistically significant ($p = 0.02$ for survival and $p = 0.009$ for percentage of patients cancer-free). All patients injected with M-FP showed no evidence of toxic effects or signs of autoimmunity during the 12–15-year follow-up. **Discussion:** The preliminary evidence indicates that M-FP is beneficial in the overall survival of early-stage breast cancer patients. This long-term clinical follow-up of patients strongly supports the necessity for a large Phase III study of direct M-FP injection in early-stage breast cancer patients, to evaluate immunotherapy as an adjuvant treatment for breast cancer.

KEYWORDS: breast cancer ▪ C-type lectin ▪ dendritic cell targeting ▪ immunotherapy ▪ mannan ▪ mannan–MUC1 ▪ mannose receptor ▪ mannose receptor targeting ▪ MUC1 ▪ MUC1 clinical trial ▪ vaccine

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MUC1 is a glycoprotein overexpressed in adenocarcinomas and, in particular, in breast cancer [1,2]. Over the last 20 years, numerous preclinical studies demonstrated that MUC1 is immunogenic, depending on the MUC1 vaccine formulation. MUC1-based vaccines quickly entered into human clinical trials with immune responses and some clinical responses being reported [3]. Twenty years ago, we demonstrated that targeting the mannose receptor [4] on macrophages and dendritic cells (DCs) leads to strong Th1 or Th2 cellular and/or humoral immune responses, dependent on the chemical nature of the ligand mannan [5]. Targeting antigens to receptors expressed on DCs, has shown a recent upsurge of interest in developing enhanced vaccines for diseases, including cancer [6,7].

We were the first to report that MUC1 was safe in humans as a peptide conjugated to diphtheria toxoid – we soon went into human clinical trials using oxidized mannan–MUC1 (M-FP), which indicated that M-FP was safe in humans and in advanced cancer patients immunity was induced [8,9]. An *ex vivo* approach using M-FP demonstrated that strong cellular immunity was induced in advanced adenocarcinoma patients [10]. A double-blinded placebo versus M-FP trial was conducted in early breast cancer patients. The 5–8-year follow-up study of early breast cancer patients vaccinated with M-FP was published in 2006 (ISRCTN71711835) [11]. The patients were selected and treated at the Prolipsis Medical Centre (Athens, Greece). M-FP was produced under GLP conditions at the Austin Research Institute (now Burnet Institute; Melbourne, Australia), where the 5-year immunological analysis was performed after peripheral blood mononuclear cells and sera were sent from the Prolipsis Medical Centre. The study was initiated once ethics approval had been granted by the National Drug Administration of Greece (EOF; 26 September 1997, no. 27581). The preparation of M-FP, the inclusion and exclusion criteria, the immunotherapy and follow-up schedule and full reference list were previously published [11]. We now report the 12–15-year clinical follow-up of these patients.

Future Medicine part of fsg

Patients & vaccine schedule

The 31 enrolled patients were postmenopausal women treated surgically with or without local radiation for stage II estrogen receptor-positive breast carcinoma, and, no more than four metastatically involved axillary lymph nodes. All patients received adjuvant therapy with tamoxifen 20 mg daily for 5 years. All 31 patients were enrolled in a double-blind study, in which 16 patients had received M-FP and 15 patients had received placebo [11]. All patients' blood samples were taken every 2 weeks, seven times and then at the 6th and 9th month. Follow-up of all the patients was scheduled for clinical and laboratory evaluation every 6 months for 5 years (as previously published), and yearly thereafter for clinical evaluation only, as described herein. The first patient was vaccinated on 13 December 1997 and the last patient 17 July 2000. The follow-up of all patients is presented in **Tables 1 & 2**.

Up to 15-year, clinical follow-up of patients

On 31 December 2012, 22 patients were living with a follow-up ranging from 132–181 months. Of note, as of 15 August 2013, there have been no changes in patient health/recurrence status.

In the vaccine M-FP study group of 16 patients, 12 were alive, free of disease; one developed leukemia 118 months after surgery and died 1 year later, without metastatic disease; one developed bone metastases 141 months after surgery, still living with disease; and one developed bone metastases 95 months after surgery and died 14 months later. As reported in our initial paper [11], nine out of 13 patients who had received M-FP developed anti-MUC1 antibodies – initially IgM, which seroconverted to IgG. Interestingly, patient six, who developed bone metastases 95 months after surgery and died 14 months later, had not developed anti-MUC1 antibodies. In the current follow-up study, we have not measured cellular or humoral immune responses, apart from the initial immunity measured after vaccination. No specific correlation of outcome with immune responses has been measured, however, it was clear from the original publication [11] that, of the patients receiving M-FP, nine of 13 had measurable antibodies to MUC1 and four out of ten had MUC1-specific T-cell responses; none of the placebo-treated patients showed an immune response to MUC1. It is suggestive that humoral and cellular immunity has played a role in the outcome of the vaccinated patients.

In the placebo study group of 15 patients, four were alive free of disease; two were alive free of disease at 120 and 130 months, respectively, after surgery (we are trying to trace them) and nine developed metastatic disease, six of them died and three are still living with disease. As reported in our initial paper [11], there were no detectable anti-MUC1 antibodies in all 16 patients.

In terms of type and time of recurrences, in the vaccine M-FP study group, two patients developed bone metastases that appeared 95 and 141 months (mean: 118 months) after surgery; in the placebo study group, nine patients developed metastases, four bone, one skin and four visceral – the time of recurrences ranged from 7 to 180 months (mean: 65.8 months) after surgery.

One potential problem with administering a 'self' antigen such as MUC1 is the development of autoimmunity to normal tissues and immune cells expressing MUC1. Surprisingly, this does not appear to be a problem in any of the trials to date, by our group and others [12–16]. In the current study, no patient at any stage, reported any adverse event; indeed in this and in other studies using M-FP, no adverse events have ever been noted [8–11].

Statistical analysis of protective efficacy against relapse, was determined by plotting the data for the placebo and M-FP group as Kaplan–Meier survival curves using the PRISM program. All of the patients with relapses and no relapses were analyzed, with time of observation ranging from 132 to 181 months. The placebo and M-FP curves were compared using the log rank test and found to differ significantly with $p = 0.02$ for survival (**Figure 1A**) and $p = 0.009$ for percentage of patients cancer-free (**Figure 1B**).

Discussion

The M-FP 'vaccine' appears to confer protection from breast cancer recurrence – both in the short term (5-year follow-up) [11] and, as now shown, in the long term (up to 15-year follow up). Clearly, the M-FP vaccine shows promise and warrants inclusion as a harmless adjuvant therapy, in the current management of patients with breast cancer. The major drawback of the study is the small number of patients in both arms of the study – a large Phase III trial needs to be carried out.

As noted in our 2006 paper [11] and others, oxidized mannan targets the antigen (MUC1) to C-type lectin receptors, including the mannose receptor and, via stimulation of Toll-like receptor-4, of APCs (DCs and macrophages) elicits both T-cell and antibody immunity [17]. Presumably, this immunity removes metastatic cells. Of relevance, we note a study where the occurrence of MUC1 antibodies without

Table 1. Follow-up of patients receiving oxidized mannan–MUC1.

n	Age (years)	Date of surgery	Type of Tx	Tumor size (cm)	Total nodes	Met nodes	Grade	ER	MUC1	Date of first vaccine	Side effects	DOR (months); location	Last follow-up	Months from surgery
1	58	01/12/1997	Partial mast., RT, Tam	1.0	17	1	II	+	+++	21/01/1998	Mild skin redness	–	12/2012	181
2	72	14/01/1998	Total mast., Tam	2.0	12	1	I	+	–	03/02/1998	Mild skin redness	Free of disease	12/2008 Death (cardiac)	130
3	52	24/08/1998	Partial mast., RT, Tam	1.3	20	2	II	+	++	16/11/1998	–	–	12/2012	172
4	53	22/08/1998	Partial mast., RT, Tam	1.5	23	2	III	+	+	30/11/1998	–	–	12/2012	172
5	53	03/09/1998	Total mast., Tam	3.0	21	4	†	+	+++	23/09/1998	–	–	12/2012	171
6	58	10/11/1998	Total mast., Tam	1.5	22	3	II	+	+++	30/11/1998	Mild skin redness	10/2006 (95); bone	11/2008 Death	119
7	78	08/12/1998	Bil., Total mast., Tam	Right: 1.2 Left: 1.2	18 16	0 4	I I	+ +	++	28/12/1998	–	–	12/2012	168
8	62	27/01/1999	Partial mast., RT, Tam	1.0	8	1	II	+	+++	16/02/1999	Mild skin redness	09/2010 (141); bone	12/2012	167
9	61	01/04/1999	Partial mast., RT, Tam	2.0	18	1	I	+	+++	26/04/1999	–	–	12/2012	164
10	58	21/04/1999	Partial mast., RT, Tam	2.0	17	3	II	+	+++	07/05/1999	–	–	12/2012	164
11	59	16/04/1999	Total mast., Tam	1.4	19	1	I	+	+++	26/06/2000	–	–	12/2012	164
12	53	13/07/1999	Partial mast., RT, Tam	2.3	29	1	†	+	+++	19/08/1999	Mild skin redness	–	12/2012	161
13	59	10/11/1999	Total mast., Tam	0.7	10	1	†	+	+++	22/11/1999	–	06/2010; leukemia	06/2011 Death	138
14	63	09/02/2000	Partial mast., RT, Tam	1.2	13	1	II	+	+++	01/03/2000	–	–	12/2012	154
15	55	10/02/2000	Partial mast., RT, Tam	2.5	23	3	II	+	+++	25/02/2000	–	–	12/2012	154
16	65	29/06/2000	Partial mast., RT, Tam	0.8	17	1	II	+	–	17/07/2000	Mild skin redness	–	12/2012	150

†The histologic type was invasive lobular adenocarcinoma with no grading.
+: Moderate; ++: Strong; +++: Very strong; Bil.: Bilateral; DOR: Date of recurrence; ER: Estrogen receptor; Mast.: Mastectomy; Met: Metastasis; RT: Radiotherapy; Tam: Tamoxifen; Tx: Treatment.

Table 2. Follow-up of patients in the placebo group.

n	Age (years)	Date of surgery	Type of Tx	Tumor size (cm)	Total nodes	Met nodes	Grade	ER	Date of first vaccine	Side effects	DOR (months); location	Last follow-up	Months from surgery
17	62	11/12/1997	Total mast., Tam	2.5	27	1	II	+	28/01/1998	Mild skin redness	10/10/2003 (70); liver	Death 03/2005	86
18	54	14/12/1997	Partial mast., RT, Tam	2.0	20	1	II	+	10/01/1998	–	14/07/1999 (7); local 2006 (96); lung	Death 06/2007	101
19	55	16/12/1997	Partial mast., RT, Tam	1.5	17	1	II	+	06/03/1998	–	–	12/2012	180
20	60	13/12/1997	Partial mast., RT, Tam	2.3	24	2	II	+	23/12/1997	–	–	12/2012	180
21	62	20/01/1998	Total mast., Tam	2.4	27	2	II	+	13/03/1998	–	–	12/2012	179
22	61	13/03/1998	Total mast., Tam	1.8	23	2	I	+	29/04/1998	–	14/03/2013 (180); skin on operated Br	12/2012	177
23	62	01/04/1998	Partial mast., RT, Tam	1.5	12	1	II	+	08/07/1998	Mild skin redness	14/11/2006 (103); bone	12/2012	176
24	54	29/04/1998	Partial mast., RT, Tam	0.5	17	1	III	+	22/07/1998	Mild skin redness	–	12/2012	176
25	58	10/11/1998	Total mast., Tam	2.2	21	3	II	+	30/11/1998	Mild skin redness	13/11/2000 (24); bone	Death 11/2003	59
26	51	17/12/1999	Partial mast., RT, Tam	2.0	11	1	II	+	10/03/1999	–	28/09/2000 (19); bone	Death 11/2004	68
27	71	10/06/1999	Total mast., Tam	3.2	26	1	II	+	10/01/1999	–	05/12/2007 (102); lung	Death 01/2008	102
28	70	30/06/1999	Partial mast., RT, Tam	2.0	20	3	II	+	13/07/1999	–	–	06/2009	162
29	54	02/12/1999	Partial mast., RT, Tam	2.5	12	1	II	+	10/01/2000	–	–	09/2010	156
30	81	08/12/1999	Total mast., Tam	2.5	14	1	II	+	10/01/2000	–	27/07/2006 (79); supracti local bone	12/2012	156
31	58	24/02/2000	Partial mast., RT, Tam	2.0	13	1	II	+	20/03/2000	Mild skin redness	16/11/2000 (9); liver	Death 04/2004	25

+: Moderate; Br: Breast; DOR: Date of recurrence; ER: Estrogen receptor; Mast.: Mastectomy; Met: Metastasis; RT: Radiotherapy; Tam: Tamoxifen; Tx: Treatment.

immunization in early breast cancer patients (stages I and II) were associated with significant benefit in terms of disease-specific survival [18,19]. Thus, the presence of antibodies and (T-cell immunity), contribute to the elimination of micrometastasis or early recurrence in breast cancer patients.

Evaluation of the immunological treatment, should be taken into consideration of the disease stage. If the disease is in a very-early stage, the risk of recurrence is low and the follow-up time needed for evaluation is long. If the disease is in an advanced stage, then the risk for recurrence is high, but the immunological status of the patient in such a condition will respond poorly.

In this protocol, the patients of the study and placebo group were comparable in stage II where the expected recurrences are approximately 30%, of which 75% will appear within the first 5 years after surgery. In the study group receiving the vaccine, two of 16 patients developed metastases (12.5%). In the placebo group, nine out of 15 patients developed metastases (60%). Furthermore, in the vaccine study group the first metastases appeared 95 months and the second 141 months (mean: 118 months) after surgery, whereas in the placebo group, four out of eight developed metastases within the first 24 months after surgery and five patients developed late metastases after 72 months. The time of recurrence ranged from 7 to 180 months (mean: 51.6 months) after surgery. We note that three of the subjects who appeared to respond to the vaccine (although one subsequently died of leukemia) had lobular cancer, perhaps leading to the suggestion that these cancers may be more responsive than others. However, the numbers are too small to make definitive conclusions, even though lobular carcinomas, will be noted in future studies with larger numbers. When the lobular carcinoma patients were removed from the vaccine group, the statistics were still significant, with p = 0.04 for survival and p = 0.017 for percentage of patients cancer-free.

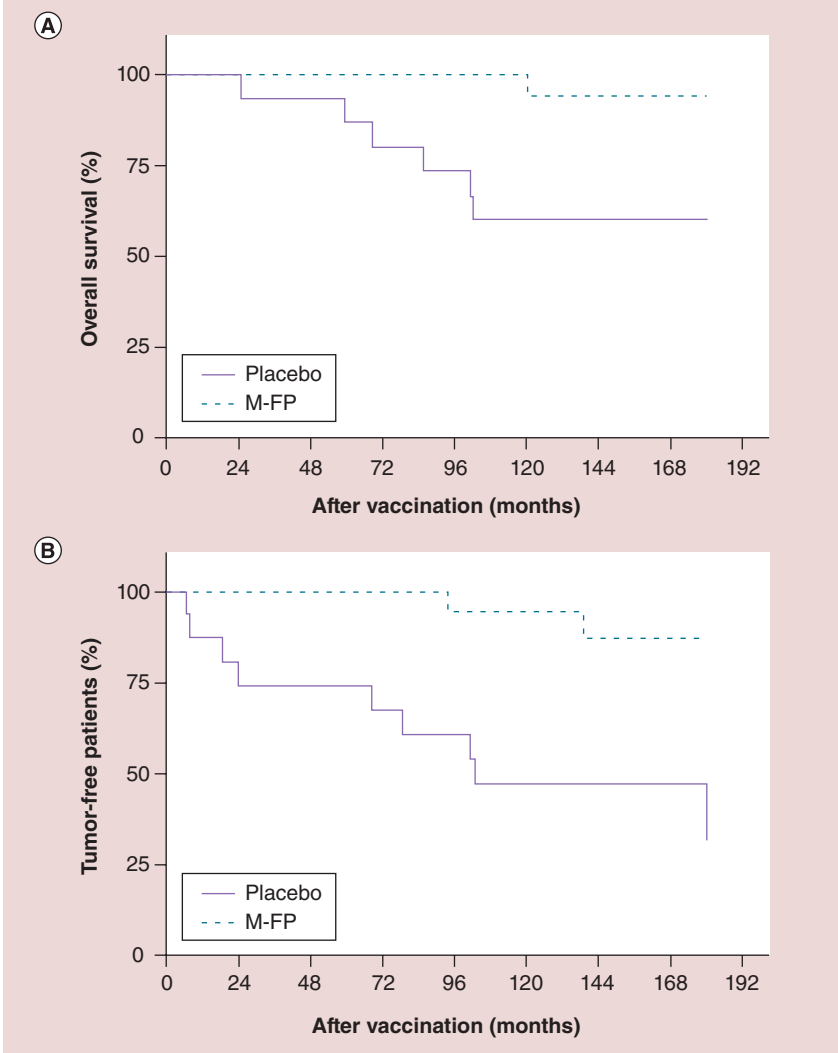

Figure 1. Kaplan–Meier survival curves of all 31 patients. The PRISM program was used to construct the curves for the placebo and M-FP immunized patients. **(A)** Survival curve and **(B)** percentage of patients cancer-free.
M-FP: Oxidized mannan–MUC1.

Acknowledgements

V Apostolopoulos would like to thank VAConsulting Services and Melissa Altona for their help in completing this article.

Financial & competing interests disclosure

This work was supported by the New Idea Breast Cancer Funds, Hellenic Funds, Bosom Buddies Breast Cancer Foundation and Prolipsis Medical Center Funds. At the time of the trial, V Apostolopoulos was an NH and MRC R Douglas Wright Fellow 223316. V Apostolopoulos, GA Pietersz and IFC McKenzie were also supported by the Austin Research Institute and S Vassilaros, A Tsibanis and A Tsikkinis were supported by the Prolipsis Medical Center. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Vaccination with oxidized mannan–MUC1 leads to improved survival in patients with early-stage breast cancer.
- Of 16 patients who received the vaccine, two recurrences occurred in up to 15-year of follow-up. In the placebo arm, nine of 15 had recurrences in this time (two lost to follow up) – the expected rate of recurrence of stage II breast cancer.
- A Phase III study is required in the appropriate number of patients.
- Targeting antigens to APCs (macrophages and dendritic cells) via C-type lectin receptors, including the mannose receptor, results in efficient stimulation of immunity in patients with cancer and confers protection against cancer recurrence.

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