Beta-interferon, retinoids and tamoxifen in metastatic breast cancer: Long-term follow-up of a phase II study

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Abstract. Based on a series of *in vitro* data, including the additive and/or synergistic antiproliferative effect of interferon and tamoxifen on breast cancer cell lines, and on clinical reports, we designed a pilot phase II study to test the activity and toxicity of simultaneous administration of ß-interferon (ß-IFN), retinoids (R) and tamoxifen (TAM) as a salvage therapy in a group of patients with metastatic breast cancer (MBC). Herein we describe the outcome of this cohort of patients after a median follow-up of 150 months. Sixty-five stage IV breast cancer patients, 13 pre-treated with hormones, 38 with chemotherapy and 15 with both, received, as a salvage therapy, TAM, ß-IFN and R. Among 65 evaluable patients, 36 achieved a clinical response (55.5%) (95% c.i. 42-67.7%). Toxicity was moderate and mainly hepatic. Median progression-free and overall survival, which did not show any statistically significant difference in patients with different estrogen and progesterone receptor content, were 43 months and 47.9 months, respectively. In conclusion, the study shows that long-term treatment with TAM, ß-IFN and R in MBC is feasible, has moderate toxicity and seems to give a long-term benefit, irrespective of the receptorial status.

Introduction

The primary purpose of treatment of metastatic breast cancer (MBC) is palliative, in order to ameliorate quality, and perhaps also duration of life without any realistic hope for cure (1). Tamoxifen (TAM), a potent antiestrogen, has been the most widely accepted form of endocrine therapy for advanced disease (2). In patients (Pts) with estrogen and progesterone receptor (ER, PgR)-positive tumors, a response rate in the range of 50-60%, and a median progression-free survival of ~7 months may be expected (2). In recent years, the introduction of aromatase inhibitors (AIs) has improved the clinical outcome of breast cancer endocrine therapy. In randomized trials, anastrazole (3), letrozole (4) and examestane (5) have shown a better progression-free survival (PFS) compared with TAM in MBC. However, even in responders, invariably resistance to hormones will develop and the disease relapses (6). With the aim of improving response of MBC to hormonal therapy, in a previous study we combined TAM with ß-interferon (ß-IFN) and retinoids (R) (7). Prompted by the encouraging results obtained with the clinical study, we verified, *in vitro*, the growth inhibitory action of R alone or combined with ß-IFN on both ER-positive and -negative breast cancer cell lines. Our results clearly indicated that R inhibited the growth of both ER^+ and ER-human breast cancer cell lines and that the combination of R with ß-IFN induced an antiproliferative effect more pronounced than that produced by each of the two single agents, being the inhibitory effect synergistic in estrogen-sensitive cells (8).

Other authors obtained similar results with different models (9). The rationale for using interferons was due to the capacity that these agents have to inhibit the growth of both ER-positive and ER-negative breast cancer cell lines (10), being ß-IFN more active (11), and to sensitize estrogen-responsive cells to the antiproliferative action of TAM (12). The observed increase in cell growth inhibition could be due to the enhancement of ER which may allow an increased anti-estrogen-receptor interaction (13). Retinoids as well as TAM induce the secretion of transforming growth factor-ß that inhibits the growth of most epithelial cells (14). In some breast cancer cells, they have been shown to enhance the growth inhibitory action of various anti-estrogens (15). They are also able to increase ER in both TAM-sensitive and resistant sublines of breast cancer cell line MCF-7 and to enhance PgR level in T47D cells in which the expression of these receptors is independent of estrogen control (16,17).

Patients with advanced breast cancer have been reported to have a variety of functional abnormalities of the immune system, which may differ in magnitude from one subject to another one and which may be related to the extent of disease (18). As a result, the immuno-inhibitory influence of the tumor might extend far beyond the tumor microenvironment and become a systemic effect, especially in Pts with advanced and

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metastatic disease. Both ß-IFN and R may have an important role in improving the immune function. It has been shown that ß-IFN upregulates the expression of breast cancer tumor antigens, and R increase the percentage of peripheral blood lymphoid cells expressing surface markers for T-helper cells (19,20).

Herein we describe a further follow-up of a phase II pilot study, previously published (7), that included ß-IFN, R and TAM as a salvage treatment of MBC.

Patients and methods

Patients. In the present study, we report on 65 Pts, affected by metastatic inoperable, histologically proven advanced breast cancer, with evidence of progressive disease. Details on Pts recruitment and entry criteria have been previously described (7). Forty-nine Pts were entered onto the protocol, from March 1988 to July 1992. Additional 16 Pts were recruited from July 1992 to December 1994. The extension of Pts accrual increased the median follow-up time to 150 months. Written informed consent, approved by the Institutional Review Board, was obtained from each patient.

Treatment plan. The dose of TAM, given until progression, was established at 30 mg/day because 28 patients had been pretreated with 20 mg and had progressed and because there have been studies suggesting that the higher dosage of TAM might have a better antitumor effect (21,22). ß-IFN was administered at the dose of $1x10^6$ IU/m^2 subcutaneously 3 times a week and retynil palmitate, 15,000 IU orally twice per day. During the second year, and thereafter, in order to decrease toxicity, therapy was continued in responders, with ß-IFN $1x10^6$ IU/m^2 subcutaneously once a week and retynil palmitate, 15,000 IU orally once a day for 5 days/week, 2 weeks each month until progression. When fever and constitutional symptoms occurred, ß-IFN administration was preceded by 500 mg acetaminophen. If hematological toxicity occurred, administration of therapy was delayed until platelet counts were ≥$100x10^9$/l and absolute granulocytes were ≥$1x10^9$/l. If gastrointestinal toxicity occurred, the administration of ß-IFN and R was delayed until optimal dose could be tolerated. All women underwent an outpatient treatment and received therapy at home. At disease progression with TAM, an aromatase inhibitor was administered, followed by chemotherapy.

Table I. Characteristics of patients.

Characteristics	No.	%
No. of patients	65	100
Age, years		
Median	62	
Range	28-75	
Performance status (ECOG)		
0-1	41	63
2	22	34
3	2	3
Disease-free interval		
Median 28 months	47	72
Stage IV at diagnosis	18	28
Estrogen receptors		
ER^+	30	46
ER^-	11	17
Unknown	24	37
Progesterone receptors		
PgR^+	20	31
PgR^-	16	25
Unknown	29	44
Previous treatment		
Surgery	64	98
Chemotherapy	53	82
Hormonotherapy	28	43
Radiotherapy	35	54
Menopausal status		
Premenopause	7	11
Postmenopause	58	89
Dominant site of disease		
Viscera	25	38
Bone	27	42
Soft tissue	13	20

Statistical considerations. The study was designed to test the hypothesis that the hormonal therapy adopted might be effective in the treatment of MBC Pts who had been pretreated with hormones and had progressed. Simon's optimal two-stage design was used (23). According to this design, the first stage required a confirmed response in at least 8 responders out of 24 Pts. This criterion would rule out an undesirably low response probability of 0.30 (P0), in favor of a desirable response probability of 0.50 (P1), with a 5% probability of accepting a poor agent (α=0.05) and a 10% probability of rejecting a good agent (ß=0.1) before proceeding to the second stage. In the second stage, if 24 or more Pts achieved a confirmed response on a total of 63 assessable Pts, than the primary end-point would have been met. The time to progression was defined as the time between the start of therapy and any relapse or the appearance of a second primary cancer or death, whichever occurred first. Overall survival (OS) was measured from study entry to death, or study entry to December 2007, for censored Pts. Statistical analysis was performed with SAS statistical software (version 9.1, 2003 SAS Institute Inc., Cary, NC), PFS and OS were determined using the Kaplan-Meier method (24). All comparisons of Pts characteristics, response rates and toxicity profiles were performed using Pearsons χ^2 contingency table analysis. Analysis of data was performed in February 2008.

Results

All Pts had symptoms from their disease. The median age was 62 years (range: 28-75) with seven pre-menopausal and

Table II. Toxicity.

	(WHO grade)			
	0 No. (%)	1 No. (%)	2 No. (%)	Total No. (%)
Hematologic				
Leucopenia	59 (91)	6 (9)	0 (0)	65 (100)
Neutropenia	59 (91)	6 (9)	0 (0)	65 (100)
Thrombocytopenia	57 (88)	8 (12)	0 (0)	65 (100)
Anemia	62 (95)	3 (5)	0 (0)	65 (100)
Gastrointestinal				
Hepatic	54 (83)	10 (15)	1 (2)	65 (100)
Diarrhea	61 (94)	4 (6)	0 (0)	65 (100)
Triglycerides	57 (88)	8 (12)	0 (0)	65 (100)
Cutaneous	54 (83)	8 (12)	3 (5)	65 (100)
Fever	58 (89)	7 (11)	0 (0)	65 (100)
Autoimmune reactions	55 (85)	10 (15)	0 (0)	65 (100)

58 post-menopausal women. Median follow-up was 150 months. Median disease-free interval was 28 months for 47 Pts, while 18 Pts had stage IV disease at diagnosis. Initial stage of Pts at the moment of diagnosis was: stage I, 10 Pts; stage II 19 Pts; and stage III 18 Pts. Three Pts had bilateral breast cancer. ER and PgR, determined by immunohistochemical assay, were strongly positive (>50% of cells stained) in 30 and 20 Pts, respectively; negative in 11 and 16, and unknown in 24 and 29 Pts, respectively. Primary treatment had been modified radical mastectomy for 46 Pts; segmental mastectomies for 18 Pts; and radiotherapy for one patient. Twelve Pts (18%) had received adjuvant chemotherapy. Twenty-eight Pts (43%) had been pre-treated with hormones, 18 with TAM alone and 10 with TAM plus medroxyprogesterone acetate and aminogluthetimide. Five Pts had responses for a median time of 10 months, four had disease stabilization; all Pts receiving TAM had progressed. Forty-seven Pts (72%) had received one or two lines of chemotherapy for metastatic disease. Fifteen had been treated with both hormones and chemotherapy. Pts characteristics are listed in Table I.

Activity. Among 65 evaluable Pts, objective overall remission was observed in 36 women (55.5%) (95% c.i. 42.5%-67.7%) (Table III). Fourteen Pts achieved a complete response (21.5%), (95% c.i. 12.3%-33.5%); 22 a partial response response (34%), (95% c.i. 22-5%-46.6%). Disease stability was observed in 14 Pts (21.5%), (95% c.i. 12.3%-33.5%), and progression in 15 Pts (23%), (95% c.i. 13.5%-35.2%). Autopsy was performed in two Pts; pathology confirmed the complete response in brain and liver. Median PFS and OS were 43 and 47.9 months, respectively. There was no statistically significant difference in PFS and OS, between patients with high, low or unknown ER content (data not shown). Similarly, there was no statistically significant difference in PFS (P=0.76) and OS

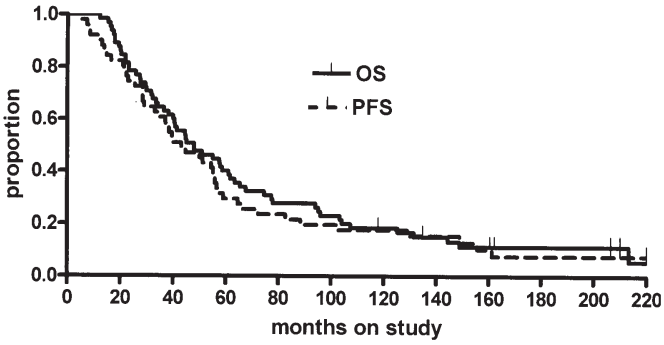

Figure 1. Progression-free survival (PFS). Events 60: (92.3%), censored 5: 7.7%, median PFS: 43 months. Overall survival (OS). Events 58: 89.2%, censored 7: 10.8%, median OS: 47.9 months.

Table III. Response to therapy.

	No. (%)	95% CI:
CR	14 (21.5)	12.3-33.5%
PR	22 (34.0)	22.5-46.6%
SD	14 (21.5)	12.3-33.5%
PD	15 (23.0)	13.5-35.2%
R.R.	36 (55.5)	42.5-67.7%

No., number of patients; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CI: confidence intervals.

(P=0.68) between patients pretreated with hormonal therapy or chemotherapy. After a median follow-up of 150 months, 7 Pts (10.8%) were alive. Responses were obtained in 18 Pts who had received chemotherapy, in 10 Pts who had progressed with TAM and in 8 Pts who had received TAM plus medroxyprogesterone acetate and aminogluthetimide. Sites of response were viscera in 16 Pts, bone in 11 Pts and soft tissue in 9 Pts. Responses in bone and soft tissue were evenly distributed, with six Pts in each group. Progressions occurred with pulmonary carcinomatous lymphangitis in 18 Pts; 19 Pts died with cerebral metastases and 21 with disseminated bone disease and malignant hypercalcemia.

Serial biopsies were performed on cutaneous lesions in four Pts; ER content increased, with respect to the previous determinations, by 20, 20, 30 and 100%, respectively. PgR showed no significant variation in three Pts, while in one patient they became positive (40%), being negative before entering the trial. The receptors became negative in one of these Pts when she failed, after six months of a partial response. A statistically significant improvement was observed in $CD4^+/CD8^+$ ratio in the 46 Pts who had shown a clinical benefit. One and three years later, the ratio increased by 16% and 23%, respectively. Twenty-five Pts who had relapsed received a salvage chemotherapy with carboplatin combination with a good tolerance. Twelve Pts (18%) with ER^+ tumors developed controlateral breast cancer during the follow-up time.

Toxicity. Toxicity of therapy was acceptable (Table II) and compliance was optimal. Initially, low grade fever was observed in 11% of Pts, that was avoided, afterwards, administering ß-IFN at night and preceding it with 500 mg acetaminophen. Grade 1 skin toxicity occurred in eight Pts (12%). Manifestations of autoimmune reactions, such as thyroiditis and reacutization of psoriasis, were observed in 15% of Pts. Elevation of triglycerides (twice baseline value) was observed in 8 Pts. Hepatic toxicity (low grade abnormality of hepatic sonogram and liver enzymes elevation 3 times baseline value) was observed in 11 Pts (17%). Liver biopsy, performed in two, showed fatty infiltration of 30% and 60% of hepatocytes, respectively, without abnormalities of porto-biliary space, after 23 and 13 months of therapy, respectively. Both Pts indulged in alcohol consumption. Retinoids were discontinued with no further problem. In order to avoid excessive liver toxicity, retynyl palmitate, on the second year of therapy, was given 5 days/week, two weeks each month.

Discussion

In recent years, outstanding progress has been made in the treatment of MBC, with the introduction of new chemotherapeutic agents, hormones and biological agents. However, the prognosis of MBC remains poor. For almost two decades, TAM has been considered the first line hormonal treatment of advanced breast cancer in post-menopausal Pts. Premenopausal Pts, with an endocrine responsive disease, have also a benefit from TAM after adjuvant chemotherapy (25). The preminence of TAM has been challenged by the AIs, anastrazole, letrozole and examestane. All three AIs markedly suppress plasma estrogen levels via inhibition of the peripheral conversion of androgens to estrogens. In randomized trials, they showed a better clinical outcome with respect to TAM in MBC (3-5). Nevertheless, a pooled analysis of published randomized trials of first line therapy with AIs versus TAM, in hormone-responsive MBC, showed an advantage of treatment with AIs in terms of PFS and clinical benefit but not of OS or objective response (26).

When the present study was initially started, the options for Pts with hormone-responsive MBC progressing on TAM were limited to progestins and aminogluthetimide. For this reason, we tried to find an approach that could improve the efficacy of TAM. Our aim was to render breast cancer cells more susceptible to hormonal therapy and to improve the immune function with the use of ß-IFN and R. Sica *et al* demonstrated that ß-IFN may induce the increase in ER in mammary cancer cell lines and, in agreement with other authors, they showed that ß-IFN treatment induce increase in both ER and PgR in cutaneous metastases of breast cancer patients (27,28). The receptor increase may be potentially linked to an enhanced hormone-sensitivity.

Furthermore, R have been reported to induce a series of effects on breast cancer cell lines. Besides the anti-proliferative action, they have been shown to promote apoptosis in both ER^+ and ER^- models (15,29). Products of retinoic acid metabolism blocking agents induce breast cancer cell differentiation (increased expression in cytokeratin and ER) and inhibit breast cancer cell growth both *in vitro* and *in vivo* (30). Moreover, it has been demonstrated that a novel retinoic acid metabolism blocking agent may reverse the resistance that develops with prolonged exposure to AIs (31). Finally, R stimulate the expression of IFN-regulated genes in IFN-resistant cells, wich suggests that combination treatment with R and IFNs may increase IFN-stimulated gene expression in IFN-resistant tumors, leading to augmented antitumor effects (32).

One of the reasons of failure of cancer therapy is due to the fact that the immune system is compromized in Pts with advanced breast cancer (18). Such a dysfunction may appear early in the course of disease and may worsen after cancer therapy (33). ß-IFN and R might have a key role in the functional improvement of cell-mediated immunity. Interferon has the capacity to enhance the tumor-associated antigens on a spectrum of freshly isolated human adenocarcinoma cells (34). Additionally, an important action of R on the immune system has been recently focused; the facilitation of the differentiation of immature myeloid suppressor cells ($Gr\text{-}1^+$ $CD115^+$), which are responsible for the development of tumor-induced T-cell anergy in tumor-bearing hosts (35), with an improvement of the immune response (36,37).

In our study, 41% of responses have been obtained in Pts pre-treated with hormones. Eleven of them were refractory to TAM and our findings are in agreement with data from Buzzi *et al* (38) concerning the possibility of overcoming of resistance to the anti-estrogen with IFN. Using ß-IFN in combination with TAM, 59% of responses were obtained in Pts pre-treated with chemotherapy showing a stabilisation of disease with deteriorating markers.

Clinical response seems not to be related to the receptor status of Pts, even if the number of observations was not adequate to make a statement regarding receptor significance. It is well known that receptor content may change from primary tumor to metastatic disease, but it can not be excluded that receptor status underwent some variations over time due to the different treatments. It can be hypothesized that IFN, R, or both, as shown in studies performed *in vitro*, determined a receptor enhancement, which could occur not only in those cells considered ER^+ but also in the ER^- ones.

Our Pts had, from the start of biological therapy, a median PFS and OS of 43 and 47.9 months, respectively. Even if it is not correct to compare data from different patient populations, Pts with hormone responsive tumors, from the MD Anderson (1) series, had a median PFS and OS of 14.3 and 28.6 months, respectively.

The major toxicity of our regimen was hepatic and it was observed in eight Pts. Two of them, with negative hepatitis markers, could have had increased liver toxicity of retinyl palmitate by alcohol abuse. Both Pts were asymptomatic and had a mild elevation of liver enzymes. The observation that 15% of our Pts had some form of autoimmune disease indicates in the response we observed some kind of immune mechanism might be involved.

Even if the role played by the single agent is difficult to clarify, the combination of TAM, ß-IFN and R seems to be an effective treatment modality for MBC. No previous data either pre-clinical or clinical exist concerning this three-drug combination. Nevertheless, our findings are in agreement with results by other authors concerning the effectiveness of

the association of IFN and TAM in human cancer cells (9-11). Moreover, they are in accordance with a study by our group showing that 13-cis retinoic acid and ß-IFN have a synergistic effect in terms of growth inhibition in a CG-5 estrogen-sensitive breast cancer cell line (8).

The similarity of retinoic acid receptors and steroid hormone receptors, which are involved in the mediation of antiestrogen action should be kept in mind. Eighteen percent of our Pts, with ER^+ tumors, developed a new cancer in the controlateral breast after a median time of 9 years. This supports the idea that ER^+ MBC, even in the presence of a complete response can recur and therefore a life-long surveillance should be maintained with a contemporary therapy administration.

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