ORIGINAL ARTICLE

Evaluation of the safety and efficacy of the first-line treatment with somatostatin combined with melatonin, retinoids, vitamin D3, and low doses of cyclophosphamide in 20 cases of breast cancer: a preliminary report

Giuseppe Di Bella [1], Fabrizio Mascia [1], Alessandro Ricchi [1], Biagio Colori [2]

1 Di Bella Foundation, Bologna, Italy
2 Rizzoli Institute, Scientific Research and Care Institute, Bologna, Italy

Correspondence to: Dr. Giuseppe Di Bella, MD.
Di Bella Foundation
Via G. Marconi 51, Post code 40122, Bologna, Italy.
TEL: +39 051 239662; E-MAIL: posta@giuseppedibella.it

Submitted: 2013-10-03 Accepted: 2013-11-05 Published online: 2013-12-03

Key words: **breast cancer; somatostatin; melatonin; retinoids; vitamin D3; Di Bella Method; first-line treatment; biological therapy; growth factors**

Neuroendocrinol Lett 2013; **34**(7):660–668 **PMID:** 24464005 NEL340713A04

Abstract

OBJECTIVE: The current strategies for the treatment of breast cancer are essentially based on surgery, preceded and/or followed by chemotherapy often supplemented by radiotherapy and/or the administration of hormonal therapy and monoclonal antibodies. Their combined use has made it possible to increase an overall survival but they are still penalized by adverse effects and toxicity. The marked anti-cancer effects of biological molecule such as somatostatin, melatonin, retinoid, vitamin D3 and prolactin inhibitors have been studied and documented for several decades. Their integrated and synergic action have been demonstrated, but only a few studies have as yet been carried out on their combined application in humans. The aim of the present investigation was to evaluate both the objective clinical response and toxicity of the biological multimodal treatment named *Di Bella Method* (DBM).

MATHERIAL AND METHODS: The clinical data from a total of 20 women with a certified diagnosis of breast cancer,defined disease stage, and who independently decided to follow the DBM as first-line treatment, were retrospectively reviewed.

RESULTS: The mean age of the patients was 51 years (min 30; max 73). Twelve (12) patients (60%) presented an early stage disease, while the other 40% had a locally advanced/metastatic stage. An overall clinical benefit was achieved in 75% of cases, with 55% of complete response and 20% of partial response. For metastatic patients, the overall survival rate was 71%. The main toxicity effects included leukopenia, gastrointestinal phenomena and drowsiness.

CONLUSIONS: The preliminary results of this report confirm the positive action of the biological treatment in terms of efficacy and survival, showing a more than favorable profile of tolerability.

INTRODUCTION

In the last few decades, numerous biological molecules, including Somatostatin (SST) and its analogues, Melatonin (MLT) and various classes of vitamins, such as retinoids, vitamin D3 and tocopherols, have been shown to be potentially useful both in the prevention and treatment of breast cancer (Seitz *et al.* 2013; Sanchez-Barcelo *et al.* 2012; Tang & Gudas 2012; Mehta *et al.* 2012; Frati *et al.* 2011; Fulan *et al.* 2011;). Numerous studies *in vitro* carried out on different cell lines have shown their marked anti-cancer activities, clarifying on one hand the putative mechanisms of action and on the other paving the way for the achievement of encouraging results in clinical practice (Proietti *et al.* 2012; Margheri *et al.* 2012; Cescon *et al.* 2012; Ostendorf *et al.* 2012; Suhail *et al.* 2012; Zhang *et al.* 2012; Proietti at al. 2011; He *et al.* 2009; Watt *et al.* 2009; Lee *et al.* 2008). However, only a very limited number of long-term clinical studies have been carried out in humans, especially as regards the association of these molecules in a multitherapic context that enhances their synergism and interaction. We report below an observational retrospective study carried out on 20 patients affected by breast cancer, who received the biological therapy (*Di Bella Method,* DBM) based on the combined use of low doses of cyclophosphamide and estrogen inhibitors together with molecules with a confirmed anti-cancer effect, such as somatostatin/octreotide, melatonin, prolactin inhibitors, Retinoids, Vitamins E, C, and D3, Calcium and extracellular matrix (ECM) components (Di Bella 2001).

PATIENTS AND METHODS

Eligibility criteria

Only patients with an *Eastern Cooperative Oncology Group* (ECOG) status ≤3, with a histological diagnosis of breast cancer and with disease characteristics measurable according to *Response Evaluation Criteria in Solid Tumors* (RECIST) were assessed (Patrick *et al.* 2000). Another criterion was that the patients had not undergone to any *standard therapeutic protocols* (surgery, poli-chemotherapy, radiation therapy, monoclonal antibodies), and that, after informed consent, they agreed to administrate the first-line biological treatment.

The patient sample described above was divided into two groups, according to their disease stage:

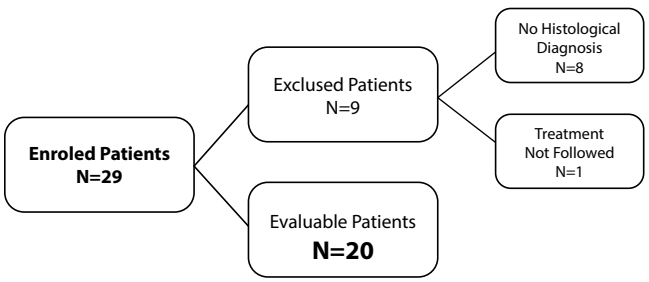
- ***Group A:*** Patients in early stage breast cancer (stages 0, I, IIA, IIB);
- ***Group B:*** Patients with locally advanced/metastatic stage disease (stages IIIA, IIIC, IV).

Treatment

All the patients received a daily combination of somatostatin/octreotide, melatonin, retinoids solubilised in alfa tocopherol acetate, dopamine sub-type 2 receptor (D_2R) agonists, oestrogen inhibitors and minimal doses of cyclophosphamide. Specifically, the components were administered as follows: gradually increasing oral doses of all trans retinoic acid (ATRA, 1 453 488.372 IU), axerophtol palmitate (909000 IU) and beta-carotene (3 334 000 IU) solubilized in alfa tocopheryl acetate (1×10^6 IU); an oral dose of dihydrotachysterol (15 200 IU) together with retinoids; increasing doses of somatostatin (1 mg a day for the first 7 days, increasing to 3 mg a day at 21 days)**;** tetracosactide acetate (1 mg), initially administered intramuscularly every 7 days, constantly monitoring blood pressure and blood sugar, after 30 days 1/2 vial/week; slow-release octreotide (20 mg) every 3 weeks intramuscularly; melatonin*:* 10 mg in the morning, at midday, and in the evening with meals, plus 40 mg before going to bed (average total daily dose = 70 mg); cabergoline orally during the main meal 1 mg (1\2 tablet), twice a week; bromocriptine (2.5 mg) orally, half a tablet morning and evening**;** cyclophosphamide (50–100 mg) *orally,* gradually increasing doses, starting with 1 tablet with the main meal and increasing to 1 tablet morning and evening after 1 week; L-ascorbic acid (Vitamin C) *orally,* gradually increasing doses: 1/2 spoonful (2 g = 4×10^3 IU) in a glass of water at midday and in the evening with the meal together with Calcium lactate gluconate + calcium carbonate equal to 1 000 mg of calcium in the same glass; chondroitin sulphate (500 mg), one tablet with meals in the morning, at midday and in the evening. More details as regards methods of administration and the respective doses are shown in the Table 1.

Evaluation of the response to treatment of the target lesions (Efficacy): Statistical and analytical methods

The criteria for evaluation of the objective response refer to the guidelines adopted by the World Health Organization (*WHO handbook*) and by the *Response Evaluation Criteria In Solid Tumors* (RECIST). These are classified as: Overall Response **(OR)**; *Complete Response* **(CR)**; *Partial Response* **(PR)**; *Progression of Disease* **(PD)**; *Stable Disease* **(SD),** expressed as absolute frequency (n), relative frequency (%), and 95% confidence interval (95% CI). Radiological exams (MNR, CT-PET total body and breast ultrasound) every 4 months were routinely performed. *Overall Survival*



Flow chart: patient enrolment criteria.

(OS) has been evaluated as primary end-point. This latter analyses were performed using the Kaplan–Meier method, with a 95% CI (R© software package; version 2.15.12, 2012).

Safety and toxicity evaluation

Only the adverse events that could be correlated to the treatment (degrees of correlation*: possible, probable or certain)* expressed as absolute frequency (n) and relative frequency (%), were considered when evaluating the toxicity; as described by the National Cancer Institute (NCI-CTC) criteria (http://www.eortc.be/services/doc/ctc/).

Note: this is a study on the combined use of drugs that have already passed all the tests of reliability and proven antitumor activity. Therefore, because all the products are already widely tested and whose use is approved by the international health organizations, but in this context are only used in a new combination, it was decided not to submit to any ethical committee. The therapeutic methodology used in such investigation is therefore strictly designed on the basis of the concept of *evidence-based medicine* (EBM) (Sackett *et al.* 1996). All the other prerogatives of this investigation are also in accordance with the fundamental principles of the Declaration of Helsinki. Therefore, all patients gave their informed written consent.

RESULTS

A total of 29 patients accrued over a 5 year period (from March 2006 to December 2012) were consecutively treated with a biological therapy (DBM) and monitored for 26 months (min 9, max 51). Twenty (20) of these patients satisfied the eligibility criteria and were retrospectively reviewed (see flowchart). Table 2 shows the clinical characteristics of the patients at baseline: the median age was 51 years (range: 30–73 years). The main histotype of the primary lesion was *Infiltrating Ductal Carcinoma* (IDC, 15 cases, 75%), while lung, lymph nodes and bones represented the sites of secondary lesions (10%, 60% and 30% respectively). The molecular biological profile has been assessable only in 60% of the patients (12 cases). The disease stages at the time of evaluation were as follows: early stage disease (*Group A)*: Stage 0, 2 cases (10%); Stage I, 2 cases (10%); Stage II, 8 cases (40%). The Gleason score grad-

Tab. 1. DBM therapeutical regimen.

Drug	Chemical Information	Dosage	Route of administration	Frequency
Somatostatin	14 aa peptide	3 mg	subcutaneous	Daily (at night, 12 hours infusion)
Octreotide LAR	Octreotide Acetate 8 aa	20 mg	Intramusculus	Every 20 days
Melatonin	Melatonin 12% Adenosine 51% Glycine 37%	70–100 mg	per os	Daily
Retinoids *	All-Trans-Retinoic acid	0.5 g 1 453 488.372 IU	*per os*	Daily (3 times)
	Axeroftole-Palmitate	0.5 g 909 000 IU		
	Beta-Carotene	2 g 3 334 000 IU		
	Alfa Tocopheryl Acetate	1 000 g 1×10^6 IU **		
Vitamin C	L-Ascorbic Acid	2–4 g 40×10^3–80×10^3 IU	per os	Daily
Vitamin D_3	1,25-diOH-Tachysterol	15 200 IU	per os	Daily (3 times)
Synachten	Tetracosactide Acetate (ACTH)	1 mg	intramuscolar	Once a week
PARLODEL®	Bromocriptine	2.5 mg ****	per os	Daily
DOSTINEX®	Cabergoline	0.5 mg		Twice a week
ENDOXAN®	Cyclophosphamide	50 mg	per os	Daily
Calcium	Lactate gluconate + carbonate	2 g	per os	Daily
Oestrogens Inhibitors	Anastrozole/exemestane Triptoreline/Leuprorelin acetate	1 mg	Parenteral	Monthly

* These molecules are mixed in solution form, a formulation which allows maximum bioavailability. The daily dose is calculated on the basis of body weight decimals; **** Can be used together with or instead of Bromocriptine.

ing were: G1 20%; G2 40%, G3 13.3% and n.a. (26.7%). Locally advanced/Metastatic disease (*Group B*): Stage IIIA, 3 cases (15%); Stage IIIC, 2 cases (10%); Stage IV, 3 cases (15%). Of these patients, 4 cases (50%) were grade G3. Taken together, an overall objective response (OR) [Complete response (CR) + partial response (PR)] was observed in 75% (11+4 cases; 53.1–88.8; 95% CI) of the patients, with a CR in 55% of cases (n=11; 30–70; 95% CI). In addition, 85% (16 cases; 58–92; 95% CI) of the patients achieved an objective clinical benefit (CR+PR+SD) (Table 3). The 2 years Overall survival (OS) rate of the patients was 100% (Figure 1). *Group A (Early Breast Carcinoma, NA=12).* An OR (CR+PR) was achieved in 91% of the patients (7+4 cases; 64–98; 95% CI), with CR in 58% of the cases (n=7, 32–81; 95% CI). In addition, all the patients achieved a clinical benefit (CR+PR+SD) (Table 4). The 2 years Overall survival (OS) rates of the patients were 100% (Figure 2). *Group B (Locally Advanced/Metastatic Carcinoma, NB=8)*. The 2 years OS rate of the patients was 71% (Figure 3). The OR (CR+PR) was 50% (3+1 cases; 21–78; 95% CI) of the patients, with CR in 37.5% of the cases (Table 5). In addition, the 62.5% of the patients achieved a clinical benefit (CR+PR+SD).

Tab. 2. Clinical baseline *in situ* Ductal carcinoma.

Median Age	51	Abs. Freq.	Rel. Freq (%)
Min	30		
Max	73		
ECOG (PS)	Grade 0	3	15
	Grade 1	7	35
	Grade 2	5	25
	Grade 3	5	25
Histotype	IDC	15	75
	DCIS	3	10
	Other	2	15
Molecular Profile**	PR+ (>80%)	12	100
	ER+ (>50%)	7	58
	Her/neu+	5	25
	n.a**	8	66
Histology Grade	G1	3	20
	G2	7	40
	G3	5	13.3
	n.a	5	26.7
Staging	0	2	10
	I	2	10
	II	8	40
	IIIA	3	15
	IIIC	2	10
	IV	3	15
Secondary lesions site	Lung	1	10
	Bone	3	30
	Lymphnode	6	30

DCIS: *in situ* Ductal carcinoma; IDC infiltrating ductal carcinoma; PR: Progesteron Receptor; Estrogen Receptor, Her/Neu type 2 Epidermal Growth Factor Receptor. * Total of 12 patients

Safety evaluation

The most frequent toxicity phenomena observed in the study (grade II) were the following: haematological toxicity (leukopenia, 35%), gastrointestinal toxicity (nausea, 25%), and drowsiness (40%). A reduction,

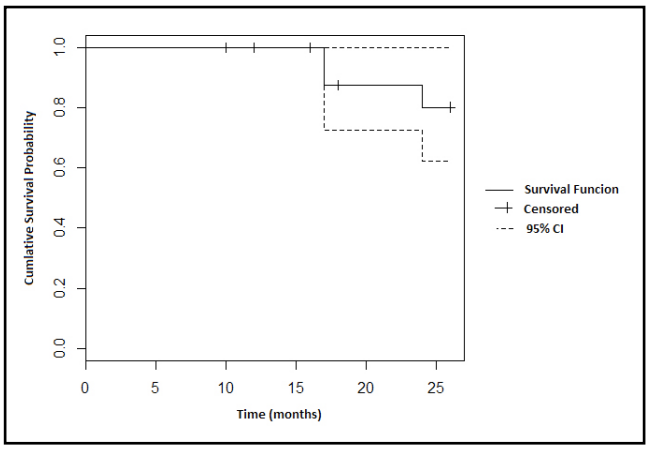


Fig. 1. Global cumulative overall survival of early + locally advanced/metastatic breast cancer cases (Groups A+B; N=20).

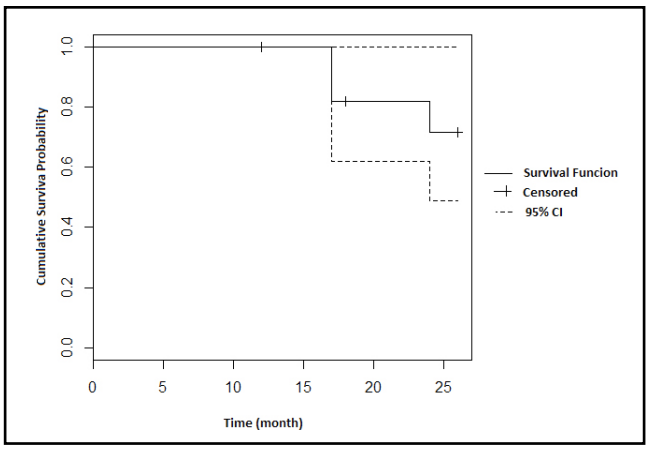


Fig. 2. Global cumulative overall survival of early stage breast cancer cases (Group A; N_A=12).

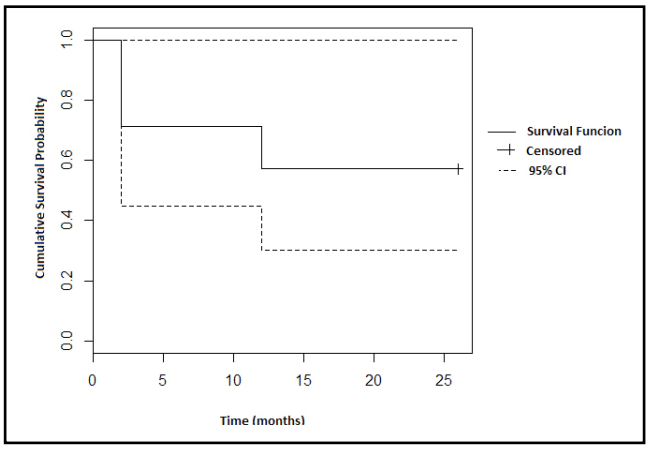


Fig. 3. Global cumulative overall survival of locally advanced/metastatic breast cancer cases (Group B; N_B=8). * Two (2) patients dead.

delay or temporary discontinuation of the treatment due to toxicity was necessary in patients with leukopenia (discontinuation of Cyclophosphamide until the leukocyte count returned to within the normal range), and in the cases of gastrointestinal effects. No deaths due to the treatment occurred (Table 6).

DISCUSSION

Rationale of the treatment, review of the literature and brief discussion of the clinical data

Numerous experimental studies and clinical investigations clearly indicate that some pituitary hormones, including the Growth Hormone (GH) and Prolactin (PRL), have a crucial role in the development and progression of human breast cancer (Schally *et al.* 2001; Kamenicky *et al.* 2010; Perry et al., 2008; Harvey 2012; Bernichtein *et al.* 2010; Faupel-Badger *et al.* 2010; Raccurt *et al.* 2002). While on one hand the ubiquitous expression of PRL and GH represents a clear confirmation of the direct, generalized and synergized mitogenic role of these hormones (Wu *et al.* 2011; Xu *et al.* 2012), the causal dose-dependent relationship between the receptorial expression of GH and the processes of tumour induction and progression has on the other hand has been also demonstrated. The distribution of GHR and GHRHR are in fact markedly superior in breast tumour tissues with respect to their physiological and peritumoral receptorial expression, further confirming the potent mitogenic role of GH with a dose-dependent proliferative index (Gebre-Medhin *et al.* 2001; Chatzistamou *et al.* 2004). The temporal mechanism of this etiopathogenetic process is currently being studied: the most probable being mechanisms of autocrine and/or paracrine signalling, on the basis of the detection of local production of GH, Insulin Growth Factor I (IGFI), and ES in breast cancer, peritumoral tissues stromal area (Kaulsay *et al.* 2001; Siriwardana *et al.* 2010). Both physiological and tumour cell proliferation take place by means of these same molecules, which a tumour cells uses to a much greater extent than normal cells. It can thus be deduced that the PRL-GH-IGF1 axis has a determining influence on neoplastic biological development. In addition, it is now confirmed that sex hormones play a key role in the etiology and progression of breast cancer, as well as in various hormone-dependent tumours such as prostate and ovarian cancer. The main mechanism at the basis of these tumours is the result of the prolonged hormonal stimulation, with serious long-term repercussions on the normal growth and function of the target tissues. Considerable experimental evidence has demonstrated that both the contribution of sex hormones and the growth factors regulated by them carry out a profound gene modulation (Zheng *et al.* 2010; Rasmussen *et al.* 2010). This means that tumor cell proliferation is closely connected to the PRL-GH axis, in association with the steroid Hormones and to GH-dependent mitogenic molecules (*Growth Factors,* GFs), such as *Epidermal Growth Factor* (EGF), *Fibroblast Growth Factor* (FGF), *Hepatocyte Growth Factor* (HGF), *Insulin Growth Factor* (IGF1-2), *Nerve Growth Factor* (NGF), *Platelet Derived Growth Factor* (PDGF), *Vascular Endothelial Growth Factor* (VEGF), and *Transforming Growth Factor* (TGF), as well as to GFs produced by the gastrointestinal tract (*Vasoactive*

Tab. 3. Global effectiveness with MDB in Breast Cancer (Groups A+B).

Resp. Rate	Cases Abs.Fr	Rel. Fr. (%)	95% CI
CR	11	55	34;74
PR	4	20	8;41
SD	2	10	3;30
P	3	15	5;36

Response Rates (N=20) CR: Complete remission; PR: Partial Remission; SD: Stable disease; P: Progression.

Tab. 4. MDB Effectiveness in early stage Breast Cancer.

Resp. Rate	Cases Abs.Fr	Rel. Fr. (%)	95% CI
CR	7	58	32;80
PR	4	33	14;61
SD	1	8	1;35
P	0	0	//

Response Rates (GroupA; N_A=12) CR: Complete remission; PR: Partial Remission; SD: Stable disease; P: Progression.

Tab. 5. MDB Effectiveness in Locally-Advanced/Metastatic Breast Carcinoma.

Resp. rate	Cases Abs.Fr	Rel. Fr. (%)	95% CI
CR	3	37.5	14;70
PR	1	12.5	2.4;47
SD	1	12.5	2.4;47
P*	3	37.5	14;70

Response Rates (Group B; N_B=8). CR: Complete remission; PR: Partial Remission, SD: Stable disease, P: Progression

Table 6. Grades II toxicities phenomena.

	Ab. Freq.	Rel. Freq.	Active molecule	Grade of correlation
Haematological Leukopenia	7	35	Endoxan©	*Probable*
Gastrointestinal Nausea/Vomiting	5	25	somatostatin	Certain
Neurological Drowsiness	8	40	Melatonin	Probable

Intestinal Peptide, VIP) (Li *et al.* 2011; Frank *et al.* 2008; Fürstenberger *et al.* 2003; Laban *et al.* 2003; Milewicz *et al.* 2011; Carver *et al.* 2010; Haines *et al.* 2009; Smith *et al.* 2011; Moody *et al.* 2003). It therefore seems evident that the use of biological antagonists of GH and PRL, such as Somatostatin, its analogues and D_2R agonists; not only downregulate both the expression and the secretion of mitogenic GFs, but also extend their negative regulation to the signalling pathways of the respective receptorial targets, with sub-sequent antiproliferative and antiangiogenic effects. This concept of a "multi-targeted biological approach", is slowly emerging through the increasingly frequent research, although still with very few models *in vivo* (Tejeda *et al.* 2006; Jacobson *et al.* 2010; Schally *et al.* 2008). The suppressive effect of SST and its analogues on the serum levels of IGF1 is both direct, through inhibition of the IGF gene, and indirect, by suppression of the GH and thus of its hepatic induction of IGF1 (Pollak 1997). It has been observed that breast tumours express the receptorial subclasses SSTR1-5 (Kumar *et al.* 2005, Cameron *et al.* 2003). This representing a further rational indication for the use of SST, already fully justified by the aforementioned negative regulation on GH, of the GH–correlated GF and oestrogen regulation. The inhibitory activity of SST, is directed to another mitogenic growth factor, the EGF, through multiple mechanisms: by blocking of the dose-dependent signalling (inhibition of tyrosine phosphorylation), its gene down-regulation along with its plasma concentration (Watt *et al.* 2009, Hofland *et al.* 1995, Ruscica *et al.* 2012). The same mechanisms have been included within the retinoid anticancer action. Expecially as regards retinoic acid compounds (Salvatori *et al.* 2011). This interference is shown to reinforce by the concomitant administration of MLT and Vitamin D3 *in vitro* (Proietti *et al.* 2011). In addition, Angiogenesis and neoangiogenesis, necessary conditions for tumour progression, as well as the cascade of monocytes, the paracrine release of interleukin 8 (IL-8)and the contribution of GFs (whose synergism is essential), are specific molecular targets negatively regulated by Somatostatin and its analogues (Ruscica *et al.* 2012). The inhibition of angiogenesis induced by SST is synergically reinforced by MLT, Retinoids and vitamin D3 (Kim *et al.* 2013; Lissoni *et al.* 2001; Sogno *et al.* 2009; Picotto *et al.* 2012). Furthermore, the local conditions of hypoxia/anoxia and acidosis promote angiogenesis, and are mostly corrected by the improvement in the blood-tissue exchanges induced by the differentiating components of the DBM. At the same time, the cytostatic, antiproliferative, and antimetastatic effects of Somatostatin are synergically increased by the other components of the DBM. An additional contribution is provided by the daily administration of low doses (50–100 mg/die *per os*) of Cyclophosphamide (Endoxan®). As well as drastically reducing the known anitblastic/myelosuppressive effects, this dosage induces a marked turnaround of its mechanisms of action: triggering of the mitochondrial-dependent apoptotic cascade, anti-angiogenetic action by drastically down-regulating the VEGF gene expression (Loven *et al.* 2013, Pasquier *et al.* 2010). Numerous preclinical investigations have also demonstrated the mechanisms of action of MLT. The use of such indole extends to all histotypes of breast cancer due to its high membrane receptorial/nuclear distribution (Oprea-Ilies *et al.* 2012; Rögelsperger *et al.* 2011). Since the molecule is associated with the signalling pathways of both the physiological and neoplastic epithelial development, this substance has the properties to selectively neutralize the proliferative signals of estrogens and negatively modulate their local biosynthesis (Hill *et al.* 2011; Girgert *et al.* 2009). The administration of low doses of second generation aromatase inhibitors (Anastrozole©), already used in clinical practice, combined with MLT, SST and Retinoids, negatively regulates the hormone-dependent processes of proliferation of breast tumours (Alvarez Garzia *et al.* 2013; Margheri *et al.* 2012; Wang *et al.* 2012; Knower *et al.* 2012; Ciolino *et al.* 2011). The literature has therefore confirmed anticancer anctivity throught their differentiating, antiproliferative, antiangiogenetic and antimetastatic mechanisms of action, of all the compounds covered by the DBM both *in vitro* and *in vivo*. This retrospective study confirms the objective response of breast cancer to the concomitant treatment of the aforesaid biological molecules. Another issue worth mentioning is that the objective response obtained in 15 cases was achieved *without recourse to conventional chemo-radio therapy and/or surgery*, especially considering that recurrence and post-operative metastases are the main cause of mortality correlated to breast cancer, by inducing a systemic dissemination (Naumov *et al.* 2002; Demicheli *et al.* 1996; Fisher *et al.* 1989; Tyzzer *et al.* 1916; Marie *et al.* 1910) and that the use of massive doses of antiblastic agents and of radiation therapy not only increases the mutations but also provides a fertile ground for the activation of factors that promote the growth and progression of hormone-dependent tumors (Sun *et al.* 2012; Lagadec *et al.* 2012). The combined use of the DBM molecules in local advance/metastatic patients allowed a complete survival rate at the first year of follow-up. Furthermore, in the 8 cases observed, the OS is currently at several months (24). The objective response, without severe toxicity, achieved by the progressive reduction and disappearance of the initial eteroplastic lesions, the axillary adenopathies, is unequivocal proof of the efficacy of this treatment and confirms the preliminary positive results already published on the use of the DBM in lymph proliferative disorders (Di Bella *et al.* 2013; Todisco *et al.* 2001), in advanced stage lung cancer and in cervical-facial tumours (Di Bella *et al.* 2012; Norsa *et al.* 2007; Norsa *et al.* 2006;). Without toxicity and without in any way reducing working activities, the DBM avoids surgical

trauma and the significant side effects of the usual chemotherapy and radiation therapy protocols. The early application of the DBM as first-line therapy, in patients not weakened by the toxic, mutagenic and immunodepressive effects of chemo- and radiotherapy. Thus greatly facilitated the possibilities of achieving an objective response, both promoting survival and improving a better quality of life.

CONCLUSIONS

The rationale for the use of biological molecules to treat breast cancer, supported by the clinical results reported above, is proof of the logic and efficacy of the MDB's multi-therapy concept as a biological treatment for tumours: the synergic interaction of its components supports and enhances the vital reactions and anticancer homeostasis, allowing them to counter the anarchy of the neoplastic processes of the tumour's microenvironment. In short, the aim of the biological therapy is to counter the progression of the neoplastic phenotype: a) b*y Inhibiting the neoplastic proliferation* by means of apoptosis/necrosis and deprivation of hormones and cell growth factors; b) *by contrasting the marked mutagenic tendency* by direct activation of DNA repair system and through epigenetic cell reprogramming; c) *by blocking tumour progression*, by halting the formation of blood vessels (*Neoangiogenesis-Lymphangiogenesis*) and of cell motility (*migration*), essential for the spread of tumour cells to distant sites; d) *by providing defence* against neoplastic aggression by reinforcing the natural defence mechanisms (innate and acquired immunity). Tumors can in fact be considered an aimless deviation of cellular homeostasis, meaning that it is necessary to restore the abnormal biochemical reactions to normal status by the reinforcement and modulation of all the means physiologically considered essential to sustain life. The documented antiangiogenic synergism of all the components of the DBM, together with the antiproliferative effect of SST and PRL and ES inhibitors and the differentiating, immunomodulating, trophic and homeostatic effects of the other components of the DBM, achieved this result while avoiding on one hand the severe toxicity and sometimes permanent damage of the usual medical treatments for cancer, and on the other notably increasing the PS and quality of life and survival. In conclusion, we believe it useful and necessary to report this observational study in order to encourage greater interest in the scientific community. We also believe that the few clinical studies carried out on the multitherapy and synergic use of these biological molecules should be extended to the various forms of cancer in order to provide a documented confirmation. Future randomized and double-blind clinical studies investigating this treatment should be encouraged.

REFERENCES

1 Alvarez-García V, González A, Martínez-Campa C, Alonso-González C and Cos S (2013). Melatonin modulates aromatase activity and expression in endothelial cells. Oncol Rep. **29**(5). 2058–64.
2 Bernichtein S, Touraine P and Goffin V (2010). New concepts in prolactin biology. J Endocrinol. **206**: 1–11.
3 Cameron Smith M, Orlando C, Serio M and Maggi M (2003). Somatostatin receptors and breast cancer. J Endocrinol Invest. **26**: 125–30.
4 Carver KC, Piazza TM and Schuler LA (2010). Prolactin enhances insulin-like growth factor I receptor phosphorylation by decreasing its association with the tyrosine phosphatase SHP-2 in MCF-7 breast cancer cells. J Biol Chem. **285**: 8003–12.
5 Cescon DW, Ganz PA, Beddows S, Ennis M, Mills BK, and Goodwin PJ (2012). Feasibility of a randomized controlled trial of vitamin D vs. placebo in women with recently diagnosed breast cancer. Breast Cancer Res Treat. **134**: 759–67.
6 Chatzistamou I, Schally AV, Kiaris H. *et al.* (2004). Immunohistochemical detection of GHRH and its receptor splice variant 1 in primary human breast cancers. Eur J Endocrinol. **151**: 391–6.
7 Ciolino HP, Dai Z and Nair V (2011). Retinol inhibits aromatase activity and expression in vitro. J Nutr Biochem. **22**: 522–6.
8 Common Terminology Criteria for Adverse Events (CTCAE) and Common Toxicity Criteria (CTC) (http://ctep.can cer.gov/protocolDevelopment/electronic_applications/ctc.htm).
9 Demicheli R, Abbattista A, Miceli R, Valagussa P, Bonadonna G (1996). Time distribution of the recurrence risk for breast cancer patients undergoing mastectomy: further support about the the concept of tumor dormancy. Breast Cancer Res Treat. **41**(2). 177–85.
10 Di Bella G: The Di Bella Method (DBM)(2010). Neuro Endocrinol Lett. **31**: 1–42.
11 Di Bella G and Colori B (2012). The Di Bella Method (DBM) improved survival, objective response and performance status in a retrospective observational clinical study on 23 tumours of the head and neck. Neuro Endocrinol Lett. **33**: 249–56.
12 Faupel-Badger JM, Sherman ME, Garcia-Closas M *et al.* (2010). Prolactin serum levels and breast cancer: relationships with risk factors and tumour characteristics among pre- and postmenopausal women in a population-based case-control study from Poland. Br J Cancer. **103**: 1097–102.
13 Fisher B, Gunduz N, Coile J, Rudock C, Saffer E. (1989). Presence of a growth-stimulating factor in serum following primary tumour removal in mice. Cancer Res. **49**: 1996–2001.
14 Frank SJ (2008). Mechanistic aspects of crosstalk between GH and PRL and ErbB receptor family signaling. J Mammary Gland Biol Neoplasia. **13**: 119–29.
15 Frati A, Antoine M, Rodenas A, Gligorov J, Rouzier R and Chéreau E (2011). Somatostatin in breast cancer. Ann Biol Clin (Paris). **69**: 385–91.
16 Fulan H, Changxing J, Baina WY *et al.* (2011). Retinol, vitamins A, C, and E and breast cancer risk: a meta-analysis and meta-regression. Cancer Causes Control. **22**: 1383–96.
17 Fürstenberger G, Morant R and Senn HJ (2003). Insulin-like growth factors and breast cancer. Onkologie. **26**: 290–4.
18 Gebre-Medhin M, Kindblom LG, Wennbo H, Törnell J and Meis-Kindblom JM (2001). Growth hormone receptor is expressed in human breast cancer. Am J Pathol. **158**: 1217–22.
19 Girgert R, Hanf V, Emons G and Gründker C (2009). Membrane-bound melatonin receptor MT1 down-regulates estrogen responsive genes in breast cancer cells. J Pineal Res. **47**: 23–31.
20 Haines E, Minoo P, Feng Z *et al.* (2009). Tyrosine phosphorylation of Grb2: role in prolactin/epidermal growth factor cross talk in mammary epithelial cell growth and differentiation. Mol Cell Biol. **29**: 2505–20.
21 Harvey PW (2012). Hypothesis: prolactin is tumorigenic to human breast: dispelling the myth that prolactin-induced mammary tumors are rodent-specific. J Appl Toxicol. **32**: 1–9.
22 He Y, Yuan XM, Lei P *et al.* (2009). The antiproliferative effects of somatostatin receptor subtype 2 in breast cancer cells. Acta Pharmacol Sin. **30**: 1053–9.

23 Hill SM, Blask DE, Xiang S *et al.* (2011). Melatonin and associated signaling pathways that control normal breast epithelium and breast cancer. J Mammary Gland Biol Neoplasia. **16**: 235–45.
24 Hofland LJ, van der Burg B, van Eijck CH, Sprij DM *et al.* (1995). Role of tumor-derived fibroblasts in the growth of primary cultures of human breast-cancer cells: effects of epidermal growth factor and the somatostatin analogue octreotide. Int J Cancer. **60**: 93–9.
25 Jacobson EM, Hugo ER, Tuttle TR, Papoian R and Ben-Jonathan N (2010). Unexploited therapies in breast and prostate cancer: blockade of the prolactin receptor. Trends Endocrinol Metab. **21**: 691–8.
26 Kamenicky P, Lombès M and Chanson P (2010). [New insights in growth hormone physiology and pathophysiology]. Ann Endocrinol (Paris). **71**: S25–32.
27 Kaulsay KK, Zhu T, Bennett W, Lee KO and Lobie PE (2001). The effects of autocrine human growth hormone (hGH) on human mammary carcinoma cell behavior are mediated via the hGH receptor. Endocrinology. **142**: 767–77.
28 Kim KJ, Choi JS, Kang I *et al.* (2013). Melatonin suppresses tumor progression by reducing angiogenesis stimulated by HIF-1 in a mouse tumor model. J Pineal Res. **54**(3): 264–70.
29 Knower KC, To SQ, Takagi K (2012). Melatonin suppresses aromatase expression and activity inbreast cancer associated fibroblasts. Breast Cancer Res Treat. **132**: 765–71.
30 Kumar U, Grigorakis SI, Watt HL *et al.* (2005). Somatostatin receptors in primary human breast cancer: quantitative analysis of mRNA for subtypes 1--5 and correlation with receptor protein expression and tumor pathology. Breast Cancer Res Treat. **92**: 175–86.
31 Laban C, Bustin SA and Jenkins PJ (2003). The GH-IGF-I axis and breast cancer. Trends Endocrinol Metab **14**: 28–34.
32 Lagadec C, Vlashi E, Della Donna L, *et al.* (2012). Radiation-induced reprogramming of breast cancer cells. Stem Cells. **30**: 833.
33 Lee LT, Schally AV, Liebow C *et al.* (2008). Dephosphorylation of cancer protein by tyrosine phosphatases in response to analogs of luteinizing hormone-releasing hormone and somatostatin. Anticancer Res. **28**: 599–605.
34 Li X, Huang Y, Jiang J and Frank SJ (2011). Synergy in ERK activation by cytokine receptors and tyrosine kinase growth factor receptors. Cell Signal. **23**: 417–24.
35 Lissoni P, Rovelli F, Malugani F, Bucovec R, Conti A, Maestroni GJ (2001). Anti-angiogenic activity of melatonin in advanced cancer patients. Neuro Endocrinol Lett. **22**: 45–7.
36 Loven D, Hasnis E, Bertolini F and Shaked Y (2013). Low-dose metronomic chemotherapy: from past experience to new paradigms in the treatment of cancer. Drug Discov Today. **18**: 193–201.
37 Margheri M. *et al.* (2012). Combined effects of melatonin and all-trans retinoic acid and somatostatin on breast cancer cell proliferation and death: molecular basis for the anticancer effect of these molecules. Eur J Pharmacol. **68**: 34–43.
38 Marie P, Clunet, J. (1910). Fréquences des métastases viscérales chez les souris cancéreuses aprčs ablation chirurgicale de leur tumeur. Bull. Assoc. Fr. Etud. Cancer. **3**: 19–23.
39 Mehta RG, Peng X, Alimirah F *et al.* (2012). Vitamin D and breast cancer: Emerging concepts. Cancer Lett. pii: S0304-3835(12)00639-8. doi: 10.1016/j.canlet.2012.10.034. [Epub ahead of print].
40 Milewicz T, Ryś J, Wójtowicz A, *et al.* (2011). Overexpression of P53 protein and local hGH, IGF-I, IGFBP-3, IGFBP-2 and PRL secretion by human breast cancer explants. Neuro Endocrinol Lett. **32**: 328–33.
41 Moody TW, Chan D, Fahrenkrug J and Jensen RT (2003). Neuropeptides as autocrine growth factors in cancer cells. Curr Pharm Des. **9**: 495–509.
42 Naumov GN, MacDonald IC, Weinmeister PM, Kerkvliet N *et al.* (2002). Persistence of solitary mammary carcinoma cells in a secondary site: a possible contributor to dormancy. Cancer Res. **62**: 2162–2168.
43 Norsa A and Martino V (2007). Somatostatin, retinoids, melatonin, vitamin D, bromocriptine, and cyclophosphamide in chemotherapy-pretreated patients with advanced lung adenocarcinoma and low performance status. Cancer Biother Radiopharm. **22**: 50–5.
44 Norsa A and Martino V (2006). Somatostatin, retinoids, melatonin, vitamin D, bromocriptine, and cyclophosphamide in advanced non-small-cell lung cancer patients with low performance status. Cancer Biother Radiopharm. **21**: 68–73.
45 Oprea-Ilies G, Haus E, Sackett-Lundeen L *et al.* (2012). Expression of melatonin receptors in triple negative breast cancer (TNBC) in African American and Caucasian women: relation to survival. Breast Cancer Res Treat. **137**(3). 677–87.
46 Ostendorf GM (2012). High dosage vitamin C in breast cancer? Versicherungsmedizin. **64**: 85.
47 Patrick T. *et al.* (2000). New Guidelines to Evaluate the Response to Treatment in Solid Tumors. Journal of the National Cancer Institute, Vol. 92.
48 Picotto G, Liaudat AC, Bohl L and Tolosa de Talamoni N (2012). Molecular aspects of vitamin D anticancer activity. Cancer Invest. **30**: 604–14.
49 Perry JK, Mohankumar KM, Emerald BS, Mertani HC and Lobie PE (2008). The contribution of growth hormone to mammary neoplasia. J Mammary Gland Biol Neoplasia. **13**: 131–45.
50 Pollak M (1997). The potential role of somatostatin analogues in breast cancer treatment. Yale J Biol Med. **70**: 535–9.
51 Proietti S, Cucina A, Reiter RJ and Bizzarri M (2010). Molecular mechanisms of melatonin's inhibitory actions on breast cancers. Cell Mol Life Sci. **70**(12). 2139–57.
52 Proietti S, Cucina A, D'Anselmi F, *et al.* (2011). Melatonin and vitamin D3 synergistically down-regulate Akt and MDM2 leading to TGFβ-1-dependent growth inhibition of breast cancer cells. J Pineal Res. **50**: 150–8.
53 Raccurt M, Lobie PE, Moudilou E, *et al.* (2002). High stromal and epithelial human gh gene expression is associated with proliferative disorders of the mammary gland. J Endocrinol. **175**: 307–18.
54 Rasmussen LM, Frederiksen KS, Din N, Galsgaard E, Christensen L, Berchtold MW and Panina S (2010). Prolactin and oestrogen synergistically regulate gene expression and proliferation of breast cancer cells. Endocr Relat Cancer. **17**: 809–22.
55 Rögelsperger O, Wlcek K, Ekmekcioglu C *et al.* (2011). Melatonin receptors, melatonin metabolizing enzymes and cyclin D1 in human breast cancer. J Recept Signal Transduct Res. **31**: 180–7.
56 Ruscica M, Arvigo M, Steffani L, Ferone D and Magni P (2012). Somatostatin, somatostatin analogs and somatostatin receptor dynamics in the biology of cancer progression. Curr Mol Med. **13**(4): 555–71.
57 Sackett DL *et al.* (1996). "Evidence based medicine: what it is and what it isn't". BMJ. **312**(7023): 71–2.
58 Salvatori L, Ravenna L, Caporuscio F, *et al.* (2011). Action of retinoic acid receptor on EGFR gene transactivation and breast cancer cell proliferation: Interplay with the estrogen receptor. Biomed Pharmacother. **65**: 307–12.
59 Sanchez-Barcelo EJ, Mediavilla MD, Alonso-Gonzalez C and Reiter RJ (2012). Melatonin uses in oncology: breast cancer prevention and reduction of the side effects of chemotherapy and radiation. Expert Opin Investig Drugs. **21**: 819–31.
60 Sanchez-Barcelo EJ, Mediavilla MD, Alonso-Gonzalez C and Rueda N (2012). Breast cancer therapy based on melatonin. Recent Pat Endocr Metab Immune Drug Discov. **6**: 108–16.
61 Seitz S, Buchholz S, Schally AV *et al.* (2013). Targeting triple-negative breast cancer through the somatostatin receptor with the new cytotoxic somatostatin analogue AN-162 (AEZS-124). Anticancer Drugs. **24**: 150–157.
62 Schally AV, Comaru-Schally AM, Nagy A *et al.* (2001). Hypothalamic hormones and cancer. Front Neuroendocrinol **22**: 248–91.
63 Schally AV and Nagy A (2003). New approaches to treatment of various cancers based on cytotoxic analogs of LHRH, somatostatin and bombesin. Life Sci. **72**: 2305–20.
64 Schally AV (2008). New approaches to the therapy of various tumors based on peptide analogues. Horm Metab Res. **40**: 315–22.

65 Siriwardana G, Bradford A, Coy D and Zeitler P (2006). Autocrine/paracrine regulation of breast cancer cell proliferation by growth hormone releasing hormone via Ras, Raf, and mitogen-activated protein kinase. Mol Endocrinol. **20**: 2010–9.
66 Smith J, Axelrod D, Singh B and Kleinberg D (2011). Prevention of breast cancer: the case for studying inhibition of IGF-1 actions. Ann Oncol. **22**: i50–2.
67 Sogno I, Venè R, Sapienza C, Ferrari N, Tosetti F, Albini A (2009). Anti-angiogenic properties of chemopreventive drugs: fenretinide as a prototype. Recent Results Cancer Res. **181**: 71–6.
68 Suhail N, Bilal N, Khan HY *et al.* (2012). Effect of vitamins C and E on antioxidant status of breast- cancer patients undergoing chemotherapy. J Clin Pharm Ther. **37**: 22–6.
69 Sun Y, Campisi J, Higano C *et al.* (2012). Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. Nat. Med. **8**: 1359–1368.
70 Tang XH and Gudas LJ (2011). Retinoids, retinoic acid receptors, and cancer. Annu Rev Pathol. **6**: 345–64.
71 Tyzzer EE (1913). Factors in the production and growth of tumor metastases. Cancer Res. Clin. Oncol. **13**: 546–547.
72 Tejeda M, Gaál D, Hullán L, Hegymegi-Barakonyi B and Kéri G (2006). Evaluation of the antitumor efficacy of the Somatostatin structural derivative TT-232 on different tumor models. Anticancer Res. **26**: 3477–83.
73 Todisco M, Casaccia P and Rossi N (2001). Cyclophosphamide plus somatostatin, bromocriptin, retinoids, melatonin and ACTH in the treatment of low-grade non-Hodgkin's lymphomas at advanced stage: results of a phase II trial: Cancer Biother Radiopharm **16**: 171–7.
74 Voss MJ, Möller MF, Powe DG *et al.* (2011). Luminal and basal-like breast cancer cells show increased migration induced by hypoxia, mediated by an autocrine mechanism. BMC Cancer. **2**: 158.
75 Wang J, Xiao X, Zhang Y *et al.* (2012). Simultaneous modulation of COX-2, p300, Akt, and Apaf-1 signaling by melatonin to inhibit proliferation and induce apoptosis in breast cancer cells. J Pineal Res. **53**: 77–90.
76 Watt HL, Kharmate GD and Kumar U (2009). Somatostatin receptors 1 and 5 heterodimerize with epidermal growth factor receptor: agonist-dependent modulation of the downstream MAPK signalling pathway in breast cancer cells. Cell Signal. **21**: 428–39.
77 Watt HL, Kharmate G and Kumar U (2008). Biology of somatostatin in breast cancer. Mol Cell Endocrinol. **286**: 251–61.
78 Wu ZS. *et al.* (2011). Tumor expression of human growth hormone and human prolactin predict a worse survival outcome in patients with mammary or endometrial carcinoma. J Clin Endocrinol Metab. **96**: E1619–29.
79 Xu J, Sun D, Jiang J, Deng L, Zhang Y *et al.* (2012). The Role of Prolactin Receptor in GH Signaling in Breast Cancer Cells. Mol Endocrinol. **27**(2): 266–79.
80 Zhang Y, Zhang H, Wang X *et al.* (2012). The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles. Biomaterials. **33**: 679–91.
81 Zheng RH, Guan XX, Zhang XP, *et al.* (2010). [The correlation between the expression of PRL-R and ER/PR in breast cancer]. Nan Fang Yi Ke Da Xue Xue Bao. **30**: 596–8.