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

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

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 molecules 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).

MATERIAL 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.

CONCLUSIONS: 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 multitherapeutic 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 (D2R) 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, 1453 488.372 IU), axerophtol palmitate (909000IU) and beta-carotene (3 334000 IU) solubilized in alfa tocopheryl acetate (1×10^6 IU); an oral dose of dihydro-cholesterol (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 = 4×10^6 IU) in a glass of water at midday and in the evening with the main 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

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**Flow chart: patient enrolment criteria.**
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(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.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Chemical Information</th>
<th>Dosage</th>
<th>Route of administration</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatostatin</td>
<td>14 aa peptide</td>
<td>3 mg</td>
<td>subcutaneous</td>
<td>Daily (at night, 12 hours infusion)</td>
</tr>
<tr>
<td>Octreotide LAR</td>
<td>Octreotide Acetate 8 aa</td>
<td>20 mg</td>
<td>Intramusculus</td>
<td>Every 20 days</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Melatonin</td>
<td>12%</td>
<td>70–100 mg</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
<td>51%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>37%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinoids</td>
<td>All-Trans-Retinoic acid</td>
<td>0.5 g</td>
<td></td>
<td>Daily (3 times)</td>
</tr>
<tr>
<td></td>
<td>Axerofole-Palmitate</td>
<td>1.453488.372 IU</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beta-Carotene</td>
<td>0.5 g</td>
<td>909000 IU</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alfa Tocopherol Acetate</td>
<td>2g</td>
<td>3334000 IU</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>L-Ascorbic Acid</td>
<td>2–4 g</td>
<td>per os</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40x10^3–80x10^3 IU</td>
<td></td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>1,25-dioH-Tachysterol</td>
<td>15200 IU</td>
<td>per os</td>
<td>Daily (3 times)</td>
</tr>
<tr>
<td>Synachten</td>
<td>Tetracosactide Acetate (ACTH)</td>
<td>1 mg</td>
<td>intramuscular</td>
<td>Once a week</td>
</tr>
<tr>
<td>PARLODEL⁺</td>
<td>Bromocriptine</td>
<td>2.5 mg</td>
<td>per os</td>
<td>Daily</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOSTINEX⁺</td>
<td>Cabergoline</td>
<td>0.5 mg</td>
<td>per os</td>
<td>Twice a week</td>
</tr>
<tr>
<td>ENDOXAN⁺</td>
<td>Cyclophosphamide</td>
<td>50 mg</td>
<td>per os</td>
<td>Daily</td>
</tr>
<tr>
<td>Calcium</td>
<td>Lactate gluconate + carbonate</td>
<td>2 g</td>
<td>per os</td>
<td>Daily</td>
</tr>
<tr>
<td>Oestrogens Inhibitors</td>
<td>Anastrozole/exemestane</td>
<td>1 mg</td>
<td>Parenteral</td>
<td>Monthly</td>
</tr>
<tr>
<td></td>
<td>Triptoreline/Leuprolexin acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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 IIC, 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).

**Safety evaluation**

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

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

<table>
<thead>
<tr>
<th>Median baseline in situ Ductal carcinoma.</th>
<th>51</th>
<th>30</th>
<th>73</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min</td>
<td>Max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOG (PS)</td>
<td>Grade 0</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Grade 1</td>
<td>7</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>5</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>5</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Histotype</td>
<td>IDC</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>DCIS</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Molecular Profile**</td>
<td>PR+ (&gt;80%)</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>ER+ (&gt;50%)</td>
<td>7</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Her/neu+</td>
<td>5</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>n.a**</td>
<td>8</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Histology Grade</td>
<td>G1</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>G2</td>
<td>7</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>5</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>n.a</td>
<td>5</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>Staging</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>I</td>
<td>2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>IICC</td>
<td>2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Secondary lesions</td>
<td>Lung</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Bone</td>
<td>3</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Lymphnode</td>
<td>6</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

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

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**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=12).**

**Fig. 3. Global cumulative overall survival of locally advanced/metastastic breast cancer cases (Group B; N=8).** * Two (2) patients died.
delay or temporary discontinuation of the treatment
due to toxicity was necessary in patients with leuko-
penia (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 investiga-
tions clearly indicate that some pituitary hormones,
including the Growth Hormone (GH) and Prolactin
(PRL), have a crucial role in the development and pro-
gression 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; Rac-
curt et al. 2002). While on one hand the ubiquitious
expression of PRL and GH represents a clear com-
firmation of the direct, generalized and synergized mito-
genic role of these hormones (Wu et al. 2011; Xu et al.
2012), the causal dose-dependent relationship between
the receptor 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 physiologi-
cal and peritumoral receptor 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 cur-
rently being studied: the most probable being mecha-
nisms of autocrine and/or paracrine signalling, on
the basis of the detection of local production of GH, In-
sulin Growth Factor I (IGF), and ES in breast cancer,
peritumoral tissues stromal area (Kaulsaly 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 influ-
ence on neoplastic biological development. In addition,
itis 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 pro-
longed 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 tumour
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), Fibro-
blast 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

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

<table>
<thead>
<tr>
<th>Resp. Rate</th>
<th>Cases</th>
<th>Abs.Fr</th>
<th>Rel. Fr. (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>11</td>
<td>55</td>
<td>34;74</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>4</td>
<td>20</td>
<td>8;41</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>2</td>
<td>10</td>
<td>3;30</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>3</td>
<td>15</td>
<td>5;96</td>
<td></td>
</tr>
</tbody>
</table>

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

### Table 4. MDB Effectiveness in early stage Breast Cancer.

<table>
<thead>
<tr>
<th>Resp. Rate</th>
<th>Cases</th>
<th>Abs.Fr</th>
<th>Rel. Fr. (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>7</td>
<td>58</td>
<td>32;80</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>4</td>
<td>33</td>
<td>14;61</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>8</td>
<td>1;35</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>0</td>
<td>//</td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Resp. rate</th>
<th>Cases</th>
<th>Abs.Fr</th>
<th>Rel. Fr. (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>3</td>
<td>37.5</td>
<td>14;70</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>1</td>
<td>12.5</td>
<td>2.4;47</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
<td>12.5</td>
<td>2.4;47</td>
<td></td>
</tr>
<tr>
<td>P+</td>
<td>3</td>
<td>37.5</td>
<td>14;70</td>
<td></td>
</tr>
</tbody>
</table>

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

### Table 6. Grades II toxicities phenomena.

<table>
<thead>
<tr>
<th>Ab. Freq.</th>
<th>Rel. Freq.</th>
<th>Active molecule</th>
<th>Grade of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematologial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>7</td>
<td>35</td>
<td>Endoxan®</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
<td>5</td>
<td>25</td>
<td>somatostatin</td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drowsiness</td>
<td>8</td>
<td>40</td>
<td>Melatonin</td>
</tr>
</tbody>
</table>
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 D2-R 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. Espe- cially 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 (Projetti et al. 2011). In addition, Angiogenesis and neoangiogenesis, necessary conditions for tumour pro- gression, as well as the cascade of monocytes, the para-crine 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 compo- nents of the DBM. At the same time, the cytostatic, antiproliferative, and antimetastatic effects of Soma- tostatin are synergically increased by the other compo- nents 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 antileptic/myelosuppressive effects, this dosage induces a marked turnaround of its mechanisms of action: triggering of the mitochondrial-dependent apoptotic cascade, antiangiogenic 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-Illes 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 prolifer- ative 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-depen- dent 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 activity through their differentiating, antiproliferative, antiangiogenic 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 mortal- ity correlated to breast cancer, by inducing a systemic dissemination (Naumov et al. 2002; Demicheli et al. 1996; Fisher et al. 1989; Tytzer et al. 1916; Marie et al. 1910) and that the use of massive doses of antithec- tic 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 pro- gression 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 pro- gressive reduction and disappearance of the initial etioplastic 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) by 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 (Neangiogenesis-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 angiogenic synergy 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 multi-therapy 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.

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