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Dose intensification of mitoxantrone in combination with levofolinic acid, fluorouracil, cyclophosphamide and granulocyte colony stimulating factor support in advanced untreated breast cancer patients. A multicentric phase II study of the Southern Italy Oncology Group

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Fifty-five consecutive patients with metastatic breast cancer (MBC) ($n = 57$) were treated with a combination of levofolinic acid (*l*-FA) 100 mg/m^2 plus 5-fluorouracil (5-FU) 340 mg/m^2 i.v. on day 1–3, cyclophosphamide (CTX) 600 mg/m^2 i.v. on day 1 and mitoxantrone (DHAD) 12 mg/m^2 i.v. on day 1. DHAD dose was progressively escalated by 2 mg/m^2/cycle up to 18 mg/m^2 in the absence of dose-limiting toxicities. Granulocyte colony stimulating factor (G-CSF) was given s.c. in order to prevent neutropenia. DHAD dosage could be increased to 18 mg/m^2 in 66 out of 317 cycles of chemotherapy (21%). In most patients the dose-limiting toxicity was represented by myelosuppresion. A statistically significant correlation was found between median white blood cell (WBC) or absolute neutrophil count (ANC) nadir and DHAD dose level. Moreover, a statistically significant correlation was observed between the number of chemotherapeutic cycles, nadir ANC and WBC, and the occurrence of anemia and thrombocytopenia of increasing severity. These data suggest the occurrence of progressive cumulative bone marrow toxicity. Although patients who reached different DHAD levels showed differences in mean dose intensity, such differences were not statistically significant. No correlation was found between the increase in dose intensity and type, rate or duration of objective responses. In patients with metastatic breast cancer the overall response rate was 72% (95% CL 57–84%) with a 18% complete response rate. Median duration of response was 12 and 11 months, respectively, for complete and partial responses. Projected median survival of the whole series of patients with MBC was 18 months. These data demonstrate that the combination of 5-FU with *l*-FA, CTX and DHAD is very active against MBC. G-CSF use allows the increase DHAD dosage up to 18 mg/m^2/cycle, but its use may be linked to the occurrence of sometimes severe cumulative hematological toxicity.

Key words: Breast cancer, cyclophosphamide, 5-fluorouracil, granulocyte colony stimulating factor, levofolinic acid, mitoxantrone.

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Introduction

Despite the proven magnitude of benefit of adjuvant systemic therapy in reducing the risk of recurrence,[1] a significant number of patients with early stage breast cancer will develop metastatic disease and ultimately die of advanced breast cancer.

Although metastatic breast cancer (MBC) is a chemosensitive disease with response rates ranging from 40 to 75%, it still must be considered a non-curable illness and the role played by systemic chemotherapy is still palliative.[2] Cure rates have plateaued in the last two decades, so that the need for new chemotherapeutic regimens able to maximize antineoplastic activity while limiting side effects has led investigators to look for new therapeutic strategies. Among these new approaches are the testing of new drugs, biochemical modulation of already known drugs and the use of increased amounts of these drugs. This latter aim can be achieved because of the recent availability of human recombinant hematopoietic growth factors in the clinic. In fact a number of controlled clinical studies have demonstrated that granulocyte colony stimulat-

ing factor (G-CSF) is able to significantly reduce the incidence and the degree of life-threating neutropenia following chemotherapy and therefore lower the frequency of infections.[3–5]

Among antineoplastic agents recently employed in clinical practice, mitoxantrone (DHAD), an antraquinone drug, has demonstrated a good therapeutic efficacy in MBC. Moreover it has been reported to be less cardiotoxic than anthracyclines in experimental and clinical studies.[6,7] The mechanism of action of DHAD, even though not completely known, consists of its capacity to interact with DNA and RNA, thereby inhibiting the synthesis of nucleic acids. Inhibition of RNA synthesis is responsible for blocking the transcription and the activation of cytoplasmatic and nuclear RNA. When employed as a single agent, DHAD has been reported to yield a 30–35% overall response rate in previously untreated patients with MBC.[9,11,12] In prospective randomized studies comparing DHAD to anthracyclines, DHAD has shown a clinical activity only slightly lower than that reported for anthracyclines,[8,9] but with a lower incidence of alopecia, nausea/vomiting and cardiotoxicity.[8,10]

Based on encouraging results achieved in colorectal cancer, the association between 5-fluorouracil (5-FU) and levofolinic acid (*l*-FA) was also tested in MBC, and Margolin *et al.* reported a 36% objective response rate among 45 patients with a good tolerability.[13] These interesting results led some investigators to test *l*-FA + 5-FU in combination with DHAD. The combination of 5-FU + *l*-FA and DHAD achieved very interesting results as reported by Hainsworth *et al.*[14] and Jones *et al.*[15] with a 65 and 45% objective response rate, respectively, achieved in 31 and 53 patients with MBC. In these studies DHAD was employed at doses of 12 and 10 mg/m^2, respectively.

In view of these considerations, in September 1993 we started a multi-institutional phase II study with the association of cyclophosphamide (CTX), biochemically modulated by folinic acid and escalating doses of DHAD with G-CSF support.

Patients and methods

Elegibility criteria

Elegibility requirements included: histologically proven MBC; measurable lesions according to the WHO criteria[16] located outside of previous irradiation fields; age between 18 and 65 years; Karnofsky performance status greater than 60, life expectancy greater than 3 months; blood cell counts and blood chemistries within normal limits; left ventricular ejection fraction (LVEF), measured by cardiac ultrasound, not more than 10% below the normal limit of the participating Institutions. Patients had also to be willing to participate in the trial and an informed consent form had to be signed.

Patients were not included if they had a past or current history of malignant neoplasm other than MBC except for basal cell skin cancer or curatively treated *in situ* carcinoma of the cervix. Clinically detectable CNS involvement, a history of pre-existing heart disease, determined by clinical signs of cardiac failure and/or coronary artery disease, left ventricular hypertrophy, severe pleural effusion, ascites or the presence of blastic bone lesions as the only site of disease were all considered as exclusion criteria.

Previous adjuvant chemotherapy, except for anthracyclines or anthrachinonic containing regimens, was accepted but the treatment had been withdrawn at least 12 months before entry into the study. Hormonal therapies were also permitted, but they had to be discontinued at least 4 weeks prior to entry into the study.

Pretreatment staging procedures

Before entry into the study all patients had to have a complete history, physical examination, ECG and cardiac ultrasonography to determine LVEF. The staging procedures to assess the extent of disease included chest X-ray, computed tomography (CT) of the thorax, ultrasound or CT of the abdomen and isotopic ^{99}Tc bone scan. Before each course of chemotherapy a full physical examination was carried out, including a complete blood count and a serum chemistry screening. Cardiac function was assessed at baseline and then after two cycles with ECG and cardiac sonogram.

Schedule and treatment plan

The chemotherapy regimen consisted of *l*-FA 100 mg/m^2, 5-FU 340 mg/m^2 both administered i.v. on day 1–3; CTX 600 mg/m^2 and DHAD 12 mg/m^2 both administered i.v. on day 1. G-CSF 5 μg/kg was given s.c. for eight consecutive days starting from day 5 of each course. The planned cycle length was 21 days if neutrophils were greater than 2000/ml and platelets were greater than 100 000/ml, with the possibility to shorten the intercycle time and thus to

recycle earlier if hematological parameters make it possible. If hematological parameters did not reach adequate levels on time, treatment was delayed until day 28. Patients without a complete hematological recovery after 2 weeks of delay were withdrawn from study. Interim blood cells count was performed between days 10 and 12 of each cycle in order to evaluate a dose escalation of DHAD in the subsequent cycle by one dose level (2 mg/m^2) until the maximal dosage of 18 mg/m^2 was reached. DHAD dosage was not further increased if one of the following occurred: nadir grade 4 leukopenia with absolute neutrophil count (ANC) less than 500/ml and/or grade 3 thrombocytopenia, and/or more than 20% decrease of previous cycle hemoglobin value or any extra-hematological toxicities greater than grade 3, except for alopecia. Treatment was discontinued if LVEF fell more than 15% below the patient's own baseline during treatment.

Objective response (OR) evaluation was carried out according to the WHO criteria,[16] so that complete response (CR) was defined as the total disappearance of all clinical, radiological and laboratory evidence of disease for at least 4 weeks. Partial response (PR) was defined as a 50% or greater reduction in the sum of the product of the largest perpendicular diameters of indicator lesions for at least 4 weeks. Stable disease (SD) was defined as a less than 50% decrease or a less than 25% increase in the size of tumoral deposits and progressive disease (PD) was defined as a greater than 25% increase in the size of indicator lesions or the appearance of any new lesion.

Patients were first evaluated after three cycles of chemotherapy. However, patients with (PD) or death before completion of the third cycle were considered as treatment failure (PD) according to an intent-to-treat analysis. Response duration was calculated from the start of chemotherapy until the date when progression was observed clinically, or last follow-up evaluation or death. Survival was calculated from the first day of chemotherapy to the time of death or last follow-up. Dose intensity was calculated dividing the amount of drug (mg/mq) by the time expressed in weeks between the first and the last day of chemotherapy administration.[17]

The duration of chemotherapy we used depended on the type of objective response achieved after the first three cycles: the treatment was stopped in case of PD, while in the presence of PR or SD chemotherapy was continued up to six cycles and stopped if a CR was recorded; if after six cycles a PR was recorded, other two cycles of therapy were administered.

Statistical analysis

All data were centrally collected at the Oncology Institute of Bari. Objective response were evaluated as relative rates with their 95% confidence limits (95% CL). A univariate analysis of survival data according to the product-limit estimate (Kaplan–Meier) was performed. Comparisons in survival distribution were made by the log-rank test. The Kruskall–Wallis test was used to test the hypothesis that the time to progression was equal in the response group; the Matched Sample Sign Test was performed to evaluate the equality of medians.

Results

Patients characteristics

From September 1993 and August 1995, 55 women entered into the study. The main demographic and clinical characteristics of enrolled patients are given in Table 1. The median age of the patients was 55 years and 38 patients were postmenopausal. Thirty-eight patients (69%) had dominant visceral metastatic disease and 17 patients had extra-visceral dominant disease. There were also three patients affected by inflammatory breast cancer. Thirty-six patients (65%) had disease spread in more than one site. Hormonal receptor status was positive in 18 patients, negative in 12 patients and unknown in the remaining 25 patients. Pretreatment included sur-

Table 1. Patient characteristics

Patient characteristics	Number (%)
Enrolled	55
Evaluable	50
Median age (range)	55 (29–71)
Median PS	90
Menopausal status	
pre	17 (31)
post	38 (69)
Hormonal receptors	
positive	18 (33)
negative	12 (22)
unknown	25 (45)
Dominant site of disease	
viscera	38 (69)
bone	05 (9)
soft tissue	09 (16)
inflammatory breast cancer	03 (6)
Number of involved sites	
single	19 (35)
multiple	36 (65)

gery in all cases, adjuvant chemotherapy in 26 patients (47%) and hormonotherapy in 22 patients (40%).

Clinical efficacy

Fifty patients were fully evaluable for response: one patient was left out because of hematological toxicities after two cycles, while four patients refused to continue the treatment after one (three patients) and two (one patient) cycles of therapy and were lost to follow-up. Nevertheless, all five patients were included in an intent-to-treat analysis and considered as PD.

When only fully evaluable patients were considered, the overall response rate (ORR) was 72% (95% CI 57–84%). Nine patients achieved a CR (18%), 27 patients showed a PR (54%), nine cases were categorized as SD (18%) and five patients as PD (10%). According to an intent-to-treat analysis the ORR was 65% (95% CL) with a 16% CR rate.

When responses were analyzed according to the dominant site of disease (Table 2), soft tissue metastases showed the highest response rate (87%), followed by viscera (74%), while disappointing results were observed in patients with bone as the dominant site of disease (25%). Table 3 shows the response rate according single site of disease. Note the good efficacy of chemotherapy against lung and pleura metastases with 75 and 83% objective response rates, respectively.

The three patients with inflammatory breast cancer were given a mastectomy after three or four cycles with subsequent radiotherapy. Thus response duration for these patients was calculated from the first day of treatment until the date of locoregional treatment. All the three patients are alive at 17+, 28+ and 35+ months since surgery. The first of these patients developed pleural effusion with positive malignant cytology after 14 months from surgery. The median durations of CRs and PRs were 12 and 11 months, respectively.

After a median follow-up of 28 months, the median overall survival of the whole series was 18 months (Figure 1). The median survival of patients who achieved a CR was not reached, but this was 19 months for patients with PR. A favorable trend in survival was seen in the subset of patients with viscera as the dominant site of disease between CR

Table 2. Response according to dominant site of disease

Site	No. of patients	Response				CR + PR
		CR	PR	SD	PD	No. (%)
Viscera	35	06	19	07	03	25 (74)
Bone	04	—	01	02	01	01 (25)
Soft tissue	08	03	04	—	01	07 (87)
Inflammatory breast cancer	03	—	03	—	—	03 (100)

Table 3. Response according to single site of disease

Site	No. of patients	Response				CR + PR
		CR	PR	SD	PD	No. (%)
Viscera						
liver	18	2	6	8	2	8 (44)
lung	12	2	7	1	2	9 (75)
pleura	06	3	2	1	0	5 (83)
effusion	06	3	1	2	0	4 (67)
Bone	14	2	2	9	1	4 (29)
Soft tissue						
skin	02	2	0	0	0	2 (100)
breast	06	1	4	1	0	5 (83)
lymph nodes	24	12	8	3	1	20 (83)

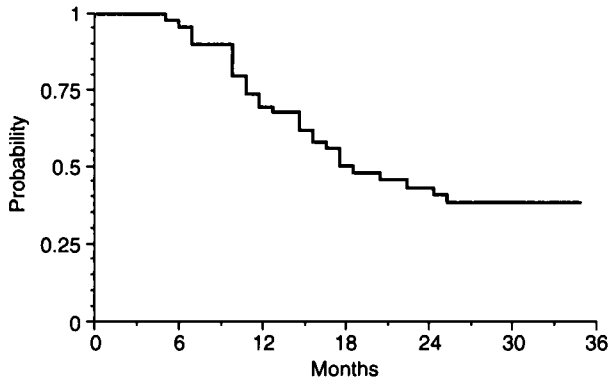

Figure 1. F-FNC Kaplan–Meier survival curve for the 50 patients (median follow-up: 28 months).

and PR patients, respectively. Until now, 30 patients have died while 20 out of 50 (40%) patients are still alive and 10 out of 36 (28%) of responding patients are still in response.

Toxicity and dose escalatation

Toxic effects, recorded according to the WHO scale both before each cycle and at nadirs, are listed in Table 4. No chemotherapy related deaths were observed.

Leukopenia, as recorded before recycling at day 21, was observed in 37 patients (67%) with only two cases of grade 3 leukopenia. At nadir grade 3–4 leukopenia was observed in 38 patients (69%). At day 21 anemia was present in 29 patients (53%) with only one case of grade 4. At nadir most patients (91%) experienced anemia of any grade and in 13 patients (24%) it was recorded as grade 3–4. In the five patients who experienced at nadir anemia grade IV, it was necessary to give blood transfusions. Grade 1–2 thrombocytopenia was seen in only seven patients at recycling. At nadir, grade 1–2 thrombocytopenia was observed in 17 patients and grade 3–4 in 10 cases (18%). Two out of four patients with grade 4 thrombocytopenia required platelet transfusions.

A PD in the median values of white blood cell, hemoglobin and platelets recorded both at nadir and before any cycle was observed as the number of chemotherapeutic cycles increased. Figures 2–4 show median values of hematological series plotted against the number of cycles of therapy. When hematological toxicity was analyzed according to the levels of DHAD dosage, a statistically significant correlation between DHAD dosages and the occurrence grade 3–4 leukopenia at nadir was observed. Grade 3–4 leukopenia occurred in 46 out of 66

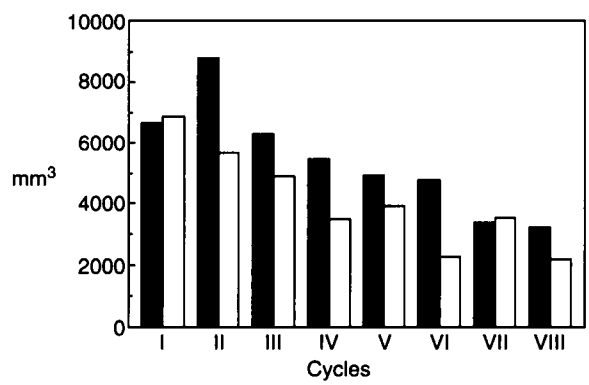

Figure 2. Toxicity: leucopenia: (■) basal and (□) nadir.

Table 4. Toxicity (WHO) (no. of patients = 55)

	I	II	III	IV	Total	III–IV
					No. (%)	No. (%)
Leucopenia						
basal	19	16	2	—	37 (67)	2 (4)
nadir	3	7	16	22	48 (87)	38 (69)
Anemia						
basal	22	6	—	1	29 (16)	1 (2)
nadir	28	9	6	3	46 (84)	9 (16)
Thrombocytopenia						
basal	6	1	—	—	7 (13)	—
nadir	13	4	6	4	27 (49)	10 (18)
Mucositis	13	10	4	—	27 (49)	4 (7)
Nausea/vomiting	18	21	2	—	41 (75)	2 (4)
Hair loss	13	21	8	—	42 (76)	8 (15)

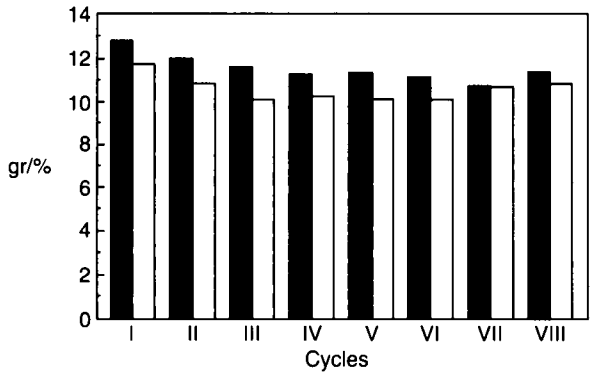

Figure 3. Toxicity: anemia: (■) basal and (□) nadir.

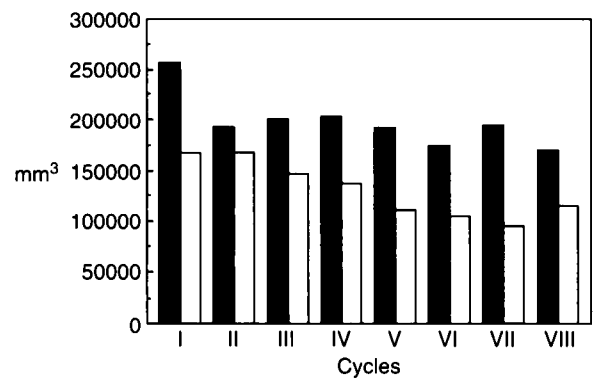

Figure 4. Toxicity: thrombocytopenia: (■) basal and (□) nadir.

(70%) cycles in which DHAD was administered at 18 mg/m^2 against 18 out of 90 cycles (20%) with DHAD 12 mg/m^2 ($p < 0.0001$). The same finding was also seen for grade 1–2 anemia that occurred in 51 out of 66 (77%) cycles at the maximum dosage and in 33 out of 90 (37%) cycles of the starting dosage of DHAD ($p < 0.0001$).

Nausea/vomiting and stomatitis were generally mild. Nausea/vomiting was recorded in 41 patients (75%) with only two patients showing grade 3 (4%). Stomatitis was observed in 27 patients (49%) with grade 3 in only 7% of cases. No substantial differences in the frequence of nausea/vomiting and stomatitis were detected as DHAD dosage increased. Hair loss affected 42 patients, but only in eight patients it was of grade 3. Only one case of grade 1 cardiac toxicity was recorded: it occurred in a patient with inflammatory breast cancer who underwent, after four cycles of chemotherapy, radical mastectomy plus radiotherapy on the chest wall.

In total, 317 cycles were administered: in 221 cycles (70%) DHAD dosage could be increased (12 mg/m^2 = 90 cycles, 14 mg/m^2 = 80 cycles, 16 mg/m^2 = 73 cycles, 18 mg/m^2 = 66 cycles). In the remaining eight cycles DHAD was reduced at 10 mg/m^2 because of nadir grade 3–4 leukopenia (six cases), grade 4 anemia (one case) or persistant grade 3 stomatitis (one case). In 49 patients (89%) DHAD dosage was increased, while the time interval between courses could be reduced in 38 patients (69%) for a total of 77 cycles (24%). The median duration of interval among courses was 23.6 days. Statistical analysis performed in an attempt to correlate response rate with DHAD dose intensity was not significant. The median cumulative dosage of DHAD was 90.48 mg/m^2; median dose intensity of DHAD was 5.32 mg/m^2/week, which represents a 34% increase over the starting dosage.

Discussion

Hortobagy,[18] reviewing the results of seven randomized clinical trials of MBC to evaluate the relationship between dose, response rate and survival, could not reach a definitive conclusion. In fact, in four of these trials there was a substantial difference in favor of higher dose, although this reflected a survival advantage in only two studies. By contrast, in a similar retrospective analysis of chemotherapy in patients with metastatic disease, Hryniuk and Bush[19] demonstrated a relationship between dose intensity and treatment outcome.

Because of its documented activity as a single agent and its comparable efficacy to anthracyclines, DHAD was used in several combination chemotherapeutic regimens and recently also in dose-escalation studies with colony stimulating factors as rescue from hematological toxicity.[20–23] In a dose-finding study in which DHAD, in combination with 5-FU + *l*-FA, was progressively increased with s.c. G-CSF support, the maximum tolerated dose of DHAD was 24 mg/m^2.[23] In this study the dose-limiting toxicity of DHAD was represented by myelosuppression and a response rate of 62% was observed in 21 evaluable patients with three patients showing a CR. However, collected data from several studies[24–28] with the F-FNC combination with fixed doses of DHAD (12 mg/m^2) showed 82 objective responses in 159 patients for an ORR of 52%, with a median duration of response ranging from 5 to 13 months.

The purpose of this phase II multicenter study was to find an effective and safe combination chemotherapy able to achieve a higher response rate, longer disease control and prolonged survival in patients with MBC. The ORR achieved in this

multi-institutional phase II study was 72% (95% CL 57–84%) with a median duration of CR and PR of 12 and 11 months, respectively. These data are in the range of activity reported in the medical literature with similar regimens. However, it should be stressed that the evaluation of a possible positive impact of higher doses of DHAD on response rate was not in the aims of this non-comparative phase II trial.

In a previous study,[29] that differed from the present one only in the substitution of DHAD with epirubicin, we achieved an ORR of 72% in a series of 61 evaluable patients with 14 (23%) CRs. Median duration of response was 10 months and 13 months, respectively, for CRs and PRs, while, after a median follow-up of 18 months, projected median survival for the whole series of patients with MBC was 20 months. Myelosuppression was the most frequent side effect with a significant decrease in median WBC levels as the number of cycles increased. With regard to cardiotoxicity there was one case of myocardial infarction and one case of a 25% fall in LVEF, and three patients experimented a 10% reduction of LVEF. All patients had grade III hair loss. It is thus clear that no statistical significant differences in response rate, duration of objective response and toxicity are present between this study and the latter one. On the other hand, the present study seems to show a favorable trend in survival for responder patients, especially for complete responders in the setting of patients with viscera as the dominant site of disease.

In this study we employed *r*-methyl-G-CSF for 8 days instead of 13 days as we did in the past trial. Despite the reduction in the number of days of G-CSF treatment we were able to increase the dose of mitoxantrone. Regarding the clinical use of G-CSF, although this could ameliorate chemotherapy induced bone marrow toxicity, leukocytopenia and anemia increased according to DHAD dose escalation and from cycle 1 to 6 within each given dose level. This finding, however, did not compromise the possibility to administer increased doses of DHAD safely even on an outpatient clinic setting. Comparing the two studies, the conclusions are that the F-FNC combination had an equally efficacy as the F-FEC regimen but with less incidence of cardiotoxicity, hair loss and mucositis.

In conclusion, the dose-limiting toxicity of DHAD in combination with CTX and 5-FU + *l*-FA is myelosuppression. The above reported results suggest that the use of s.c. G-CSF is effective in allowing the use of higher doses of DHAD in such combination regimens in a significative fraction of patients. However, intensive chemotherapy with G-CSF support may sometimes cause severe cumulative toxicity, i.e. myelosuppresion. Our data support the use of high-dose DHAD in combination with CTX and 5-FU + *l*-FA with G-CSF support but with a careful follow-up. Despite the fact that this combination regimen is certainly very active against MBC, its routine use cannot be recommended due to its significant cost and the incidence and severity of side effects.

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