Multicenter phase II study of chemoimmunotherapy in the treatment of metastatic melanoma

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Combining chemotherapy and immunotherapeutic agents such as interleukin-2 and interferon α-2b might improve treatment results in metastatic melanoma (MM) patients compared with chemotherapy alone. This prospective study evaluated the potential efficacy of a biochemotherapy regimen followed by maintenance biotherapy for the treatment of MM. Twenty-two patients with stage IV melanoma were treated for 5 consecutive days with cisplatin at 20 mg/m^2, vinblastine at 1.6 mg/m^2, and dacarbazine at 160 mg/m^2. Pegylated interferon α-2b at a dose of 50 μg every week, subcutaneous interleukin-2, 1.8 MIU, and oral 13-*cis*-retinoic acid (13-*cis*-RA) at 0.5 mg/kg were given 5 days/week for 3 weeks each month during the period of chemotherapy administration. Maintenance biotherapy was continued in patients who had a complete or partial response or disease stability (clinical benefit) after six courses of biochemotherapy. The primary endpoint was response; secondary endpoints were the evaluation of the immunologic parameters, toxicity, progression-free survival, and overall survival. Twelve patients (54.5%) achieved a response, and seven (31.8%) maintained stable disease for at least 6 months with maintenance biotherapy. The median progression-free survival and overall survival were 23.3 and 45.7 months, respectively. The most important toxicities from chemotherapy were grades 3 and 4 neutropenia and thrombocytopenia in 41 and 18% of patients, respectively, whereas grade 2 autoimmune reactions were observed in 21% of patients after maintenance biotherapy. A prolonged enhancement of immunologic function was observed in the 19 patients treated with maintenance therapy. A regimen of six cycles of biochemotherapy followed by maintenance immunotherapy is well tolerated, and shows significant activity in patients with MM. *Anti-Cancer Drugs* 19:201–207

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Introduction

Although early diagnosis and appropriate surgery for malignant cutaneous melanoma provide high cure rates, therapeutic possibilities after the disease becomes metastatic are limited.

In the last three decades, little progress has been made in the systemic treatment of melanoma [1]. Sporadic cures for advanced melanoma with immunotherapy using high-dose interleukin-2 (IL-2) have been described [2]. The addition of biotherapy to standard dacarbazine-based chemotherapy has improved the clinical outcome, but at the cost of significant toxicity [3]. Metastatic melanoma (MM) is, however, often characterized by an unpredictable clinical outcome. Often cellular-mediated immunity is not sufficient to control the disease for a number of reasons.

The tumor can elude the host immune surveillance, especially if a state of tolerance versus the tumor is induced [4,5]. Tolerance is increased by the presence of immature myeloid cells that are frequently seen in patients with MM [6]. In addition, an impaired function of the major histocompatibility complex class I antigen-processing and presentation pathway is observed in melanoma patients [7].

IL-2 promotes T-cell proliferation, the generation of cytotoxic T-lymphocytes (CTLs), the activation of T-lymphocytes and B-lymphocytes, and enhances the activity of natural killer cells (NK) [8]. Maintenance of self-tolerance through the termination of T-cell responses is another recognized role of IL-2 [9]. Indeed, prevention of autoimmunity and limiting of inflammation is achieved through the amplification of T-regulatory cells (T_{reg}) [10].

An overview of randomized trials has shown that better response rates and prolonged survival can be achieved in MM with regimens that include α-interferon (IFN-α) therapy [11]. A refinement in the immunotherapeutic armamentarium is the pegylated form of IFN-α (PEG-IFN). With its distinct pharmacokinetic advantages, this drug can be given on a convenient weekly schedule, and its activity is similar to that of conventional IFN-α

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monochemotherapy in stage IV melanoma [12]. From preclinical data, there is evidence that combined treatment with IFN-α and retinoids can result in enhanced antiproliferative and differentiative effects compared with either single agent alone [13,14]. Moreover, retinoids facilitate the differentiation of immature myeloid-suppressor cells ($Gr\text{-}1^+CD115^+$), which are responsible for the development of tumor-induced T-cell anergy in tumor-bearing hosts [15], with an improvement of the immune response [16,17].

In a previous study [18], we demonstrated that in the tumor-bearing host, the combination of low-dose IL-2 and 13-*cis*-retinoic acid (13-*cis*-RA) enhanced some of the prognostically relevant immunologic parameters such as lymphocyte and NK cell counts, as well as the $CD4^+/CD8^+$ ratio. This improvement led to a substantial advantage in progression-free survival (PFS) and overall survival (OS), with respect to historical controls, in patients with metastatic renal cell carcinoma and other tumor types [19–22].

The aim of this study was to evaluate the efficacy and toxicity of a chemoimmunotherapy regimen for the treatment of patients with MM. Chemotherapy was given in association with biotherapy (IL-2, 13-*cis*-RA, and PEG-IFN), which was continued in patients who achieved clinical benefit from the treatment.

Methods

Patient eligibility

Patients were eligible if they had histologically confirmed MM, along with the following characteristics: (i) if they were chemotherapy-naive, (ii) had at least one measurable lesion, (iii) a good Eastern Cooperative Oncology Group performance status (2), (iv) age above 18 years, (v) life expectancy of 3 months or more, and (vi) no concurrent medical illnesses. Previous adjuvant IFN therapy was allowed. Patients were required to have a satisfactory baseline bone marrow function, hepatic function, and renal function. Patients with malignancies other than curatively treated skin and cervical cancers, with severe comorbid conditions, or who lacked the ability to comply with the protocol requirements, were excluded. This phase II study, conducted in accordance with the Declaration of Helsinki and the European Union Guidelines on Good Clinical Practice, was approved by each local ethics committee, and written informed consent was obtained from each patient.

Pretreatment evaluations

A medical history survey, clinical examination, complete blood cell count, assessments of plasma urea and creatinine levels, electrolyte measurements, liver function tests, and lactate dehydrogenase measurements were carried out before treatment. Electrocardiogram, computed tomographic scans of the chest, abdomen, and brain, and radiographs of the abnormal areas of the bone scan uptake were also carried out, within the 1 month preceding the chemotherapy. Before each successive course of treatment, all patients had plasma urea, electrolytes, serum creatinine, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, and bilirubin measurements taken. In addition, a blood cell count was repeated weekly. Peripheral blood samples for immunologic study were drawn from all patients at baseline and before each cycle of immunotherapy. Counts of NK cells, lymphocytes, $CD4^+$ and $CD8^+$ cells, and $CD4^+/CD8^+$ ratio determinations were obtained from whole blood.

Chemoimmunotherapy

Outpatient treatment was performed according to the following 5-day schedule, which was repeated every 4 weeks: 15-min intravenous administration of dexamethasone (20 mg) and a $5HT_3$ antagonist in 100 ml of saline, and a 1-h administration of 20-mEq KCl and 4-mEq $MgSO_4$. Vinblastine at 1.6 mg/m^2, dacarbazine at 160 mg/m^2, and cisplatin at 20 mg/m^2 were administered daily, with hydration for 5 consecutive days every 4 weeks. Antiemetic prophylaxis and antidiarrhea therapy were given according to local protocols. Granulocyte colony-stimulating factor and erythropoietin were used if a grade 4 neutropenia or grade 3 anemia resulted from the previous course of chemotherapy. After the administration of chemotherapy, patients underwent treatment with self-administered subcutaneous IL-2 at a dose of 1.8×10^6 IU daily at bedtime, 5 days/week, in addition to oral 13-*cis*-RA at 0.5 mg/kg of body weight administered with meals for 5 days/week for 3 weeks of each month [18]. PEG-IFN was given at a dose of 50 μg every week. Using primarily the lower abdomen and the upper and lower extremities, injections sites were rotated daily. Patients began a new course of chemotherapy and a 3-week course of immunotherapy after 1 week of rest.

At the conclusion of six courses of chemoimmunotherapy, patients who were not progressing continued to receive the same maintenance immunotherapy for 3 weeks each month. Immunotherapy was given for 2 weeks every month in the second year. Afterwards, therapy was continued for 5 days each month or more frequently in accordance with the immune competence of the patient. Patients exhibiting evidence of disease progression were removed from the study, treated with salvage chemotherapy, and were included in the analysis on an intention-to-treat basis.

Dose modifications

If grade 3 or 4 myelosuppression occurred, drug doses were decreased by 10%; in the case of grade 3 or 4 gastrointestinal toxicity, the drugs were withheld, and

subsequent doses were decreased by 20% after the toxicity resolved. Drug doses were never increased.

Statistical considerations

The primary endpoint of the study was to assess the overall response rate (ORR). The number of patients required for the study was calculated according to a Simon optimal design [23]. The first stage required at least one or more of nine patients to have a confirmed response, to rule out an undesirably low response probability of 0.05 (P0) in favor of a desirable probability of 0.25 (P1), with a 5% probability of accepting a poor agent ($a = 0.05$) and a 20% probability of rejecting a good agent ($b = 0.20$), before proceeding to the second stage. In the second stage, 17 assessable patients could be added if a total of two or more patients showed a response, as the primary endpoint would then have been met. The results of the immunologic parameters were expressed as the mean ± standard deviation of four determinations made in three different experiments, and the differences were determined using a repeated-measures analysis of variance. Post-hoc comparisons were performed using Tukey's honestly significant difference test.

The PFS was defined as the time between the start of chemotherapy and any relapse, appearance of a second primary cancer, or death, whichever occurred first. The OS was measured from study entry to death, or December 2006 for censored patients. Statistical analyses of PFS and OS were carried out using the Kaplan–Meier method [24]. All comparisons of patient characteristics, response rates, and toxicity profiles were performed using Pearson's χ^2 contingency table analysis. The log-rank test was used to compare patients showing clinical benefit with patients who had progressive disease. Statistical analysis was performed with SAS statistical software (version 8.12, 2000; SAS Institute Inc., Cary, North Carolina, USA).

Results

Patient characteristics

Between December 2001 and December 2006, 22 consecutive patients were entered into the study. Seven patients had received an IFN-based adjuvant therapy, whereas 15 patients had stage IV disease at presentation. The median Eastern Cooperative Oncology Group performance status was 1. All patients who had received at least two cycles of chemoimmunotherapy were assessable for the safety analysis, response, PFS, and OS. After a median follow-up of 41.8 months (minimum 14.5 months), the 22 evaluable patients had received 112 cycles of chemotherapy (median of five cycles per patient; range 3–6 cycles). In contrast, 185 courses of immunotherapy (median of 9.7 cycles per patient; range 2–21 cycles) had been administered to the 19 patients who had obtained a clinical benefit. Patient characteristics are listed in Table 1. Six patients had two or more metastatic sites. The location of the metastatic sites included soft tissue (41%), liver (23%), lung (18%), and bone (14%). Nine patients, with nodal metastases, had local recurrence with lymphedema, and were not judged suitable for surgical resection. Seventeen patients had a median disease-free interval of 10 months (range 2.8–100) from primary surgery. No patient had received any form of chemotherapy or immunotherapy for metastatic disease.

Antitumor activity

Tumor response was determined by independent external radiologic review, according to Response Evaluation Criteria In Solid Tumors criteria. An ORR of 54.5% was observed [95% confidence interval (CI): 32–76%]. A complete response was observed in one patient (4%; 95% CI: 1–22%); partial responses in 11 patients (50%; 95% CI: 28–71%); disease stability in seven patients (31.8%; 95% CI: 14–55%); and progressive disease in three patients (13.6%; 95% CI: 3–35%). We observed only one complete response in the five patients with liver metastases who had survived a median period of 8 months. Disease was better controlled in the four patients with lung metastases for a median time of 16 months; whereas, 13 patients with soft tissue disease had a median survival of 41 months.

Table 1 Patient characteristics

Characteristics	No.	%
No of patients	22	100
Sex		
Male	9	41
Female	13	59
Age, years		
Median	65	
Range	30–77	
Performance status (ECOG)		
0	7	32
1	12	55
2	3	13
Site of metastases		
Regional lymph nodes	9	41
Distant skin	1	4
Bones	3	14
Lung	4	18
Liver only	5	23
Multiple visceral sites	6	27
AJCC M category		
M1a	10	45
M1b	4	18
M1c	8	36
Previous adjuvant interferon therapy		
Yes	7	32
No	15	68
PS/LDH combination		
PS = 0 and LDH < ULN	7	32
PS > 0 or LDH > ULN	9	41
PS > 0 and LDH > ULN	6	27

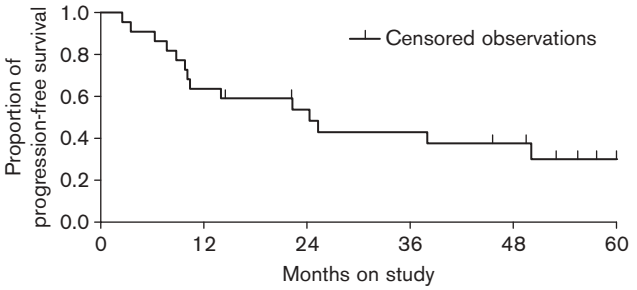
AJCC, American Joint Committee on Cancer; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PS, performance status; ULN, upper limit of normal.

Overall median PFS was 23.3 months (range 2.5–60 + months) (Fig. 1). Overall median OS was 45.7 months (range 3.8–60 + months) (Fig. 2). The 2-year survival rate was 57%.

Immunologic activity

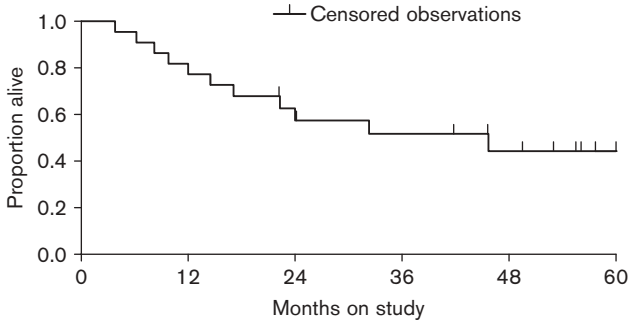
Baseline values of immunologic parameters (lymphocytes, NK, and $CD4^+/CD8^+$ ratio) were not statistically different in patients who had a clinical benefit and patients who had disease progression. A statistically significant improvement was observed in lymphocyte and NK cell counts and in the $CD4^+/CD8^+$ ratio in all 19 patients who had achieved clinical benefit from chemotherapy. In particular, lymphocytes increased by 32% after three cycles of chemobiotherapy in the 19 patients who achieved clinical benefit, but remained unchanged in patients who had disease progression. The baseline lymphocyte count of $1834 \pm 223 \times 10^9$/l, increased to a mean value of $2424 \pm 204 \times 10^9$/l ($P < 0.05$). It was impressive to see the 54%, increase in the NK cell count after the same period of time, changing from a mean value of $343 \pm 44 \times 10^9$/l to a mean value of $533 \pm 68 \times 10^9$/l, ($P < 0.0001$) (Table 2, Fig. 3). The $CD4^+/CD8^+$ ratio improved by 29% after the first 3 months of therapy. The baseline value of 1.9 ± 0.2 increased to a value of 2.5 ± 0.2 ($P < 0.05$). After 1 year of biotherapy, responding patients had no statistically significant change in the value of lymphocytes, NK counts, and the $CD4^+/CD8^+$ ratio. The three patients with disease progression had either no change or a worsening of all these immunologic parameters after 3 months of biochemotherapy.

Fig. 1



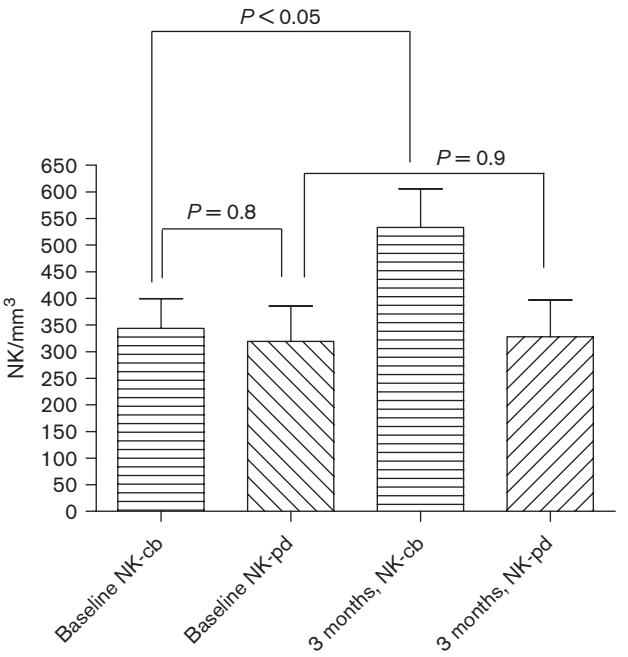
Progression-free survival (PFS). Events, 14 (52%); censored, 8 (48%); median PFS, 23.3 months (range 2.5–60 +).

Fig. 2



Overall survival (OS). Events, 11 (50%); censored, 11(50%); median OS, 45.7 months (range 3.8–60 +).

Fig. 3



NK changes. cb, clinical benefit; NK, natural killer cells; pd, disease progression.

Table 2 Immunologic changes

	Patients with a clinical benefit			Patients with progression		
	Baseline	3 months	*P* value	Baseline	3 months	*P* value
Lymphocyte/mm^3	1834 ± 223	2424 ± 204	$P<0.05$	1780 ± 194	1722 ± 270	$P=0.8$
Natural killer/mm^3	343 ± 44	533 ± 68	$P<0.05$	319 ± 66	311 ± 68	$P=0.9$
$CD4^+/CD8^+$ ratio	1.9 ± 0.2	2.5 ± 0.2	$P<0.05$	1.9 ± 0.8	1.9 ± 0.5	$P=0.8$

Toxicity

All 22 patients were assessed for toxicity (Table 3). No treatment-related deaths were observed. Grades 3–4 neutropenia occurred in nine patients (41%), grades 1 and 2 anemia in 16 patients (73%), and grade 3 or 4 thrombocytopenia in four patients (18%). Grade 2 or 3 diarrhea was seen in nine patients (41%). Grades 1 and 2 mucositis were seen in seven patients (32%). Grade 2 sensitive neuropathy occurred in three patients (14%). Grade 3 alopecia was observed in 73% of patients. The toxicity of the maintenance therapy included elevated triglycerides (twice the baseline value) in four patients (21%). Hepatic toxicity was observed with the administration of 13-*cis*-RA (low-grade abnormality of liver enzymes) in six patients (32%). Grade 1 skin toxicity occurred in 10 patients (53%). Of the patients treated with IL-2/13-*cis*-RA, 43% also had fever of grade 1 or 2. Mild hypothyroidism occurred in two patients, whereas grade 2 autoimmune reactions were observed in 21% of patients.

Discussion

Patients with MM have a short median survival of 6–12 months [25,26], with only a small minority surviving for more than 5 years [27]. High-dose IL-2 can achieve long-term control of the disease [28]. Another immunotherapeutic regimen that has been shown to be active in the treatment of metastatic melanoma is IFN-α: in fact, this biologic response modifier yields, in combination with other agents, an ORR of 24% (range, 10–46%) in untreated patients with metastatic disease [11]. The addition of chemotherapy to biotherapy has improved the outcome for poor-prognosis MM patients presenting with brain metastases, in 5% of cases: the response rates were enhanced up to 46.8% [29]. In a randomized trial of biochemotherapy versus chemotherapy alone, the combined therapy produced a slightly improved response rate and PFS, without an associated improvement in OS [30]. Vaccine therapy given to selected patients with American Joint Committee on Cancer stage IV melanoma after resection of metastatic disease can result in long-term survival [31]. The administration of the monoclonal antibody ticilimumab, which blocks CTLA-4, produces antitumor responses in patients with MM and is correlated with reductions in T_{reg} cells. Moreover, a constitutive secretion of IL-10 and an increase in IL-2 production are observed as a positive correlation between CTLA-4 and glucocorticoid-induced tumor necrosis factor receptor transcripts [32].

Even with the progress that has been made, however, the OS of patients with MM has not changed much, recently.

The objective of this study was to evaluate the efficacy and tolerability of a dacarbazine, cisplatin, or vinblastine chemotherapy regimen combined with biologic response modifiers. Chemotherapy was administered for debulking purposes. In contrast, the role of IL-2 was to increase the immune response by improving some of the prognostically relevant immunologic parameters such as lymphocyte and NK cell counts, as well as the $CD4^+/CD8^+$ ratio [18]. The function of 13-*cis*-RA was to facilitate the differentiation of immature myeloid-suppressor cells ($Gr\text{-}1^+CD115^+$), by enhancing the immune response [15]; whereas, the role of PEG-IFN was to increase the antigenic strength of the tumor-associated antigens [33].

Previous investigations have explored the use of retinoids in advanced melanoma with conflicting results. Triozzi *et al.* [34] found the combination of isotretinoin and IFN-α active and with an acceptable toxicity profile, for patients with limited tumor burden and disease confined to the skin and lymph nodes. Other investigators [35,36] who administered retinoids at higher doses found the combination of 13-*cis*-RA and IFN-α to be inactive in the treatment of metastatic melanoma and to be associated with substantial toxicity.

We also substituted the pharmacologically advantageous PEG-IFN for the 3-weekly injections of IFN-α-2b. In fact, with its distinct pharmacokinetic advantages, and with its immunomodulatory and antitumor activity in patients with MM, PEG-IFN has been shown to have a similar efficacy as nonpegylated IFNs, with similar safety and tolerability and with the advantage of less frequent administration [12,34–36]. The favorable pharmacokinetic profile of PEG-IFN, with a delayed clearance and increased area under the curve compared with native IFN-α, supports once-weekly administration.

Table 3 Toxicity according to WHO

	2		3		4	
	No.	%	No.	%	No.	%
WHO grade chemotherapy ($n=22$)						
Hematologic						
Leukopenia	3	14	5	23	2	9
Neutropenia	2	9	6	27	3	14
Thrombocytopenia	3	14	2	9	2	9
Anemia	7	32	0	0	0	0
Infection	3	14	0	0	0	0
Gastrointestinal						
Oral	4	18	0	0	0	0
Nausea and vomiting	2	9	0	0	0	0
Diarrhea	6	27	3	14	0	0
Neuropathy	3	14	0	0	0	0
Cutaneous						
Skin	5	23	0	0	0	0
Alopecia	2	9	16	73	0	0
WHO grade immunotherapy ($n=19$)						
Hepatic	2	11	0	0	0	0
Skin	2	11	0	0	0	0
Triglycerides	0	0	0	0	0	0
Fever	4	21	0	0	0	0
Hypothyroidism	0	0	0	0	0	0
Autoimmune reactions	4	21	0	0	0	0

WHO, World Health Organization.

Moreover, immunologic therapy was withheld on weekends.

In our patients who were being treated with maintenance immunotherapy, we observed a continuous improvement of established immunologic parameters such as lymphocyte and NK cells. In-vitro studies have shown that the presence of $CD4^+$ and $CD8^+$ cells is required for tumor rejection. The depletion of $CD4^+$ cells *in vivo* decreases the tumoricidal activity of $CD8^+$ cells [12,37–39]. Even if the measurement of lymphocyte subsets from peripheral blood is a rather unsophisticated method for assessing the function of the immune system, the $CD4^+/CD8^+$ ratio, nevertheless, constantly increased with the amount of administered immunotherapy (Table 2). The administration of low-dose IL-2 to patients (with minimal residual disease) who had achieved clinical benefit from chemotherapy might have the same effect as that seen in in-vitro experiments in which lymphocytes are incubated with tumor cells. Following the injection of IL-2 into the patients, the host immune-effector cells might act as lymphocyte-activated killer cells in the presence of minimal residual disease.

Additionally, it has been shown that high-dose IL-2 results in a significant decrease in T_{regs} in patients who achieve an objective clinical response to IL-2 therapy [12,37–39]; retinoids might also help in the differentiation of the $Gr\text{-}1^+CD115^+$ immature myeloid-suppressor cells to improve the immune response [15,16]. The decrease in T_{regs} was, in fact, demonstrated by the appearance of autoimmune reactions in 21% of our patients.

In general, this maintenance biotherapy regimen was well tolerated, making it suitable for prolonged administration. The most significant toxicity observed from the chemotherapy was grade 3 or 4 neutropenia in 41% of patients. In contrast, outpatient toxicities were almost exclusively of grades 1 and 2, and allowed the patients to resume their normal daily activities. With the chronic administration of biotherapy, other immune phenomena were observed (including thyroid abnormalities, the worsening of quiescent psoriasis, and the erythema nodosum syndrome) in 21% of patients, suggesting chronic immune activation.

Nine patients with disease progression were salvaged with a capecitabine-based chemotherapy administered with the same biologic response modifiers. This strategy was adopted to generate an antigen-specific immune reaction capable of sustaining prolonged antitumor activity. In fact, the antigen-specific killing ability of human CTL lines *in vitro* is not affected by 5-fluorouracil, which is the final product of the prodrug capecitabine [37–40]. Particularly good remissions were observed in three patients with bone disease.

Two thirds of the patients included in our trial belonged to the good-prognosis group, whereas only one third belonged to the poor-prognosis group. Indeed, the majority of responses were observed in patients with soft tissue or lung metastases.

We conclude that the administration of low-dose IL-2, PEG-IFN, and 13-*cis*-RA on an intermittent schedule and repeated in the long term, following chemotherapy with vinblastine, dacarbazine, and cisplatin, is feasible and has an acceptable toxicity profile. Prompted by the results of this study, we are presently planning a randomized phase III study in which patients with a clinical benefit from biochemotherapy are randomized to IL-2 + PEG-IFN and 13-*cis*-RA or observation.

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