Chemoimmunotherapy in the treatment of metastatic gastric cancer

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Docetaxel, capecitabine and 5-fluorouracil have been shown to be active in the treatment of metastatic gastric adenocarcinoma. Consistent with this finding, the aim of this study was to test this combination in a clinical trial. Forty-one patients with metastatic gastric adenocarcinoma and a median age of 64 years were recruited for the study. The treatment was based on the administration of docetaxel 60 mg/m^2 every 4 weeks, leucovorin 200 mg/m^2, 5-fluorouracil 400 mg/m^2 bolus, and capecitabine 1000 mg/m^2 twice daily on days 1 and 2 every 2 weeks. Patients achieving a clinical benefit were treated, as maintenance immunotherapy, with low-dose interleukin-2 and 13-*cis*-retinoic acid. The primary end point of this phase II study was the response rate. The secondary end points relied on the evaluation of the immunological parameters, toxicity, and progression-free survival and overall survival. The overall response rate in the 41 evaluable patients was estimated to be 49%. Median progression-free and overall survival was 9.5 and 21.1 months, respectively. Grade 3 and 4 hematological toxicities were neutropenia and thrombocytopenia in 44 and 5% of patients, respectively. A sustained improvement of evaluated immunological parameters with a negligible toxicity profile was observed in the 27 patients treated with interleukin 1-2/13-*cis*-retinoic acid. Docetaxel in combination with leucovorin, 5-fluorouracil and capecitabine followed by low-dose interleukin 1-2 and 13-*cis*-retinoic acid is well tolerated, and shows a significant activity in patients qwith metastatic gastric adenocarcinoma.
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Introduction

Stomach cancer is decreasing, and its incidence is now in the fourth place behind cancers of the lung, breast and colon [1]. Even with its declining prevalence, it remains the second most common cause of death from cancer in the world and in Italy [1].

Unfortunately, in the western world owing to the absence of screening procedures, the diagnosis of gastric cancer is usually made when the disease has reached an advanced stage. Once the disease becomes metastatic [metastatic gastric adenocarcinoma (MGC)], it is incurable and has a dismal prognosis, with a 5-year survival rate of less than 4% [2]. In recent years, even with the introduction of new compounds, the median survival time has not had a drastic improvement and continues to remain below 12 months. With the purpose of improving efficacy and tolerability, several relatively new drugs, with promising activities, such as docetaxel (D) have been investigated in the treatment of advanced gastric cancer.

Early clinical trials have confirmed the activity of single-agent D in patients with MGC with responses 17–24% in chemotherapy-naive patients [3] and 17% in pretreated patients [4].

Capecitabine (C) is an orally absorbable drug that is preferentially converted to 5-fluorouracil (5-FU) within tumors, exploiting the higher levels of thymidine phosphorylase found in areas of poor perfusion and hypoxia [5]. It is the ideal drug for ambulatory administration, because it is given orally and, mimicking the pharmacokinetics of 5-FU continuous infusion, it does not have all the inconveniences of continuous infusions. Moreover, 5-FU and C have a different pharmacokinetics profile. In fact, 5-FU given in bolus or as a continuous infusion behaves almost like two different drugs [6]: short exposure to 5-FU preferentially inhibits RNA synthesis, whereas the protracted infusion inhibits thymidilate synthase and DNA synthesis.

Clinical trials of C in combination with the taxanes have been based on the observed upregulation of thymidine phosphorylase in preclinical studies [5].

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The risk of relapse in patients with advanced epithelial tumors, even in those with a complete response to chemotherapy, is high for the presence of cancer stem cells that form what is called minimal residual disease (MRD). MRD, which is composed of tumor cells with a low proliferative rate, resistant to chemotherapy, may emerge from the dormant condition to form a new tumor mass through an 'angiogenic switch' [7].

The immune system has a profound influence on the control of regrowth of MRD and on metastatic tumor spread. T cells from the tumor-bearing host play the fundamental role in recognizing and eliminating tumor cells; failure of this mechanism owing to inhibition of signal transduction is one of the major factors of tumor escape from immune system control [8].

An additional factor of tumor escape is determined by the accumulation of immature myeloid cells that inhibit T cells' function in cancer patients [8].

Interleukin-2 (IL-2) increases T-cell proliferation and the generation of cytotoxic T lymphocytes. It also causes the activation of T and B lymphocytes, and enhances the tumoricidal activity of natural killer (NK) cells [9]. Another key function of IL-2 is the termination of T cell response and maintenance of self-tolerance [10]. In fact, as most tumor antigens are self-proteins, the immune system inhibits the generation of a robust immunity via toleragenic mechanisms, such as the elaboration of T regulatory cells (T_{reg}), which function to prevent autoimmunity and limit inflammation [11].

$Gr\text{-}1^+CD115^+$ immature myeloid suppressor cells that inhibit T cell function mediate the development of tumor-induced T regulatory cells and T cell anergy in tumor-bearing host [12].

Tumor angiogenesis also plays a key role in tumor growth and invasion [7]. High levels of serum vascular endothelial growth factor (VEGF), a marker of increased angiogenesis, correlate with tumor volume, higher relapse risk and poorer survival in patients with operable gastric cancer [13].

Retinoids, which are powerful inhibitors of angiogenesis, have shown synergistic effects with IL-2 in increasing γ-interferon production, which in turn has antiangiogenic properties [14]. Another action of retinoids that has been recently demonstrated is the facilitation in the differentiation of immature myeloid suppressor cells ($Gr\text{-}1^+CD115^+$), with improvement of the immune response [15,16].

In a previous phase IB study, we found that the optimal biological doses of IL-2 and 13-*cis*-retinoic acid (RA) [17], given as maintenance therapy in patients showing a clinical benefit (CB) from chemotherapy, were as low as 1.8×10^6 IU and 0.5 mg/kg, respectively. These low doses were free of the severe toxicity described for high-dose IL-2 [18], and they improved some of the prognostically relevant immunological parameters, such as lymphocyte and NK cell counts, as well as $CD4^+/CD8^+$ ratio and produced a sustained decrease in serum VEGF. Surprisingly, 12% of patients initiating the therapy as partial responders were converted to complete responders [19], and a significant improvement in progression-free survival (PFS) and overall survival (OS) was observed, with respect to historical controls, in patients with advanced nonsmall cell lung cancer and ovarian cancer [20,21].

The primary end point of this study was to assess the overall response rate (RR) of D in combination with leucovorin (LV), 5-FU and C. The secondary end points included the evaluation of the above-mentioned immunological parameters, toxicity, and assessments of PFS and OS to determine whether additional prospective randomized studies of this combination therapy were warranted.

Patients and methods

Patient eligibility

Patients were eligible for the study if they had histologically confirmed unresectable or metastatic adenocarcinoma of the stomach. Moreover, the patients were recruited on the manifestation of the following characteristics: chemotherapy-naive, one measurable lesion, an Eastern Cooperative Oncology Group performance status ≤ 2, age > 18 years, life expectancy ≥ 3 months and no concurrent medical illness. The patients were also required to have an adequate baseline bone marrow function (absolute neutrophil count $\geq 1\,500/\mu l$, platelet count $\geq 100\,000/\mu l$), adequate baseline hepatic function [serum bilirubin ≤ 2.0 mg/dl, transaminase (aspartate aminotransferase, alanine aminotransferase) $\leq 5\times$ the upper limit of institutional normal in the presence of liver metastases] and adequate renal function (creatinine ≤ 1.5 mg/dl). Patients with malignancies other than curatively treated skin and cervical cancer, with brain metastases, with severe comorbid conditions, or lack of the ability to comply with the requirements of the protocol, were excluded. This phase II study conducted in accordance with the Declaration of Helsinki and the EU Guidelines on Good Clinical Practice was approved by the local Ethical Committee, and written informed consent was obtained from each patient.

Pretreatment evaluations

Pretreatment evaluation included a medical history, clinical examination, complete blood cell count, assessment of plasma urea and creatinine levels, electrolyte measurement, a liver function test, serum carcinoem-

bryonic antigen and carbohydrate antigen 19.9, electrocardiogram, computed tomographic (CT) scan of the chest and upper abdomen, and radiographs of abnormal areas of bone scan uptake performed within 1 month before the start of chemotherapy. Before each subsequent course of treatment, all patients had plasma urea, electrolytes, serum creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin measurements taken. In addition, a blood cell count was repeated weekly.

Peripheral blood samples for immunological study were drawn from all of the patients at baseline and before each cycle of immunotherapy. Counts of NK cells, lymphocytes, $CD4^+$ and $CD8^+$ cells, and their ratios were obtained from whole blood.

Chemotherapy

The outpatient treatment consisted of D administered at the dose of 60 mg/m^2 as a 1-h intravenous infusion on day 1, repeated every 28 days. Premedication with dexamethasone at a dose of 8 mg was given 12, 6 and 1 h before D administration, and then twice a day for 4 days after chemotherapy. LV was administered at a dose of 200 mg/m^2 in a 2-h intravenous infusion; 5-FU was given in a bolus infusion at a dose of 400 mg/m^2 on days 1, 2, 14 and 15. C was given at a dose of 1000 mg/m^2 twice daily on days 1 and 2 and repeated on days 14 and 15. The treatment was continued for six courses, or until disease progression, unacceptable toxicity or patient's refusal. Antiemetic prophylaxis was given according to local protocols.

Immunotherapy

One month after the last course of chemotherapy, patients with a CB (complete or partial response, disease stability), assessed according to Response Evaluation Criteria For Solid Tumors [22] underwent treatment with self-administered subcutaneous IL-2 at a dosage of 1.8×10^6 IU daily at bedtime, 5 days/week, and oral RA at a dose of 0.5 mg/kg body weight, administered with meals for 5 days/week for 3 weeks each month [17]. Sites of injection were rotated daily, using primarily lower abdomen and upper and lower extremities. After 1 week of rest, patients started a new 3-week course of therapy. Two months were considered to be a single cycle of therapy. After completion of 1 year of treatment, responding patients continued to receive the same therapy as maintenance for 2 weeks each month. In the third year, therapy was continued for 5 days each month. Patients exhibiting evidence of disease progression were removed from the study and treated with a salvage chemotherapy, and were included in the analysis on an intention-to-treat principle. A salvage chemotherapy consisting of pegylated lyposomal doxorubicin as second line and mytomycin C as third line was administered to fit patients.

Dose modifications

Given that this study involved three agents, dose adjustments were made for each agent if a distinction in toxicity could be made. If both agents were believed to be causing toxicity, a dose reduction was performed for both groups of agents. If grade 3 or 4 myelosuppression occurred, D dose was decreased by 10%; in case of grade 3 or 4 gastrointestinal toxicity, the dose of LV, 5-FU and C was withheld, and subsequent doses were decreased by 20% when toxicity resolved. Under no circumstances, however, were the drug doses increased.

Statistical considerations

The primary end point of the study was to assess the overall RR. A RR that exceeded 30% was considered valuable enough to pursue this combination in a phase III trial. The number of patients required for the study was calculated according to a Simon optimal design [23]. An interim analysis was carried out after the first 18 assessable patients had completed treatment. As more than two responses were observed, 23 additional patients were recruited.

The results of the immunological parameters were expressed as the mean ± standard deviation of four determinations made in three different experiments, and the differences determined using a repeated-measures analysis of variance.

The date of relapse was defined as the time between the start of chemotherapy to any relapse and the appearance of a second primary cancer or death, whichever occurred first. OS was measured from entry into the study till death, or 31 May 2006, for censored patients. Statistical analysis of PFS and OS was performed using the Kaplan–Meier method [24]. Statistical analysis was performed with SAS statistical software (version 8.12, 2000; SAS Institute, Cary, North Carolina, USA).

Results

Patients' characteristics

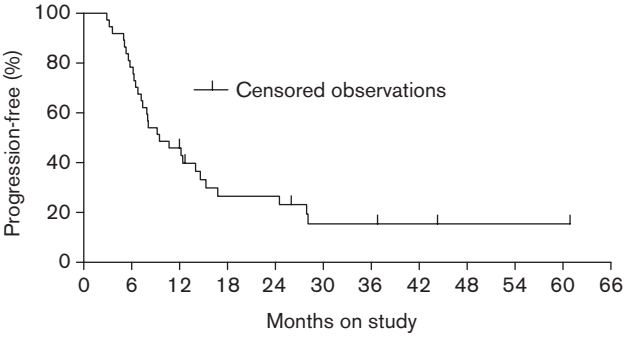
Between April 2001 and April 2005, 41 patients were entered into the study. All patients who had received at least two cycles of chemotherapy were assessable for the safety analysis, response, PFS and OS. Patient characteristics are listed in Table 1. In this predominantly male population, all tumors were localized in the gastric body. The median Eastern Cooperative Oncology Group performance status performance status was 1. Eleven patients had two or more metastatic sites. In addition, metastatic sites included liver and peritoneum in 64 and 22% of instances, respectively. Thirty-one patients (76%) were stage IV at the primary diagnosis, whereas 10 patients had a median disease-free interval of 19 months from primary surgery. No patient had received any form of chemotherapy or immunotherapy.

Table 1 Characteristics of patients

Characteristics	No.	%
No of patients	41	100
Age (years)		
Median	64	
Range	34–83	
Sex		
Males	29	71
Females	12	29
Performance status (ECOG)		
0	20	49
1	17	41
2	4	10
Type of surgery		
Total gastrectomy	16	39
Subtotal gastrectomy	15	37
Biopsy	10	24
Grading		
2	10	24
3	31	76
Stage at diagnosis		
II	3	7
III	7	17
IV	31	76
Metastatic sites		
Liver	32	64
Abdomen	11	22
Lung	2	4
Bone	2	4
Adrenals	2	4
Soft tissue	1	2

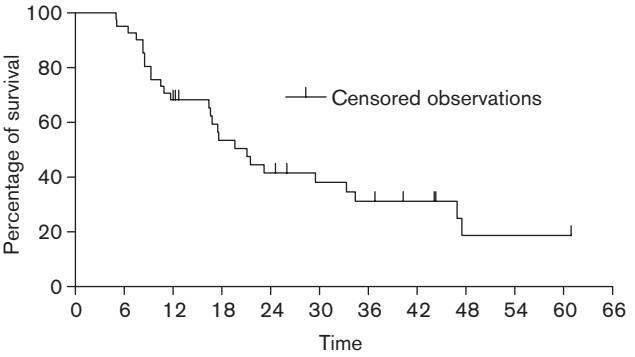
ECOG, Eastern Cooperative Oncology Group.

Fig. 1



Progression-free survival (PFS). Events 29 (71%), censored 12 (29%), median PFS was 9.5 months (range 2.9–60.9+; 95% confidence interval 10–20 months).

Fig. 2



Overall survival (OS). Events 27 (66%), censored 14 (34%), median OS was 21.1 months (range 6–60.9+; 95% confidence interval 18–28 months). Two-year survival rate was 42%.

Antitumor activity

After a median follow-up of 18 months (minimum 13 months), a total of 237 cycles of chemotherapy had been administered to the 41 evaluable patients, with a median of five cycles per patient (range 2–6). All responses were reviewed by an external radiologist. An overall RR of 49% was observed [95% confidence interval (CI) 33–65%]. A complete response was observed in four patients (10%, 95% CI 3–23%), partial response in 16 patients (39%, 95% CI 24–55%), disease stability in seven patients (17%, 95% CI 7–32%) and progressive disease in 14 patients (34%, 95% CI 20–52%). One hundred and sixty-five courses of immunotherapy had been administered to the 27 patients who had obtained a CB. Median PFS was 9.5 months (range 2.9–60.9+; 95% CI 10–20 months) (Fig. 1). Median OS was 21.1 months (range 6–60.9+; 95% CI 18–28 months) (Fig. 2). The 2-year survival rate was 42%. The median OS of patients without a CB from chemotherapy was 5.7 months.

Toxicity

All 41 patients were assessed for toxicity (Table 2). No treatment-related death was observed. Grade 3–4 neutropenia occurred in 18 patients (44%), grade 1–2 anemia in 23 patients (56%) and grade 3–4 thrombocytopenia in two patients, grade 2–3 diarrhea was seen in five patients (12%), grade 3–4 mucositis in four patients (10%), and grade 3 alopecia in 31 patients (78%). Toxicity of maintenance therapy was as follows. Triglycerides were elevated (twice baseline value) in six patients (22%). Hepatic toxicity was observed with the administration of RA (low-grade abnormality of liver enzymes) in four patients (15%); however, these patients indulged in alcohol consumption. Grade 1 skin toxicity occurred in twelve patients (44%). Twenty-two percent of patients treated with IL-2/RA also had grade 1 or 2 fevers. Mild hypothyroidism occurred in two patients. No toxicity related to immunotherapy led to dose reduction or delaying of the therapy.

Immunological activity

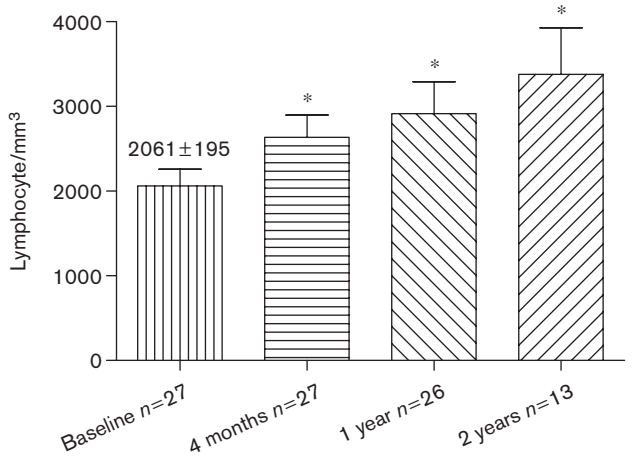
After 4 months of maintenance immunotherapy, a statistically significant improvement was observed in lymphocyte and NK cell count and in $CD4^+/CD8^+$ ratio in the 27 patients who had had a CB. After 1 and 2 years, responding patients had a further improvement of the above-mentioned immunological parameters, reaching, in all instances, statistical significance (Figs 3–5).

Table 2 Toxicity of chemoimmunotherapy according to WHO criteria

	Grade 0		Grade 1		Grade 2		Grade 3		Grade 4	
	No.	%	No.	%	No.	%	No.	%	No.	%
Chemotherapy										
Hematologic										
Leucopenia	11	27	10	24	11	27	9	22	0	0
Neutropenia	7	17	10	24	6	15	7	17	11	27
Thrombocytopenia	35	87	3	7	1	2	1	2	1	2
Anemia	18	44	20	49	3	7	0	0	0	0
Infection	39	95	2	5	0	0	0	0	0	0
Gastrointestinal										
Oral	29	70	6	15	2	5	3	8	1	2
Nausea and vomiting	23	55	15	37	3	8	0	0	0	0
Diarrhea	25	60	11	28	4	10	1	2	0	0
Nuropathy	29	70	4	10	8	20	0	0	0	0
Cutaneous										
Skin	41	100	0	0	0	0	0	0	0	0
Alopecia	0	0	0	0	10	22	31	78	0	0
Hand–foot syndrome	38	93	0	0	3	7	0	0	0	0
Immunotherapy										
Hepatic	23	85	4	15	0	0	0	0	0	0
Skin	15	56	12	44	0	0	0	0	0	0
Triglycerides	21	78	6	22	0	0	0	0	0	0
Fever	21	78	3	11	3	11	0	0	0	0
Hypothyroidism	25	93	2	7	0	0	0	0	0	0

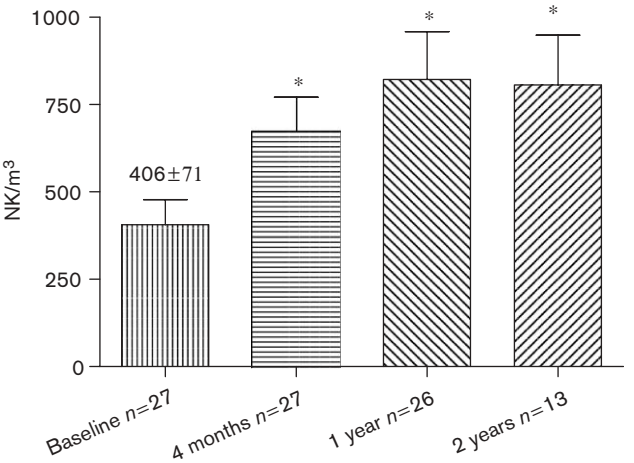
WHO, World Health Organization.

Fig. 3



Lymphocyte count after 4 months, 1 year and 2 years of treatment with interleukin-2 and 13-*cis*-retinoic acid. The difference was statistically significant with respect to baseline values. $^{*}P<0.05$.

Fig. 4



Natural killer (NK) cell count after 4 months, 1 year and 2 years of treatment with interleukin-2 and 13-*cis*-retinoic acid. The difference was statistically significant with respect to baseline values. $^{*}P < 0.05$.

In particular, lymphocytes increased by 27% after 4 months of biological therapy; after 1 and 2 years, they increased by 41 and 35%, respectively. NK, also, after 4 months, increased by 65%. The rise after 1 and 2 years was 102 and 85%, respectively. The $CD4^{+}/CD8^{+}$ ratio increased by 9% after the first 4 months of biological therapy. After 1 and 2 years, the increase was 19 and 57%, respectively.

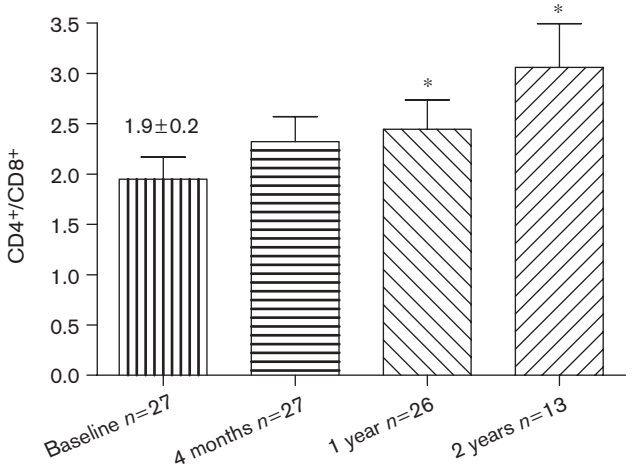
Indeed, immunotherapy not only improved lymphocytes, NK count and $CD4^{+}/CD8^{+}$ ratio, but on the contrary decreased plasma VEGF levels.

VEGF decrement was constant: the baseline value of 365 ± 45 ng/ml ($n = 24$) decreased to a mean value of 264 ± 28 ng/ml after 1 year ($n = 24$, 95% CI, 204–324). After 2 years, the value remained low (94 ± 6 ng/ml, $n = 13$, 95% CI 82–106) (Fig. 6).

Discussion

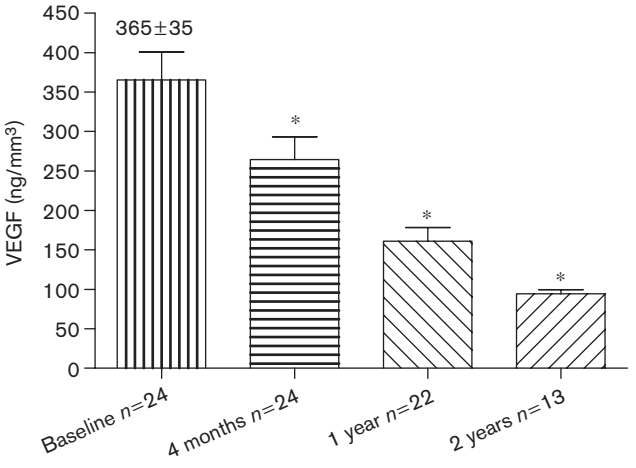
Notwithstanding the progresses in clinical oncology, the outcome of advanced gastric cancer has remained unsatisfactory, with an overall survival that rarely exceeds 9 months, despite the reasonable RR (30–70%) that can be achieved with the new treatment regimens [25].

Fig. 5



$CD4^+/CD8^+$ ratio after 4 months, 1 year and 2 years of treatment with interleukin-2 and 13-*cis*-retinoic acid. The improvement became statistically significant after 1 and 2 years with respect to baseline values. $^*P < 0.05$.

Fig. 6



Serum vascular endothelial growth factor after 4 months, 1 year and 2 years of treatment with interleukin-2 and 13-*cis*-retinoic acid. The difference was statistically significant with respect to baseline values. $^*P<0.05$.

Therefore, more effective strategies with moderate toxicity profiles are needed to prolong survival.

Taxanes that have been shown to be active agents in the treatment of gastric cancer increase programmed cell death and D appears to be more potent than paclitaxel in inhibiting angiogenesis, through inhibition of proliferation, migration and differentiation of endothelial cells [26].

The activity of D in combination with the standard reference regimen of cisplatin–5-FU (DCF) has been described in a randomized phase II study [27]. Patients were randomized to DCF or the same combination without 5-FU (DC). The RR was 43% for DCF and 26% for DC, whereas time to progression was 5.9 and 5 months, respectively. The median overall survival time was 9.6 months for DCF and 10.5 months for DC. Hematological toxicity was significant with grade 3 and 4 events in 86% of patients for DCF and 87% for DC.

A phase III randomized study compared D–cisplatin–5-FU (DCF) with cisplatin–5-FU (CF) [28]. The risk of disease progression was reduced by 32% in the DCF arm (time to progression: 5.6 vs. 3.7 months, $P = 0.0004$). Median OS was statistically significantly superior with DCF (9.2 vs. 8.6 months). As expected, toxicity was important with 10.4% of toxic deaths during treatment in DCF arm and grade 3–4 neutropenia in 82 versus 57%, respectively.

Randomized studies of C combination regimens have also been conducted. An ongoing trial (REAL-2) used a 2×2 design to evaluate several modifications of the ECF (Epirubicin, Cisplatin, 5-fluorouracil) regimen, including the substitution of C for 5-FU and oxaliplatin for cisplatin. An interim analysis of the first 80 randomized patients showed a low incidence of grade 3–4 toxicities in the arm containing C and oxaliplatin, with a 49% RR, with a trend toward superior efficacy [29].

Unfortunately, none of the above-mentioned new chemotherapy regimens have shown a better survival rate compared with the survival obtained with the old regimens. As we have seen from the previous studies, improving chemotherapy combinations, we can improve the RR, the expected time to progression and also decrease the toxicity. The OS depends not only on the efficacy of the chemotherapy regimen, but it depends also on the capacity that the immune system has to prevent the outgrowth of MRD.

The expression of metalloproteinase in gastric cancer is associated with an invasive behavior, metastatic potential and a worse prognosis; a metalloproteinase inhibitor, marimastat, has been used to maintain responses of patients with metastatic gastric cancer. A randomized phase III study of marimastat versus placebo as maintenance therapy in advanced gastric cancer has shown a modest, but significant survival advantage for the treatment arm, both in the total patient population and in the subgroup of patients who had previously received chemotherapy. The 2-year survival rate was 18% in the marimastat arm and 5% in the placebo arm [30].

In our patients treated with the maintenance immunotherapy, we witnessed a continuous improvement of all studied immunological parameters. In fact, lymphocyte, NK cell and $CD4^+/CD8^+$ ratio improved constantly with the immunotherapy administered (Figs 3–5). In addition, the median value of VEGF that was elevated after the end of chemotherapy, decreased constantly with the amount of administered immunotherapy (Fig. 6). Reports exist in the literature focusing the importance of $CD4^+/CD8^+$ ratio as a prognostic factor in melanoma [31] and renal cell carcinoma [32].

The administration of low-dose IL-2 to patients with MRD who obtain CB from chemotherapy might have the same result as in-vitro experiments in which lymphocytes are incubated with tumor cells: following injection of IL-2 in patients, the host immune effector cells, in the presence of MRD, may act as lymphocyte-activated killer cells. Moreover, it has been shown that high-dose IL-2 resulted in a significant decrease of T_{regs} in patients achieving an objective clinical response to IL-2 therapy [33] and also retinoids may help in the differentiation of the $Gr\text{-}1^+CD115^+$ immature myeloid suppressor cells, improving the immune response [15,16].

We conclude that, following chemotherapy with D, 5-FU and C, the administration of low-dose IL-2 and RA on an intermittent schedule and repetition for a long-term period is feasible, has a low toxicity profile, and results in a sustained decrease of VEGF. Prompted by the results obtained in this study, at present we are conducting a randomized phase III study in which patients with a clinical benefit from chemotherapy are randomized to IL-2 + RA or observation.

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