ABSTRACT

Purpose
Tumor cell killing by anticancer drugs may be supported by their immuno- and pharmacologic effects. Chemotherapy is in fact able to (A) upregulate tumor-associated antigen expression, including carcinoembryonic antigen (CEA) or other target molecules such as thymidylate synthase (TS); and (B) downregulate tumor cell resistance to the death signals induced by tumor antigen–specific cytotoxic T lymphocytes. This provides the rationale for combining chemo- and immunotherapy.

Materials and Methods
We describe the results of a translational phase II trial designed to evaluate the toxicity, antitumor activity and immunologic effects of gemcitabine/FOLFOX-4 (oxaliplatin, fluorouracil, and folinic acid) polychemotherapy followed by the subcutaneous administration of granulocyte macrophage colony-stimulating factor and low-dose interleukin-2 in colorectal carcinoma patients. The study involved 29 patients (16 males and 13 females with a mean age of 69 years), 21 of whom had received a previous line of treatment, and 19 had liver involvement.

Results
The treatment was well tolerated and induced very high objective response (68.9%) and disease control rates (96.5%), with an average time to progression of 12.5 months. An immunologic study of peripheral blood mononuclear cells (PBMCs) taken from 20 patients showed an enhanced proliferative response to colon carcinoma antigen and a significant reduction in suppressive regulatory T lymphocytes (CD4+CD25+Treg). A cytofluorimetric study of the PBMCs of five HLA-A(w)02.01 patients who achieved an objective response showed an increased frequency of cytolytic T lymphocyte precursors specific for known CEA- and TS-derived epitopes.

Conclusion
The results show that our regimen has strong immunologic and antitumor activity in colorectal cancer patients and deserves to be investigated in phase III trials.

INTRODUCTION
Colorectal carcinoma is the second leading cause of cancer deaths; almost 50% of the patients die because of problems related to disease progression.¹

Over the last few years, higher response rates have been achieved using the latest poly-chemotherapy regimens combining fluorouracil (FU) ± levofolinic acid (LF) with irinotecan (FOLFIRI) or oxaliplatin (FOLFOX), alone or together with
monoclonal antibodies such as bevacizumab or cetuximab. However, patients with metastatic colorectal cancer still have a poor prognosis, with an average overall survival of 20 months.

Recent progress in human immunobiotechnology has opened up new perspectives in cancer treatment: active specific immunotherapy or vaccine therapy has become a new operative treatment modality that is under large-scale investigation worldwide also for colorectal cancer. Specific immunization against a target antigen has been achieved in some patients with a number of different anticancer vaccines but they have been unsuccessful so far in controlling cancer progression for various reasons. In order to circumvent some of these difficulties, attempts have been made to combine cancer vaccines with biologic agents or cytotoxic drugs, and to test new immunization strategies.

In this context, we have previously shown that a novel, highly cytotoxic and pro-apoptotic multidrug GOLF regimen (gemcitabine [GEM] plus oxaliplatin, LF, and FU) can induce molecular and structural changes in human colon cancer cell lines. These antigenic changes make malignant cells capable of priming an efficient multi-antigenic cytotoxic T-cell (CTL) response with anti tumor activity. We have generated different CTL lines from HLA-A*02.01 donors (normal or colon cancer patients) by stimulating their peripheral blood mononuclear cells (PBMCs) in vitro with low-dose interleukin (IL) -2 and autologous dendritic cells (DCs) loaded with the mixed cell lysate of two colon cancer cell lines (WiDr and HT-29) previously exposed to different multidrug treatments including FOLFOX and GOLF. We found that the CTL lines generated using GOLF-treated colon cancer cells (GOLF-CTL lines) were strongly and specifically cytotoxic against colon cancer cell lines. These antigenic changes make malignant cells capable of priming an efficient multi-antigenic cytotoxic T-cell (CTL) response with anti tumor activity. We have generated different CTL lines from HLA-A*02.01 donors (normal or colon cancer patients) by stimulating their peripheral blood mononuclear cells (PBMCs) in vitro with low-dose interleukin (IL) -2 and autologous dendritic cells (DCs) loaded with the mixed cell lysate of two colon cancer cell lines (WiDr and HT-29) previously exposed to different multidrug treatments including FOLFOX and GOLF. We found that the CTL lines generated using GOLF-treated colon cancer cells (GOLF-CTL lines) were strongly and specifically cytotoxic against colon cancer cell lines. These antigenic changes make malignant cells capable of priming an efficient multi-antigenic cytotoxic T-cell (CTL) response with anti tumor activity. We have generated different CTL lines from HLA-A*02.01 donors (normal or colon cancer patients) by stimulating their peripheral blood mononuclear cells (PBMCs) in vitro with low-dose interleukin (IL) -2 and autologous dendritic cells (DCs) loaded with the mixed cell lysate of two colon cancer cell lines (WiDr and HT-29) previously exposed to different multidrug treatments including FOLFOX and GOLF.

The GOLF-CTL lines also showed a greater frequency of CTL precursors recognizing carcinoembryonic antigen (CEA) and thymidylate synthase (TS) epitope peptides, and were capable of specifically killing class I HLA-matching target cells (CIR-A2 cells) transfected with the CEA or TS gene (manuscript submitted for publication). The GOLF-CTL lines also showed a greater frequency of CTL precursors recognizing carcinoembryonic antigen (CEA) and thymidylate synthase (TS) epitope peptides, and were capable of specifically killing class I HLA-matching target cells (CIR-A2 cells) transfected with the CEA or TS gene (manuscript submitted for publication). On the basis of these findings, we planned a phase II trial involving advanced colorectal carcinoma patients with the aim of testing a novel chemo-immunotherapy regimen based on the sequential administration of the highly effective GOLF polychemotherapy regimen (which is able to induce cancer cell apoptosis and antigen remodeling and release) and a cytokine-based immunotherapy regimen using granulocyte macrophage colony-stimulating factor (GM-CSF; ie, to activate endogenous DCs) and IL-2 (to act as a T-cell growth factor) in order to expand antigen-presenting cell–induced, antigen-specific CTL clones. The rationale of the study is based on the following considerations: (A) chemotherapy induces apoptosis and antigen remodeling; (B) GM-CSF increases the percentage and activation of peripheral blood DCs capable of taking up, processing and presenting antigens released by chemotherapy-treated tumor cells to the effector lymphocyte precursors; (C) IL-2 sustains the immune-response by promoting the proliferation and clonal expansion of the precursors; and (D) activated antigen-specific CTLs destroy tumor cells surviving chemotherapy.

This clinical study was designed on the basis of the results of two previous phase Ib-II trials showing (A) the significant anti tumor activity of the GOLF regimen in colorectal carcinoma patients; and (B) the low level of toxicity of both the GOLF and the IG-1 regimen (a sequential combination of subcutaneous [sc] GM-CSF and low-dose IL-2). The immunobiologic investigation accompanying the IG-1 study showed that the regimen increased the percentage of peripheral blood DCs (from 0.25% to 20%) and their antigen presenting ability. The regimen also showed the ability to increase the absolute number of lymphocytes with the induction of a Th1 cytotoxic phenotype. These findings allowed us to design a treatment schedule combining the two approaches in a single chemo-immunotherapy regimen (GOLFIG-1).

The aim of this phase II trial was to investigate the anti tumor activity, toxicity and immunologic effects of GOLFIG-1 in patients with advanced colorectal carcinoma.

**MATERIALS AND METHODS**

**Study Design and Patient Characteristics**

The inclusion criteria were a histologic diagnosis of colorectal carcinoma, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, a life expectancy of more than 3 months, normal renal and hepatic function, a WBC of more than 2,500/mm3, hemoglobin more than 9 g/dl, a platelet cell count of more than 100,000/mm3, and normal cardiac function. The exclusion criteria were any major organ failure, CNS involvement, second tumors, active infectious disease, major immunosuppression due to AIDS or medical treatment with major immunosuppressive agents (such as cyclosporine for organ transplantation).

The characteristics of the 29 patients enrolled between October 2002 and July 2004 are shown in Table 1. The study was authorized by the University Committee and the Italian Ministry of Health, and all of the patients gave their written informed consent. The patients received GEM 1 g/m2 in a 30-minute intravenous (IV) infusion on day 1 before any other drug. They subsequently received LF 100 mg/m2 in a 30-minute IV infusion on days 1 and 2; FU 400 mg/m2 in a bolus injection followed by a 22-hour continuous infusion (800 mg/m2) on days 1 and 2; and oxaliplatin 85 mg/m2 in a 4- to 6-hour IV infusion before the second administration of LF and FU (FUFA) on day 2. The treatment was repeated every 15 days. GM-CSF 150 µg
Peripheral blood samples for immunologic study were drawn from all of the patients at baseline and at the end of each cycle. PBMCs were obtained by means of Ficoll-Hypaque gradient separation, and the serum samples were prepared by means of simple centrifugation; these samples were immediately frozen at –80°C until their final examination.

**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
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<td>Assessable for response</td>
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</tr>
<tr>
<td>Assessable for toxicity</td>
<td>29</td>
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<tr>
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<td>1</td>
<td>8</td>
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<td>2</td>
<td>7</td>
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<tr>
<td>Disease extension</td>
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</tr>
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<td>19</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
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**Immunologic Studies**

Peripheral blood samples for immunologic study were drawn from all of the patients at baseline and at the end of every cycle, and they were evaluated for toxicity. Response and toxicity were assessed according to the standard WHO criteria (1979).

**Results**

**Flow Cytometry**

Standard single- and double-color flow cytometric analyses were used, as previously described. Precursor frequency and epitope peptide–specific T-cell receptor expression per cell were evaluated using the Cytofluorimetric dimer assay. The kit and reagents were purchased from BD Biosciences (Erembodegem-Dorp, Belgium) and the tests were performed in accordance with the manufacturer’s instructions. The CEA peptides [CAP-1 (YLSGANLNL)], (CEAP)-1 (IQNDTGFYEA), and (CEAP)-2 (LLSVTRNDV)] and TS peptides [TS-1 (AVSEHQLLH), TS-2 (FLHHLIAEIH) and TS-3 (TSTTSLLED)] were synthesized and characterized as previously described.

**Statistical Considerations**

The study was designed to test the hypothesis that the GOLFIG-1 regimen was an active treatment for patients with advanced colorectal carcinoma. A minimum of 25 patients was required in order to maintain alpha and beta errors of 5% and 20%, respectively. Because the response rate of colorectal carcinoma patients to second line-treatment is generally less than 25%, the study was to be stopped if an objective response was not observed in three of the first 14 consecutively enrolled patients.

The between-mean differences in the immunologic results were statistically analyzed using Stat View statistical software (Abacus Concepts, Berkeley, CA). The results were expressed as the mean ± standard deviation of four determinations made in three different experiments, and the differences determined using the Student two-tailed t test for paired samples. A P value < .05 was considered statistically significant.

**Toxicity**

A total of 240 chemo-immunotherapy cycles were administered (a median of nine cycles per patient; range, 6 to 12 cycles). The most frequent adverse events were bone pain, erythema and enduration at the site of the cytokine

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**Abbreviation:** ECOG, Eastern Cooperative Oncology Group.
injection, grade 1 to 2 fever, flu-like syndrome, and grade 1 to 2 hematologic toxicity with moderate anemia, neutropenia, and thrombocytopenia (Table 2). One patient died because of a sudden internal hemorrhage few days after the first treatment cycle. One patient, who experienced grade 4 gastrointestinal toxicity with diarrhea and mucositis during the first cycle, was found to have a significant deficit in dihydro-pyrimidine-dehydrogenase (DPD), an enzyme that is involved in fluoropyrimidine catabolism. DPD deficiency increases the area under the curve of the blood concentration of FU, thus increasing drug toxicity, which often becomes lethal if administered at conventional doses. This patient continued the treatment at a 75%-reduced FU dose and also achieved an objective response. A few other cases of gastrointestinal toxicity (diarrhea, mucositis, and vomiting) were reported, and two patients required a 25% reduction in the FU dose, respectively, starting from the fifth and ninth treatment cycles. Seven patients manifested reversible grade 2 peripheral neurotoxicity. Three patients developed hypersensitivity to oxaliplatin, which was, therefore, withdrawn from their treatment regimen starting from the second, ninth, and 11th cycles, respectively.

**Clinical Response**

One patient was not assessable for response because of early death; seven achieved a complete response (CR), 13 a partial response (PR), and eight stable disease (SD). None of them showed any demonstrable disease progression during treatment. The overall response rate (CR + PR) was 68.9%, the disease control rate (CR + PR + SD) was 96.5%, and the time to progression was 12.5 months (95% CI, 6 to 18 months). Among the 21 patients who had received a previous line of chemotherapy (14 for metastatic disease and seven in and adjuvant setting), there were 13 objective responses and eight disease stabilizations. A complete remission was observed in four chemotherapy-naive patients with liver (two patients), lung, and lung/peritoneum metastases; in one patient who had received adjuvant chemotherapy (and subsequently relapsed on abdominal lymph nodes); and finally in two patients with liver and peritoneum metastases who had received a previous line of chemotherapy for advanced disease (Fig 1).

**Immunologic Study**

Cell-mediated cytotoxicity was investigated in 20 patients. Of all the possible antigens modified by the chemotherapy, we decided to study in detail the immunoresponse to CEA and TS. CEA was chosen as target antigen as it has been widely investigated in a number of immunologic studies of colon cancer patients. On the other hand, TS appeared to be an interesting new target because (A) it is the critical enzyme inhibited by FU; (B) it is indispensable for thymidine synthesis and DNA replication; and (C) TS upregulation or mutation in colon cancer cells in vivo has been associated with the occurrence of resistance to FU and a poor prognosis.

The proliferative response of the PBMCs to influenza virus and tumor-associated antigens was monitored by exposing them to various types of IRIV: an IRIV containing the plasmid backbone, the CEA gene plasmid (CEA-IRIV), and the TS gene plasmid (TS-IRIV).

The PBMCs were also incubated with WiDr cell lysate and PHA (the latter used as a positive control). Stimulation with allogenic PBMCs (data not shown) and LNCaP lysates was used as further controls. A moderate proliferative response to IRIV, CEA-IRIV and TS-IRIV, and a dramatic proliferative response to colon carcinoma cell lysate was detected in the PBMCs of patients who had received at least two treatment cycles. On the other hand, very limited proliferation occurred in the baseline and control PBMCs (Fig 2). No significant differences were observed when the control (healthy donor) PBMCs, baseline PBMCs, or

<table>
<thead>
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<th>Toxicity</th>
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<th>Grade 2</th>
<th>Grade 3</th>
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<td>%</td>
<td>No. of Patients</td>
<td>%</td>
<td>No. of Patients</td>
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<td>7 24.1</td>
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<td>1 3.45</td>
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</table>

**NOTE.** A total of 240 chemotherapy cycles were administered (median, of nine cycles per patient).
post-treatment PBMCs were exposed to PHA, LNCaP, or allogenic PBMC lysates.

We also investigated the possible existence of an antigen-specific, cell-mediated immunoresponse in drug-treated patients, a hypothesis arising from the finding that GOLF upregulates CEA and TS expression in human colon cancer cells in vitro. The PBMCs of five patients with an HLA-A*02.01+ haplotype who achieved a significant objective response were therefore examined in order to determine the precursor frequency of lymphocytes capable of recognizing CEA and TS-derived peptides. This was done using the dimer assay for previously described peptide epitopes with HLA-A*02.01 binding amino acid consensus motifs derived from TS (TS-1, TS-2, and TS-3)\(^{21}\) and CEA (CEAP-1, CEAP-2, and CAP-1).\(^{8,20}\) Moreover, PTR-4, an HLA-A*02.01 binding peptide derived from the parathyroid-related peptide (PTH-rP, which is not expressed in colon cancer cells)\(^{32}\) was used as a negative control.

The PBMCs of the patients who had received two treatment cycles showed a doubling in the frequency of the precursors for TS-1, TS-2, and CAP-1 in comparison with their baseline PBMCs, and a two- to six-fold increase in comparison with normal donor PBMCs (Fig 3A). We also observed much greater mean fluorescence intensity in the post-treatment CTL precursors specific for TS-1, TS-2, CEAP-1, CEAP-2, and CAP-1. This finding is consistent with the hypothesis that these CTLs express a larger number of epitope/peptide-specific T-cell receptors on the membrane than the controls (Fig 3B). No change in precursor frequency or T-cell receptor expression was observed for the control peptide.

**Chemo-Immunotherapy Effects on PBMC Expression of CD4\(^+\)CD25\(^+\) T Regulatory Cells, CD95\(^+\) Subsets, and the CD4\(^+\)/CD8\(^+\) T-Cell Ratio**

It has been reported that an antigen-specific immunoresponse generated by cancer vaccine may be hampered...
by the parallel occurrence of T-regulatory lymphocytes. These are a subset of T lymphocytes with a CD4$^{+}$, CD25$^{+}$, CD45Ro$^{+}$, and FoxP3$^{+}$ phenotype, whose most likely role in normal individuals is to counterbalance the occurrence of potentially dangerous immunoresponses to self-antigens. These cells are capable of suppressing the antigen-specific CTL response by means of a cytokine- and FAS-mediated induction of apoptosis.33-36

We therefore investigated the expression of CD4$^{+}$, CD8$^{+}$, CD25$^{+}$ T$_{reg}$, and CD95$^{+}$ (FAS) subsets in the PBMCs of the first 20 enrolled patients, and found that two treatment cycles significantly decreased the CD4$^{+}$CD25$^{+}$ T$_{reg}$ (from 9.4% ± 4.47% to 3.37% ± 1.92%) and CD95$^{+}$ subsets (from 14% ± 6% to 3.3% ± 2.29%), whose values became similar to those observed in normal individuals (CD4$^{+}$CD25$^{+}$ T$_{reg}$ 3.1% ± 2.2%; CD95$^{+}$, 3% ± 1.2%). These events were paralleled by the finding of an increase in the CD4/CD8 T cell ratio (from 1.27 ± 0.25 to 2.1 ± 0.24), which once again became indistinguishable from that observed in normal individuals (2.3 ± 0.3).

Taken together, these findings suggest that GOLFIG-1 chemo-immunotherapy may counter a possible state of immunosuppression.

**DISCUSSION**

A number of different cancer vaccine strategies are currently being evaluated in clinical trials involving patients with various gastrointestinal neoplasms. However, although the preliminary results indicate that they are capable of inducing an effective antigen-specific cellular and humoral response in cancer patients,9-17 no correlation has been found between successful immunization and clinical outcome.9-17 The possible reasons for this are...
related to the fact that a larger tumor burden leads to more general immune anergy and a greater likelihood of generating immuno-resistant clones. One of the major problems facing cancer therapy is that the extraordinary adaptability of tumor cells leads to drug- and radioresistance as well as to acquired resistance to the effector lymphocytes possibly generated by host vaccination. In this context, it must be remembered that common antigens such as MUC-1 or CEA are not critical for tumor cell survival, and so they can be lost under the selective pressure of a vaccine-induced antigen-specific immune response without really damaging tumor development.

However, there are various other mechanisms that may explain how neoplastic cells can avoid being recognized by the CTLs elicited by cancer vaccines. It is widely believed that, like drug- and radioresistance, immunoresistance may depend on the degree of cancer cell heterogeneity and thus on the tumor burden. One possible means of overcoming the adaptive response of tumor cells and the consequent occurrence of antigen-specific immunoresistance is the simultaneous immunization of cancer patients against multiple antigens using irradiated autologous cancer cells or tumor cells induced to express inflammatory cytokines and co-accessory molecules by means of genetic engineering, viral constructs, or heat shock proteins extracted from cancer cells and containing multiple antigen-derived peptides. A number of trials have investigated these approaches in colon carcinoma patients; some of them have led to convincing results in terms of immunologic and antitumor activity, especially when the immunologic reagents were tested in an adjuvant setting under conditions of minimal disease.

Another possible approach to overcome resistance is to reduce the tumor burden by combining immunotherapy with radio- and/or chemotherapy, which could lead to significant debulking and simultaneously affect the phenotype of tumor cells (antigen remodeling), thus making them more susceptible to vaccine-activated effectors. In an attempt to avoid the occurrence of immunoresistance, a number of empirically designed clinical studies of different malignancies have investigated the possibility of combining cytotoxic drugs with biologic agents and/or cytokines (eg, IL-2 and interferon alfa), but the results have been conflicting in terms of clinical response and survival.

We and other authors have previously described the ability of cytotoxic drugs such as triazenes, FU, VP-16 and CPT-11 to sensitize tumor cells to the cytolytic activity of antigen-specific CTLs. For example, we have shown that the exposure of various colon and breast carcinoma cell lines to sublethal doses of FU is followed by a significant increase in the expression of CEA and TS and a consequent immnosensitization to the cytotoxic activity of class-I-HLA-matching CTL lines specific for these antigens. We have also more recently shown that the GOLF regimen is capable of inducing a much greater level of necrosis and apoptosis in the same colon carcinoma cell lines in vitro, while still retaining the FU-induced overexpression of CEA and TS. GOLF treatment allowed the tumor cells to become a more efficient means of generating a strong multi-antigenic CTL response with antitumor activity when used to stimulate human PBMCs in vitro (manuscript submitted for publication), thus providing a possible model to pursue in clinical trials of chemo-immunotherapy in colorectal cancer.

The design of combined chemo-immunotherapy approaches has been criticized on the grounds that chemotherapy is immunosuppressive. This opinion is based on the fact that most cytotoxic drugs can kill granulocyte precursors in bone marrow and thus induce leukopenia, which is associated with the occurrence of bacterial and mycotic infection; however, there is no evidence that cytotoxic chemotherapy may affect an antigen-specific CTL response. We have found recently that the antigen-specific killing ability of human CTL lines in vitro is not affected by FU, oxaliplatin, or GEM if exposure to these drugs does not occur during the stimulation phase (the time interval during which CTL precursors come into contact with TAA-loaded DCs and start the proliferation; unpublished results), which suggests that chemotherapy and immunotherapy should be given sequentially rather than concomitantly. In relation to this, Novak et al have shown that GEM exposure after the inoculum of tumor cells engineered to express viral neuraminidase (NE), improved the efficacy of T-cell response and the tumor rejection rate in a xenograft mouse model and significantly inhibited the humoral response. The fact that the same effect was not observed when the tumor cells had been exposed to GEM before the inoculum, that it is improved whether GEM is administered concomitantly with an activating anti-CD40 monoclonal antibody, and that in the responsive mice there was a significant NE specific CTL response, suggests that the drug treatment somehow enhances the cross presentation of potential antigens released by drug-treated cancer cells in vivo.

In line with these considerations, the results of our clinical study clearly show that the chemo-immunotherapy regimen combining GEM + FOLFOX-4 with the sequential administration of GM-CSF and IL-2 has strong antitumor activity in advanced colorectal cancer patients. This effect is associated with a low level of toxicity. The very high clinical response rate is also associated with the occurrence of immunologic events that strongly resemble those induced by the most sophisticated cancer vaccination techniques.

The toxicity level of GOLFIG-1 was no different from that reported for FOLFOX-4 or, more recently, the
GEM + FOLFOX-4 regimen in advanced colon cancer patients.\textsuperscript{26} The only exception was the higher frequency of fever, malaise and flu-like syndrome associated with cytokine administration, which could be easily controlled by nonsteroidal anti-inflammatory-drugs.

Our clinical results showed a high rates of objective responses and disease control: the median time to progression of 12.5 months was far greater than that reported for any other regimen, including FOLFOX, FOLFIRI, FOLFIRI + bevacizumab or cetuximab,\textsuperscript{2,4} and GEM + FOLFOX-4 without cytokines (OR, 41.5%; disease control rate, 75.9%; time to progression, 7 months in patients receiving second- or third-line treatment).\textsuperscript{26} Furthermore, they take on even more significance if it is remembered that the majority of the patients were receiving second-line treatment, had multiple metastatic sites and, in some cases, an ECOG performance status of 2.

Our regimen is based on a rational combination of cytotoxic drugs and cytokines, and the immunologic results support the hypothesis that the treatment may lead to the generation of an antigen-specific immune reaction capable of sustaining prolonged anti-tumor activity. This is suggested by the fact that the post-treatment PBMCs of five responsive patients showed a significant increase in the proliferative response to colon cancer antigens including CEA and Ts, and a 2-3 times increase in the frequency of TS- and CEA-derived epitope peptide-specific CTL precursors. In addition our cytofluorimetric analysis also showed that the cell membranes of these precursors may (at least theoretically) express more antigen epitope–specific T-cell receptors, thus increasing their ability to recognize target cells.

Finally, our results show that the GOLFIG-1 regimen significantly reduced the percentage of PBMCs containing immune-suppressive CD4\textsuperscript{+}CD25\textsuperscript{+}T_{reg},\textsuperscript{33-35} and the number of cells expressing the FAS receptor (CD95), and also induced the complete restoration of the CD4/CD8 T-cell ratio, which is often reduced in advanced cancer patients showing a progressively deteriorating immune response.

In conclusion, the results of this study suggest that our combined chemo-immunotherapy regimen has strong immunologic and antitumor activity in colorectal carcinoma patients, and could be an attractive strategy to investigate in future phase III comparative trials.

Acknowledgment

We thank the technical, medical, and paramedical personnel of the Section of Oncology at the Siena University School of Medicine for their dedication to the patients and their helpful contribution to this study.

Authors’ Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

REFERENCES


