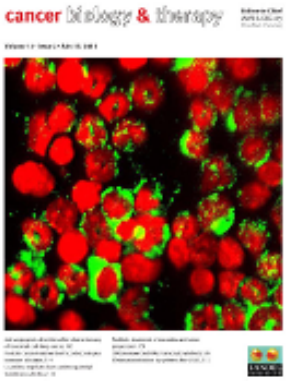

Cancer Biology & Therapy
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kcbt20

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Published online: 15 Jul 2011.

To cite this article: Pierpaolo Correale, Cirino Botta, Assunta Basile, Marco Pagliuchi, Antonella Licchetta, Ignazio Martellucci, Elena Bestoso, Serena Apollinari, Raffaele Addeo, Gabriella Misso, Ornella Romano, Alberto Abbruzzese, Monica Lamberti, Luca Luzzi, Giuseppe Gotti, Maria Saveria Rotundo, Michele Caraglia & Pierosandro Tagliaferri (2011) Phase II trial of bevacizumab and dose/dense chemotherapy with cisplatin and metronomic daily oral etoposide in advanced non-small-cell-lung cancer patients, Cancer Biology & Therapy, 12:2, 112-118, DOI: 10.4161/cbt.12.2.15722

To link to this article: http://dx.doi.org/10.4161/cbt.12.2.15722

Cancer Biology & Therapy 12:2, 112-118; July 15, 2011; © 2011 Landes Bioscience

Phase II trial of bevacizumab and dose/dense chemotherapy with cisplatin and metronomic daily oral etoposide in advanced non-small-cell-lung cancer patients

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Key words: bevacizumab, NSCLC, dose/dense-metronomic-chemotherapy, mPEBev regimen, VEGF

Abbreviations: NSCLC, non-small-cell-lung cancer; TKI, thyrosine kinase inhibitor; PFS, progression free survival; OS, overall survival; VEGF, vascular endothelial growth factor; MABD, most active biological dose; MTD, maximal tolerated dose; WBC, white blood cells; MPO, myeloperoxidase; ELISA, enzyme-linked immunosorbent assay; HADS, hospital anxiety and depression scale; TE, thrombo-embolism; FDA, Food and Drug Administration; EMEA, European Medicines Agency

Bevacizumab, is a humanized monoclonal antibody to vasculo-endothelial-growth-factor, with anticancer activity in non-small-cell-lung cancer (NSCLC) patients. Our previous results from a dose/finding phase I trial in NSCLC patients, demonstrated the anti-angiogenic effects and toxicity of a newest bevacizumab-based combination with fractioned cisplatin and daily oral etoposide. We designed a phase II trial to evaluate in advanced NSCLC patients the antitumor activity and the safety of this novel regimen. In particular, 45 patients (36 males and 9 females), with a mean age of 54 years, an ECOG ≤2, stage IIIB/IV and NSCLC (28 adenocarcinomas, 11 squamous-cell carcinomas, 2 large-cell carcinomas, 4 undifferentiated carcinomas), were enrolled. They received cisplatin (30 mg/sqm, days 1–3), oral etoposide (50 mg, days 1–15) and bevacizumab (5 mg/kg, day 3) every 3 weeks (mPEBev regimen). Patients who achieved an objective response or stable disease received maintenance treatment with bevacizumab in combination with erlotinib until progression.

Grade I-II hematological, mucosal toxicity and alopecia were the most common adverse events. The occurrence of infections (17%), thromboembolic events (4.4%) and severe mood depression (6.7%) was also recorded. A partial response was achieved in 31 (68.8%) patients, disease remained stable in 8 (17.8%) and disease progressed in 6 (13.3%) with a progression-free-survival of 9.53 months (95% CI, 7.7–11.46).

Our bio-chemotherapy regimen resulted very active in advanced NSCLC, however, the toxicity associated with the treatment requires strict selection of the patients to enroll in future studies.

Introduction

Non-small cell lung cancer (NSCLC) is the most common malignancy and the leading cause of cancer death worldwide. The majority of NSCLC patients cannot undergo curative surgery[1] while those with relapsed or advanced disease have a very poor outcome.[2] Poly-chemotherapy with platinum derivatives in combination with a second cytotoxic drug selected among gemcitabine, paclitaxel, pemetrexed or vinorelbine, represents the standard treatment for these patients.[3-6] Clinical evidence suggests an important role for maintenance therapy with pemetrexed (restricted to adenocarcinoma),[7] docetaxel[8] or with the epidermal growth factor receptor thyrosine kinase inhibitor (TKI), erlotinib[9] to improve both PFS and overall survival (OS) of advanced NSCLC patients who did not progress frontline platinum based chemotherapy.

The efficacy of standard poly-chemotherapy has been also improved by the addition of bevacizumab, a humanized

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Submitted: 03/03/11; Revised: 03/29/11; Accepted: 04/02/11
DOI: 10.4161/cbt.12.2.15722

monoclonal IgG1 to the vascular endothelial growth factor (VEGF).[10-12]

VEGF is a soluble dimeric protein family produced in hypoxic conditions, which is crucial for endothelial proliferation and neo-vessel stabilization.[11,12] Two large phase III trials, E4599 and AVAIL have shown that the addition of bevacizumab to chemotherapy improves treatment efficacy and therefore, it has been approved for the therapy of patients with metastatic non-squamous NSCLC.[13,14] Some concern has been recently raised on the true potential of bevacizumab to produce consistent advantages in terms of long term survival in fact the second study failed to demonstrate a significant benefit of bevacizumab in terms of OS.[14] Several hypotheses have been formulated to explain this finding, which include: poor pharmacological interaction of bevacizumab with cisplatin and/or gemcitabine; unbalanced selection of patients; interference of second line chemotherapy etc.; however a clear explanation has not been provided yet. As an additional consideration, both studies excluded patients with squamous cell histology because preliminary reports recorded six cases (out of 99 enrolled patients) of fatal hemorrhages, four of which recorded in patients with squamous cell carcinoma.[15]

The efficacy and safety of bevacizumab in combination with multiple chemotherapy doublets in advanced non-squamous NSCLC patients have been endured by the results of two large phase IV studies.[16-18]

The use of bevacizumab has also been evaluated for maintenance treatment of NSCLC patients who had received at least four chemotherapy courses (ATLAS, BeTa-Lung and TASK). The preliminary results of some of these studies suggest that this mAb, used in combination with the TKI erlotinib, produces a clear benefit in term of PFS and probably, also in terms of OS.[19]

In this contest, even though bevacizumab addition to chemotherapy appears to produce benefit in the treatment of advanced NSCLC, it is also clear that the most active combination and the optimal administration strategy have not been identified yet.

We have thus recently, carried-out a dose finding phase I trial aimed to investigate in advanced NSCLC patients, the toxicity, the anti-angiogenic and the biological activity of a newest bevacizumab-based biochemotherapy regimen designed on translational bases. This regimen designated as mPEBev, combines bevacizumab with fractioned cisplatin and daily oral etoposide.[20]

The mPEBev regimen was designed by taking in consideration that full dose platinum at the beginning of the cycle could induce tumor de-bulking and endothelial cell activation and proliferation, while the subsequent bevacizumab infusion, together with a prolonged oral low dose administration of etoposide (a dose/dense administration schedules), could produce a more efficient anti-angiogenic, immunobiological and antitumor effect. It was in fact, hypothesized that the anti-angiogenic and immune-modulating effects of VEGF-deprivation induced by bevacizumab, could enhance the antitumor effects of chemotherapy given by fractioned cisplatin (days 1–3) and dose/dense metronomic oral etoposide given for 15 consecutive days. At this purpose, it is known that cytotoxic drugs such as cyclophosphamide, vinorelbine and etoposide, administered with dose-dense metronomic modality (prevalently at low dose, for prolonged time, with very short inter-cycle intervals), together with a direct cytotoxic activity, also engender antitumor effects by: (1) inhibiting proliferating endothelial precursors; (2) inducing an immune-stimulating effect by killing inhibitory myeloid cells and regulatory T lymphocytes; and (3) forcing cancer cells to acquire a less aggressive phenotype (epigenetic effect).[21-27] Our study was also based on the results of a previous phase II trial which showed the low level of toxicity and the antitumor activity of a metronomic chemotherapy doublet with cisplatin and daily oral etoposide (mPE regimen) in patients with high risk advanced NSCLC.[27]

The results of the previous phase I mPEBev trial provided preliminary evidence of antitumor activity in advanced NSCLC including squamous cell histology; additionally, they allowed the identification of the most active biological dose (MABD) and the maximal tolerated dose (MTD) of bevacizumab within the mPE-Bev regimen, in a range between 5 and 7.5 mg/kg, to be used for further studies. We have designed the present phase II trial in order to evaluate the toxicity and the antitumor activity of the mPEBev regimen as front-line treatment of advanced NSCLC patients including the squamous histology. In the current trial, all of the patients received at least four mPEBev courses including 5 mg/kg bevacizumab.

Results

Demography. Between January 2008 and August 2010, 45 patients, 36 males and 9 females, with a mean age of 54 years were enrolled in the study. All of them had a histological diagnosis of NSCLC; 28 with an adenocarcinoma, 11 with a squamous cell carcinoma, 2 with a large cell carcinoma and 4 with an undefined carcinoma.

At the time of enrollment the ECOG performance status scored as 2 in 11 (24.4%) patients; 1 in 14 (31.1%) and 0 in the remaining 20 (44.4%) (**Table 1**).

All of the patients received front-line treatment according to the mPEBev regimen; those who achieved an objective response or stable disease received maintenance treatment with bevacizumab in combination with erlotinib until progression.

Toxicity. Patients received a median number of five treatment cycles. Grade I-II hematological toxicity was the most common adverse event. Grade I-II asthenia, nausea and vomiting, mucositis and alopecia were equally frequent. No early deaths were recorded in the present group of 45 patients; however, one patient, with an undefined carcinoma, under prophylactic fondaparinux treatment for preexisting episodes of atrial arrhythmia suffered a fatal episode of hemorrhage 2 weeks after the third treatment cycle. We recorded two asymptomatic cases of lung thromboembolism (TE) discovered by CT scan performed on follow-up. These episodes had no consequences and further radiological studies showed full recovery upon anticoagulant therapy. We also observed five cases of peripheral vein thrombosis occurring just after the 2nd and 3rd cycle.

In the previous phase I study, we recorded five cases of severe mood depression disturbances, thus in the present investigation we decided to monitor both depression and anxiety by performing

a psychometric analysis (HADS) in all of the enrolled patients by administering specific questionnaires at baseline and during the treatment. In this way, it was established a specific performance score for both depression and anxiety in a range between 0 and 21, with 7 used as a cut off.[28] The mPEBev regimen was associated to a greater risk of depression, in fact, the average scores recorded at baseline and 21 days after the first cycle were 7.3 (±3.2) and 10.5 (±3.3), p = 0.049, respectively. Three patients who received the mPEBev regimen suffered severe depressant symptoms (score 21); treatment was suspended to allow aggressive antidepressant medication. These patients were older than 65 years and did not show other significant side effects, brain metastases or rapid cancer progression. Conversely, in the majority of the patients, the depression symptoms were mild and self-limiting and were easily controlled by medical treatment with serotonin re-uptake inhibitors. No significant change in depression score was observed in a parallel group of 12 patients with NSCLC who received standard doublet chemotherapy only (pre vs. post chemotherapy score: 7.18 (±3.4) vs. 5.73 (±4.5); p = 0.9). In our study, we also measured possible change in anxiety, in order to exclude the possibility that the occurrence of depression could be related to a poor treatment compliance. Our test failed to demonstrate any significant treatment-related change in anxiety (baseline vs. post-treatment score: 7.33 [±4.27] vs. 7.17 [±6.32]).

We also recorded eight cases of bacterial pneumonia and five cases of non-neutropenic fever. All of them rapidly recovered upon antibiotics and anti-mycotics and could continue the treatment (**Table 2**). In these patients, we recorded a significant treatment-related decrease in neutrophil granulocyte number (**Fig. 1A**) and a defect in their functional activity measured as serum myeloperoxidase level (**Fig. 1B**). This effect was not associated to neutropenia and was not observed in a parallel group of patients who underwent to standard chemotherapy alone (data not shown).

Treatment activity. The mPEBev regimen resulted very active as frontline treatment of NSCLC patients. We recorded an objective response in 31 patients (68.8%) and disease stabilization lasting more than 60 days in further 8 (17.8%), with a disease control rate of 86.7%. Progression of disease was conversely observed in 6 (13.3%) patients. On the basis of these results, the trial fulfilled the statistical target of activity fixed at 35% suggesting that the mPEBev regimen is an active treatment for advanced NSCLC patients (**Table 3**). We also observed that the mPEBev treatment is associated to a PFS of 9.53 months (95% CI; 7.6–11.46) with 50% patients surviving at least 12 months (**Fig. 2**).

In the subset of 28 adenocarcinoma patients, in particular, we observed a PFS of 10.38 (95% CI; 7.98–12.76) months which appeared to be longer than that recorded in the 11 patients with squamous-cell histology of 8.47 (95% CI; 5.3–11.6) months. These differences did not achieve statistical significance (p value was 0.08) due to the small number of patients, nevertheless, the actuarial survival curves showed a clear trend in favor of adenocarcinoma (**Fig. 2A**). A similar finding was also observed in term of OS. In fact, our analysis recorded in patients with adenocarcinoma and squamous-cell histology an OS of 15 (95% CI; 11.92–18.07) and 11 months (95% CI; 6.67–15.3) (p = 0.15), respectively (**Fig. 2B**). Four responsive patients with locally advanced disease resulted down-staged and could undergo radical lung resective surgery. Surgical specimens showed a complete pathologic remission in three resected patients. As an additional finding, we also report three radiological responses of brain lesions

Table 1. Patient characteristics

		All patients	%
No. of patients		45	100%
Median age		54 years (range 35–73)	
Sex	Male	36	80%
	Female	9	20%
PS (ECOG)	0	20	44.4%
	1	14	31.1%
	2	11	24.4%
Histology	Adenocarcinoma	28	62.2%
	Squamous cells	11	24.4%
	Large cell carcinoma	2	4.4%
	Undifferentiated	4	8.9%
Stage	IIIB	4	8.9%
	IV	41	91.1%
Surgery	Yes	2	4.4%
	No	43	95.5%

Table 2. Toxicity (45 patients)

Adverse events	Grade 2	Grade 3	Grade 4
Hematological toxicity			
Anemia	4 (8.9%)	4 (8.9%)	
Thrombocytopenia	5 (11.1%)	1 (1.2%)	
Leucopenia	6 (13.3%)	3 (6.7%)	1 (1.2%)
Gastroenteric toxicity			
Nausea/vomiting	6 (13.3%)	2 (4.4%)	
Mucositis/stomatitis	2 (4.4%)	4 (8.9%)	2 (4.4%)
Diarrhea	2 (4.4%)		
Hemoptysis		2 (4.4%)	
Pneumonia		8 (17.8%)	
Asymptomatic lung cavitation		1 (1.2%)	
Asthenia	2 (4.4%)	3 (6.7%)	
Non neutropenic fever		5 (11.1%)	
Proteinuria	2 (4.4%)		
Thrombosis/ pulmonary embolism			2 (4.4%)
Peripheral venous thrombosis	5 (11.1%)		
Cognitive disturbance/ depression	9 (20%)	1 (1.2%)	2 (4.4%)
Hypertension	2 (4.4%)		

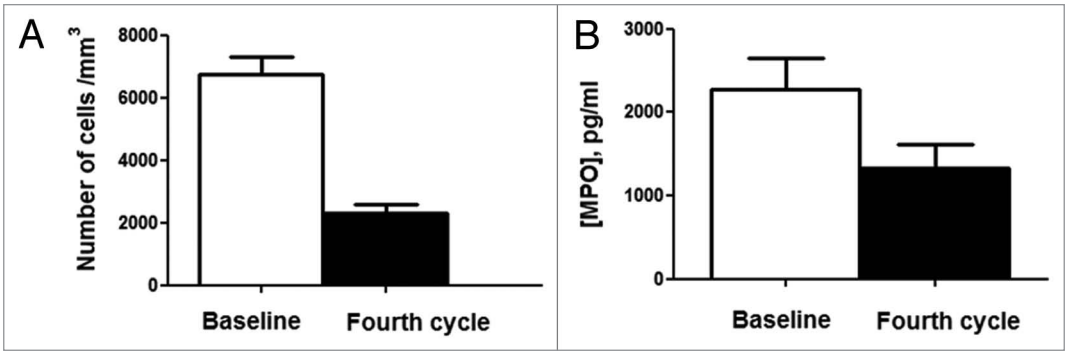

Figure 1. Patients undergone mPEBev biochemotherapy regimen present a significant reduction of in the number (A) and activity (B) of neutrophil granulocytes. Granulocyte activity was measured as serum myeloperoxidase concentration. The differences between the values measured at the first and fourth cycle were considered statistically significant. Differences reported in the (A) showed a p value = 0.025 while those reported in the (B) presented a p value of = 0.046.

Table 3. Antitumor activity

Patients						
	CR	PR	OR (CR + PR)	SD	DCR (CR + PR + SD)	PD
45	2	29	31	8	39	6
100 %	4.4%	64.4%	68.8%	17.8%	86.7%	13.3%

CR, complete remission; PR, partial response; OR, overall response; SD, stable disease; DC, disease control; PD, progression of disease.

suggesting that our combination is effective in patients with brain metastases. In our analysis finally, the occurrence of objective response or adverse events was not correlated with a particular histology.

Discussion

We report the results of a phase II trial in advanced NSCLC patients designed to confirm the safety and evaluate the antitumor activity of a novel anti-angiogenic therapeutic strategy based on the combination of bevacizumab with dose/dense metronomic doublet chemotherapy of fractioned cisplatin and daily oral etoposide (mPEBev regimen). The results of our previous phase I trial provided evidence that the mPEBev regimen exerts a powerful anti-angiogenic effect being able of inducing a significant treatment-related decrease of VEGF, angiopoietin and VEGF-transporting cells (neutrophils and platelets) combined with blood flux decline in the primary tumor site as assessed by NMR study.[20] The mPEBev regimen tested in the phase I trial, in line with results of much larger bevacizumab containing regimens,[15,29,30] did not result completely safe; the occurrence of adverse events appeared to be correlated with bevacizumab dosage with the most severe (grade III–IV) adverse events observed in the group of patients who received 7.5 or 10 mg/kg. In the current phase II trial, we confirmed a greater risk of developing pneumonia, sepsis, mood depression, peripheral vein thrombosis and lung thrombo-embolism in mPEBev treated patients. We believe that these adverse events are strictly related to the VEGF depletion operated by bevacizumab. The enhanced risk of thrombo-embolism in NSCLC as well as the effects of VEGF and bevacizumab on the vasculo-endothelial cells, blood coagulation and platelets are partially known and still under active investigation.[31]

Other authors have also risen the concern that bevacizumab may impair neutrophil granulocytes' functions. It is in fact, known that neutrophils transport 70% of VEGF amounts in the blood, and that the precursors of this blood cell lineage express VEGF-2/FLT1 receptor on their membrane whose engagement is critical for their maturation.[32,33] In line with this hypothesis we report that bevacizumab administration is followed by progressive granulocyte depletion and functional inactivation as suggested by the finding that the treatment is associated to a decrease of serum myeloperoxidase levels.

We also report the occurrence of depression in patients who received the mPEBev regimen. Our psychometric analysis revealed that the changes in mood status had to be considered an early event, mainly occurring in older patients who presented a higher depression score at baseline. This phenomenon, on the other hand, was not associated with treatment related discomfort or increase in disease related symptoms, as shown by the fact, that the treated patients did not present change in anxiety. In the majority of patients these symptoms were mild to moderate, self-limiting and easy to control with antidepressants able to inhibit serotonin re-uptake and to enhance both serotonin and VEGF in the hippocampus (sertraline).[34-37]

Brain CT scan and NMR study performed in patients with severe depression excluded the presence of metastases, major vascular lesions or cortical atrophy. All together these results suggest that the occurrence of depression is a direct effect of VEGF depletion empowered by chemotherapy administration. This effect is not surprising considering that VEGF is a highly pleiotropic molecule and recent reports indicate that this protein is necessary

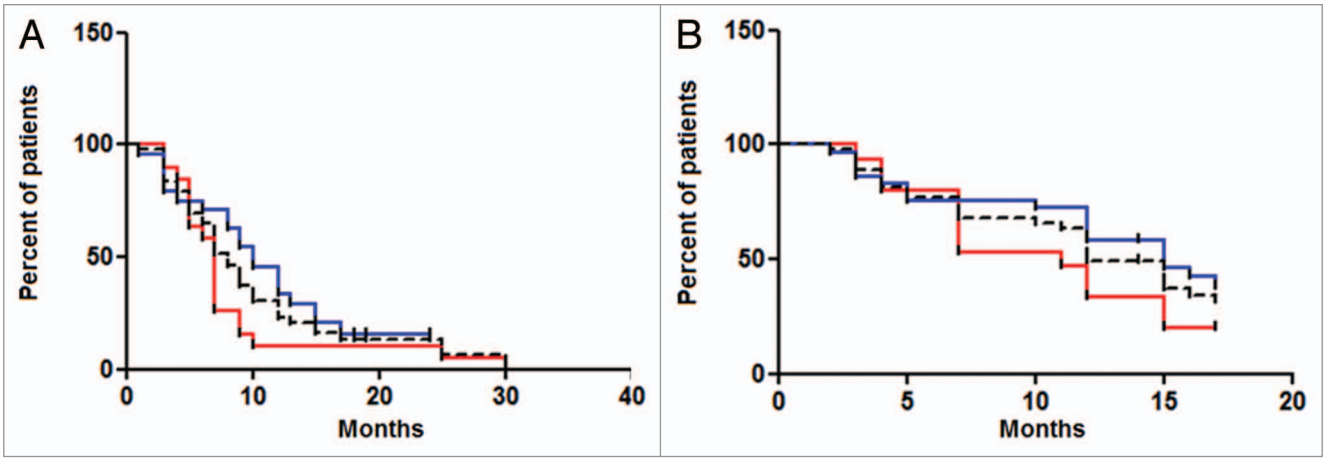

Figure 2. The progression free survival (PFS) (A) and the overall survival (OS) (B) recorded in patients enrolled in the mPEBev trial ( ). We recorded a mean PFS and OS value of 9.53 and 12 months, respectively. Patients (28) with adenocarcinoma ( ) histology showed a PFS and an OS of 10.38 and 15 months, respectively. Conversely, patients (11) with squamo-cellular ( ) histology showed a PFS and an OS of 8.5 and 11 months, respectively. Statistical differences in PFS and OS between patients with adenocarcinoma and squamous histology showed a p value of 0.08 and 0.15, respectively.

for the neurogenic and behavioral action of antidepressant in animal models.[34-37] Both peripheral and central nervous system adverse events however, have already been reported in patients receiving treatment regimens containing bevacizumab.[38] A further study is presently ongoing to evaluate this particular and complex adverse event in patients who receive combination therapies containing bevacizumab for the treatment of different malignancies.

In conclusions, the regimen resulted very active with a response rate of 68.8% which was higher than the statistical target of activity of 35% which was fixed by taking in consideration a number of studies reporting the effects of frontline polychemotherapy[3,39] alone or in combination with bevacizumab administration.[16] The antitumor activity of our combination regimen could be partially due to the metronomic administration modality of the mPE chemotherapy alone, which in a previous phase II trial in high risk NSCLC patients resulted safe and active.[27] It has to be considered that the results appear really encouraging in terms of response rate and PFS more than is OS. This might reflect the "all upfront strategy" that also includes an active maintenance therapy. The study was however not powered for survival endpoints.

As an additional observation, the current mPEBev regimen resulted also active in patients with squamous cell histology.

This finding might be relevant by taking in consideration that limited information is presently available on the effects of bevacizumab in these individuals, who account for 25–30% of all patients with NSCLC. This patient population did not show significant difference in term of objective response rate and risk of toxicity or bleeding even though they showed a trend to a worse outcome in term of progression free and overall survival compared with the adenocarcinoma group of patients. The hypothesis that bevacizumab-based therapeutic strategies may be useful in the treatment of NSCLC patients with squamous histology has already been taken in consideration by other authors and is presently under investigation in the ongoing phase III BRIDGE trial which is evaluating late bevacizumab + carboplatin and paclitaxel doublet vs. doublet alone in NSCLC patients with squamous cell histology.

The results of our study appear very promising on the basis of the relevant response rate in advanced NSCLC patients and that our study also enrolled patients with non optimal performance status (ECOG 2) and brain metastases.

These results acquire further relevance if we take in consideration those reported by the preliminary study lead by Johnson et al. and by two phase III trials (EC9446 and AVAIL) which led to the FDA and EMEA approval of bevacizumab plus chemotherapy doublets. These studies in fact, only reported, an improvement of the response rate from 15–35% of the progression free survival from 4.5–6.2 months and overall survival from 10.3–12.3 months over the chemotherapy alone.[13,14] We also need to take in account that both EC9446 and AVAIL studies only included better prognosis patients (non-squamous histology, good performance status [ECOG ≤1], no severe non neoplastic concomitant diseases, no-weight loss <5% and no brain metastases).[13,14] Additionally, the regimens investigated in these trials used a bevacizumab dosage two to three times greater than our, with consequent greater risk of adverse events and costs.

Based on these results we believe that the mPEBev regimen is very promising and that it deserves to be investigated in a further phase III trial as frontline bio-chemotherapy NSCLC cancer patients.

Patients and Methods

Study design. The study protocol EUDRACT code #BEVA2007 was performed in accordance to the good clinical practice guidelines and was approved by the Bioethics Committee of the University of Siena. All patients provided a written informed consent. The study was designed as a Simon's two-stage minimax optimal design to confirm the hypothesis that the mPEBev regimen is active in patients with advanced

NSCLC. The study was designed to test a null hypothesis of p = 0.150 vs. an alternative hypothesis = 0.350, with an expected sample size of 20.15 and a probability of early termination of 0.604. If the combination is not considered to be active, there is only a 0.046 probability (4.6%) of discharging an active treatment (the target for this value was 0.05); conversely if the regimen is found to be active, there is a 0.197 probability (19.7%) that it is actually not active. The objective response rate was the primary endpoint for this statistical analysis. The treatment under investigation should be considered non-active if less than two responses out of 15 consecutive patients were recorded in the first series and fewer than 7 out of 28 patients in the whole series. We considered the regimen as active when a response rate of at least 35% was recorded.

The inclusion criteria were: histological diagnosis of NSCLC, performance status (ECOG) from 0–2, normal renal and hepatic function, WBC count more than 2,500/mm^3, hemoglobin more than 9 g/dl, platelet cell count more than 90,000/mm^3, normal cardiac function. Enrollment of patients with brain metastatic disease was allowed if asymptomatic or responsive to radiation therapy. The exclusion criteria were: central tumors with high risk of bleeding (excavated with large necrosis and infiltration of large arterial and venous structures), second malignant tumors, active hepatitis or liver failure, chronic or acute renal failure, active infectious disease.

Treatment schedule. All the patients received every 3 weeks, iv. cisplatin (30 mg/sqm) on days 1–3 and daily oral etoposide (50 mg) on days 1–15 and iv. bevacizumab 5 mg/kg on the day 3. Maintenance treatment was administered after 4–6 chemotherapy cycles with bevacizumab (5 mg/kg) every 3 weeks and daily erlotinib (150 mg/day). At the time of study design preliminary information on the activity of this maintenance approach was available.

Clinical assessment. A complete medical history, physical examination, complete blood count and serum chemistry were performed before starting treatment and repeated every 3 weeks. Complete disease staging was undertaken at the baseline and every 2 months by computed tomography. Responses were assessed according to RECIST criteria while toxicity was reported according to CTCAE v3.0.

Myeloperoxidase assay. Myeloperoxidase (MPO) concentration was measured in patients' serum at baseline, and after four treatment cycles by using an enzyme-linked immunosorbent assay (ELISA) kit (Bender Systems, Vienna, Austria) according to the method described by Klebanoff et al. The specific activity was expressed as nmol/min/mg of total protein.

Psychometric analysis. The assessment of mood status was performed at baseline and at beginning of each treatment course by using the Hospital Anxiety and Depression Scale (HADS),[28] a self-reported test which includes two subscales, for depressive and anxiety symptoms, with seven questions each, rating from 0–3, and a total score range between 0 and 21; a higher score is indicative of worse symptoms.

Statistical analysis. Survival plots were constructed using the Kaplan-Meyer method and the survival data were analyzed by using the GraphPad Instat 3.2 statistic software.

Acknowledgments

The study was supported by a grant from the ACF-II, Siena. All of the authors wish to thank the patients who entered the study, the paramedic personnel and the residents who provided their enthusiastic support to patients and research.

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