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Impact of Bevacizumab *Versus* Erlotinib on Tumor Metrics in Patients With Previously Untreated Advanced Non-small Cell Lung Cancer: A Study by the Hellenic Cooperative Oncology Group

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Abstract. *Background: The mechanism of action of bevacizumab and erlotinib is quite different in the treatment of advanced non-small cell lung cancer (NSCLC). This study sought to compare the two targeted therapies in terms of sequential tumor response metrics. Patients and Methods: Parameters of radiological tumor response evaluation were assessed at baseline and periodically in 58 patients receiving either bevacizumab plus platinum-based chemotherapy (N=25) or erlotinib (N=33). Results: Bevacizumab-treated patients had lower longest diameter at best response compared to the erlotinib group (p=0.011). The longest diameter, tumor volume and density significantly decreased from baseline to best response for the entire cohort and bevacizumab-treated patients; no difference was found in the erlotinib group. Conclusion: Treatment with bevacizumab substantially improved tumor metrics between baseline and each cycle of treatment, as well as between baseline and best response, in patients with advanced NSCLC.*

Non-small cell lung cancer (NSCLC) remains the most lethal malignancy worldwide, accounting for the majority of cancer-related deaths in both men and women worldwide (1). Standard platinum-based chemotherapy has reached a therapeutic plateau and new agents with proven survival benefit in specific sub-populations of NSCLC include Epidermal Growth Factor Receptor tyrosine kinase inhibitors (EGFR-TKIs) (2), immunotherapy (3) and anti-angiogenic agents such as the monoclonal antibody against vascular endothelial growth factor (VEGF) bevacizumab (4). Among those agents, the taxane-derivative docetaxel, the EGFR-TKI erlotinib and bevacizumab have been among the first to gain approval in the treatment of advanced NSCLC, especially in frail patients who could not tolerate platinum-based doublet chemotherapy, and in second-line treatment, after failure of first-line chemotherapy (5).

In 2008, the Hellenic Cooperative Oncology group (HeCOG) initiated a randomized phase II study (DOPERLO NCT00783471) to determine the comparative efficacy of intermittent erlotinib/docetaxel chemotherapy, with erlotinib given for twelve consecutive days either before (group A) or after (group B) docetaxel, in chemotherapy-naive patients

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with advanced NSCLC. Docetaxel chemotherapy was considered one of the valid non-platinum-containing therapeutic options at that time and today represents one of the approved second-line treatment options after failure of first-line platinum-based chemotherapy. The trial was terminated early due to slow accrual after enrollment of 51 patients and was published in 2014 (6), showing no clinically meaningful difference between the two treatment arms.

Based on the aforementioned trial population, a parallel radiological study was conducted, evaluating sequential tumor metrics on computed tomographies (CT) performed at baseline and periodically in patients receiving either bevacizumab plus platinum-based chemotherapy or erlotinib/docetaxel. Given the differential mechanisms of action of these agents, it was hypothesized that the different parameters of radiological tumor response evaluation, namely the longest diameter, tumor volume and tumor density, would respond differently for each therapeutic category (a chemotherapeutic agent, a molecular agent and an anti-angiogenic agent). For bevacizumab, in particular, it was hypothesized that its unique mechanism of action, inducing central tumor necrosis and subsequent shrinkage (7), would lead to completely different tumor metrics compared to the other two agents. Herein, we present the final results of this analysis, showing the comparison of the two targeted therapies in terms of tumor response metrics, as evaluated in CT scans every 12-16 weeks.

Patients and Methods

We studied 111 patients with non-small cell lung cancer, who have been evaluated with computational CT scan or Magnetic Resonance Imaging (MRI) scan in several HeCOG-affiliated departments of medical oncology in Greece, using multi-slice CT scans. Diagnostic imaging and evaluation of responses performed through 5-mm (or thinner) sections in the thorax and abdomen before and after of at least 100 ml (depending of body weight) of IV contrast medium infusion, and administration of oral contrast agent. If clinically indicated, we performed brain and spinal MRI and positron emission tomography (PET-CT).

Tumor response was evaluated with the RECIST 1.1 criteria (Response Evaluation Criteria In Solid Tumors) (8), according to which there is a comparison of the sum of the longest diameters of the target lesions with concurrent evaluation of the non-target lesions in the CT scans of brain, thorax and abdomen before chemotherapy, as baseline-reference evaluation, as well as in pre-schedules re-evaluations every six weeks, until completion of 8 cycles of treatment or demonstration of tumor progression. After the completion of 8 cycles, re-evaluation was performed every three months. For each patient, the same method was used, throughout the study. Evaluation of response using the RECIST 1.1 criteria was based on the measurement of a total of 5 measurable lesions, that are recognized from the beginning of the treatment as target lesions. Evaluation of the findings was performed by two independent investigators in digital DICOM format, with the use of recent software for tumor and density evaluation (OSIRIX and HOROS, MEDICAL DICOM VIEWERS) (9-11).

However, due to the prospective nature of the DOPERLO study, some patients did not complete the planned evaluation protocol because they either did not undertake all the study scans, or did not deliver the scan results to the investigator, or they died. For the above-mentioned reasons, the number of the DOPERLO study participants was reduced to 33 patients. To increase the number of patients, we added another 54 patients that were treated with bevacizumab plus platinum-based chemotherapy. Chemotherapy was administered up to 6 cycles concurrently with bevacizumab. The latter was continued after the completion of chemotherapy as maintenance treatment until tumor progression or unacceptable toxicity.

These patients met the same criteria with those of DOPERLO, without participating in a specific protocol and their scheduled evaluation was performed every 3 months during bevacizumab chemotherapy and during maintenance. Due to the same reasons, among these 54 patients, another 29 were removed from the analysis. Hence, a total of 58 patients (33 from DOPERLO and 25 treated with bevacizumab plus platinum-based chemotherapy) were included in the final analysis.

Evaluation methods. In 43 of these patients, densitometry and volumetry were performed, both in the primary tumor and in metastatic sites. Volumetry of the whole tumor (V_T), as well as of the whole volume of four metastatic sites (V_N) was performed with the help of a specialized software. In every case, the primary cancer and metastatic tumor borders were spotted on the images (CT or MRI), and were then unified with a special software point-by-point in the periphery of the spotted tumor lines. With the use of the software, and by evaluation of parameters of magnification and thickness, the volume and density of the primary tumor location and metastasis were calculated in cubic centimeters and Housefield Units.

Statistical analysis. Descriptive statistics including counts and percentages for categorical variables and measures of location and dispersion for continuous variables were used to present patients' clinicopathological characteristics and parameters of radiological tumor response evaluation, namely the longest diameter, tumor volume and tumor density. For the calculation of the volume per cycle both the sum and the maximum of the lesions were assessed and are presented as Volume (sum) and Volume (max), respectively. Group comparisons of categorical data were performed with the Chi-square or Fisher's exact (where appropriate) test, while the Wilcoxon rank-sum test was performed to detect differences between categorical and continuous variables. The non-parametric Wilcoxon signed rank test was used to evaluate differences between two time points of interest. Overall survival (OS) was measured from the date of diagnosis to the date of patient's death (from any cause) or last contact, whichever occurred first. Survival curves were estimated using the Kaplan-Meier method and compared between treatment groups with the log-rank test. The association between type of treatment and death rates was assessed with hazard ratios estimated with univariate Cox proportional hazard regression models. Statistical significance was set at a two-sided $p<0.050$. Statistical analyses were performed using the Statistical Analyses Software (SAS; version 9.3, SAS Institute Inc., Cary, NC, USA).

Results

A total of 58 patients with a median age of 62 years (range=36-77 years) were included in the analysis. The

Table I. *Patient and tumor characteristics at diagnosis*.

	Overall N	Total	Bevacizumab	Erlotinib	*p*-Value
Age	58	61.6 (35.7,76.8)	58.5 (35.7,76.8)	62.7 (43.1,74.1)	0.52[a]
Gender	58				0.63[b]
Female		10 (17.2)	5 (20.0)	5 (15.2)	
Male		48 (82.8)	20 (80.0)	28 (84.8)	
Smoking*	50				0.67[b]
No		6 (12.0)	2 (8.7)	4 (14.8)	
Yes		44 (88.0)	21 (91.3)	23 (85.2)	
Histology*	53				0.009[b]
Adenocarcinoma		40 (75.5)	21 (91.3)	19 (63.3)	
Large cell		3 (5.7)	2 (8.7)	1 (3.3)	
Squamous cell		7 (13.2)	0 (0.0)	7 (23.3)	
Unclassified		3 (5.7)	0 (0.0)	3 (10.0)	
Grade*	40				0.060[b]
1-2		15 (37.5)	10 (52.6)	5 (23.8)	
3-4		25 (62.5)	9 (47.4)	16 (76.2)	
PS*	50				**0.041**[b]
0		34 (68.0)	19 (82.6)	15 (55.6)	
1		16 (32.0)	4 (17.4)	12 (44.4)	
Stage*	49				0.99[b]
I-III		3 (6.1)	1 (4.3)	2 (7.7)	
IV		46 (93.9)	22 (95.7)	24 (92.3)	
Surgery*	48				**0.025**[b]
No		37 (77.1)	21 (91.3)	16 (64.0)	
Yes		11 (22.9)	2 (8.7)	9 (36.0)	
Type of surgery	11				0.45[b]
Lobectomy		6 (54.5)	2 (100.0)	4 (44.4)	
Pneumonectomy		5 (45.5)	0 (0.0)	5 (55.6)	
Prior RT*	46				0.99[b]
No		41 (89.1)	21 (91.3)	20 (87.0)	
Yes		5 (10.9)	2 (8.7)	3 (13.0)	

*Data not available for all subjects. Missing values: Smoking=8, Histology=5, Grade=18, PS=8, Stage=9, Surgery=10, Prior RT=12. PS: Performance status; RT: radiation therapy. Values presented as Median (min, max) or N (column %). *p*-Values: [a]Wilcoxon rank-sum test, [b]Pearson's chi-square/Fisher's Exact test. Bold values show significance.

majority of patients were males (82.8%) with higher-grade (grade 3-4) adenocarcinomas. Twenty-five patients (43.1%) were treated with bevacizumab, while the rest (56.9%) received erlotinib and docetaxel. Baseline patient and tumor characteristics for the entire cohort and by treatment group are presented in Table I. Patients in the bevacizumab group had more frequently PS 0 as compared to those treated with erlotinib and docetaxel (82.6% *vs*. 55.6%, $p=0.041$). At a median follow-up of 4.2 years (95% CI=3.7-NR), a total of 44 patients (75.9%) had died. The median OS was 1.5 years (95% CI=1.1-2.5) for the entire cohort. Patients treated with bevacizumab had numerically slightly longer median OS as compared to those who received erlotinib (median OS: 1.7 years *versus* 1.1 years) and lower risk of death (HR=0.78, 95% CI=0.41-1.49), however significance was not reached (Log-rank $p=0.456$ and Wald's $p=0.457$, respectively) (Figure 1).

Summary statistics for the tumors' volume, density and longest diameter at baseline, first, second, third and fourth cycle were calculated for the entire study population and by treatment group (data not shown). The tumors' longest diameter was measured for all 58 patients at baseline and at the first cycle, while data regarding volume and density were available for 43 patients (74.1%) at these time points. The median longest diameter at baseline for the entire cohort was 6.55 cm, ranging from 1.50 to 26.27, and did not differ between patients treated with bevacizumab plus platinum-based chemotherapy and those who received erlotinib and docetaxel ($p=0.27$). It is of note that the values of volume, density and longest diameter did not differ between the group of patients treated with bevacizumab and those treated with erlotinib in the first, second, third or fourth cycle either.

Evaluation at best response. Table II presents summary statistics for volume, density and longest diameter at best response. The median longest diameter at best response for the entire cohort was 3.80 and was significantly lower in the bevacizumab compared to the erlotinib group (3.20 *vs*. 6.28,

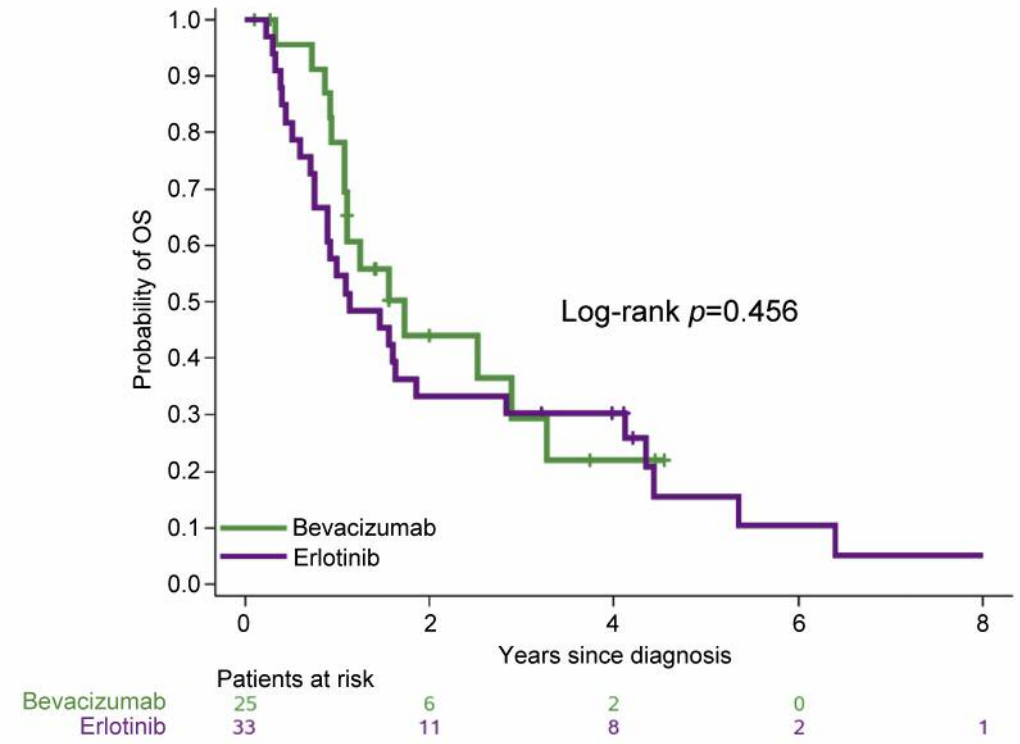

Figure 1. *Kaplan-Meier curves with respect to Overall Survival (OS) based on treatment group.*

p=0.011). Additionally, the volume at best response was significantly lower in patients treated with bevacizumab plus platinum-based chemotherapy as compared to those receiving erlotinib and docetaxel (Table II), while the density at best response was marginally significantly lower in the bevacizumab group (p=0.051).

Evaluation between baseline and first/second/third cycle. Evaluating the difference between the baseline and the first cycle's assessment regarding the volume, density and the longest diameter of the tumor, a significant decrease was observed in the tumors' longest diameter at the first cycle compared to the baseline for the entire cohort with the median longest diameter at first cycle being 4.95 (p=0.002). Similarly, the tumors' volume and density were significantly lower at the first cycle compared to baseline (p<0.001 and p=0.039, respectively) (Table III). In the entire cohort, the values of volume, density and longest diameter were significantly lower in the first cycle compared to the baseline in the group of patients who received bevacizumab (p<0.001, p=0.046 and p<0.001, respectively). In contrast, in the group of patients treated with erlotinib, none of the aforementioned parameters showed statistically significant differences between baseline and first cycle assessment. Similar results were observed when evaluating the differences in tumor metrics between baseline and second cycle for patients with available data in both time points. The longest diameter, volume and density were significantly lower in the second cycle as compared to the baseline both in the entire cohort and in the group of patients treated with bevacizumab (Table IV). In the subgroup of patients treated with erlotinib with available data at both time points, no significant difference was observed in the values of volume, density and longest diameter between baseline and second cycle.

The longest diameter significantly decreased from baseline to the third cycle in the entire cohort (median 5.78 *vs.* 4.32, p=0.001) and in the group of patients treated with bevacizumab and platinum-based chemotherapy (median 6.30 *vs.* 3.90, p=0.015). Similarly, the volume showed a statistically significant decrease from baseline to the third cycle both in the entire cohort and in the subgroup of patients treated with bevacizumab (Table V), while the median density decreased from 53.55 in baseline to 20.50 in the third cycle in the group of patients who received bevacizumab (p=0.006). Density did not differ between baseline and the third cycle assessment in the entire cohort, while no significant difference between baseline and the third cycle was observed in tumor metrics in the group of patients who received erlotinib.

Evaluation between baseline/first cycle and best response. We further evaluated the differences in tumor volume, density

Table II. *Summary statistics for tumor metrics at best response for the entire cohort and by treatment group.*

	N	Mean	Std	Median	Min	Max	*p*-Value
Volume (max)							
Bevacizumab	25	14.72	25.62	6.27	0.00	113.00	0.036
Erlotinib	18	43.72	60.24	17.97	0.00	199.00	
Total	43	26.86	45.32	7.62	0.00	199.00	
Volume (sum)							
Bevacizumab	25	15.85	25.80	6.30	0.00	113.00	**0.022**
Erlotinib	18	63.30	97.21	28.33	0.00	368.76	
Total	43	35.71	69.04	9.03	0.00	368.76	
Density							
Bevacizumab	25	–27.67	102.41	0.00	–338.00	90.20	0.051
Erlotinib	18	–10.04	133.70	28.60	–509.40	59.10	
Total	43	–20.29	115.35	13.20	–509.40	90.20	
Longest diameter							
Bevacizumab	25	3.75	2.44	3.20	0.50	8.81	**0.011**
Erlotinib	33	7.70	6.20	6.28	0.00	30.04	
Total	58	6.00	5.29	3.80	0.00	30.04	

N: Number; std: standard deviation; min: minimum; max: maximum. *p*-Values correspond to the comparison of volume, density and longest diameter between the two treatment groups. Bold values show significance.

Table III. *Comparative statistics for tumor metrics between baseline and first cycle for the entire cohort and by treatment group.*

	Baseline						First cycle						
	N	Mean	Std	Median	Min	Max	N	Mean	Std	Median	Min	Max	*p*-Value
Volume (max)													
Bevacizumab	25	56.99	86.98	20.84	2.12	378.00	25	23.64	40.10	7.62	1.12	171.00	**<0.001**
Erlotinib	18	57.08	62.38	34.74	3.20	213.00	18	47.71	60.49	21.34	1.22	199.00	0.37
Total	43	57.02	76.80	28.19	2.12	378.00	43	33.72	50.44	8.95	1.12	199.00	**<0.001**
Volume (sum)													
Bevacizumab	25	64.36	88.36	30.01	2.12	383.74	25	25.90	40.28	8.53	1.12	171.00	**<0.001**
Erlotinib	18	74.70	94.30	43.70	3.20	379.81	18	68.10	97.00	32.72	2.27	368.76	0.48
Total	43	68.69	89.93	32.77	2.12	383.74	43	43.57	71.96	12.98	1.12	368.76	**<0.001**
Density													
Bevacizumab	25	15.83	93.15	50.90	–313.50	107.00	25	–0.26	85.66	11.70	–253.90	90.20	**0.046**
Erlotinib	18	1.57	105.62	31.00	–387.90	55.30	18	–6.83	126.66	30.25	–474.70	66.60	0.40
Total	43	9.86	97.59	35.50	–387.90	107.00	43	–3.01	103.43	21.10	–474.70	90.20	**0.039**
Longest diameter													
Bevacizumab	25	6.54	3.65	5.90	2.00	16.80	25	4.75	2.31	4.00	1.60	8.81	**<0.001**
Erlotinib	33	8.71	6.34	7.47	1.50	26.27	33	8.08	6.04	7.40	1.10	30.04	0.29
Total	58	7.77	5.42	6.55	1.50	26.27	58	6.64	5.05	4.95	1.10	30.04	**0.002**

N: Number; std: standard deviation; min: minimum; max: maximum. *p*-Values correspond to the comparison of volume, density and longest diameter between the two time points in the bevacizumab group, erlotinib/docetaxel group and in the entire cohort. Bold values show significance.

and longest diameter between the best response and baseline as well as between the best response and cycles 1, 2, 3 for patients with available data in each cycle. The longest diameter, tumor volume and density were significantly lower at best response compared to the baseline both in the entire cohort and in the subgroup of bevacizumab-treated patients, while among patients treated with erlotinib even though a small decrease was observed in tumor metrics at best response compared to baseline, significance was not reached (Table VI). In contrast, the longest diameter and the tumor volume showed a significant decrease from first cycle to best response both in the entire cohort as well as in the subgroups of patients treated with bevacizumab and those treated with erlotinib (Table VII). Tumor density was also significantly lower at the best response compared to the first cycle in the entire cohort and in bevacizumab-treated patients ($p<0.001$),

Table IV. *Comparative statistics for tumor metrics between baseline and second cycle for the entire cohort and by treatment group for patients with available data at both time points.*

	Baseline						Second cycle						
	N	Mean	Std	Median	Min	Max	N	Mean	Std	Median	Min	Max	*p*-Value
Volume (max)													
Bevacizumab	20	66.29	95.26	23.18	2.12	378.00	20	18.72	30.45	3.61	0.00	113.00	**<0.001**
Erlotinib	9	57.67	73.05	12.10	3.20	213.00	9	29.45	37.85	6.69	0.00	86.71	0.074
Total	29	63.61	87.74	13.68	2.12	378.00	29	22.05	32.62	4.12	0.00	113.00	**<0.001**
Volume (sum)													
Bevacizumab	20	73.00	96.85	35.13	2.12	383.74	20	19.48	30.45	5.44	0.00	113.00	**<0.001**
Erlotinib	9	65.71	75.94	16.05	4.97	213.00	9	38.52	52.27	9.55	0.00	146.00	0.16
Total	29	70.74	89.58	18.35	2.12	383.74	29	25.39	38.61	7.33	0.00	146.00	**<0.001**
Density													
Bevacizumab	20	48.52	30.41	51.70	–32.30	107.00	20	6.37	88.78	3.30	–338.00	100.80	**0.003**
Erlotinib	9	–27.49	147.22	35.50	–387.90	55.30	9	–35.09	186.62	40.70	–509.40	66.60	0.57
Total	29	24.94	90.00	48.80	–387.90	107.00	29	–6.49	125.22	20.50	–509.40	100.80	**0.020**
Longest diameter													
Bevacizumab	20	6.74	3.77	6.05	2.40	16.80	20	4.01	2.53	3.60	0.50	10.70	**0.001**
Erlotinib	17	7.87	5.85	5.78	2.50	22.50	17	6.66	5.54	3.76	0.00	18.50	0.12
Total	37	7.26	4.80	5.90	2.40	22.50	37	5.23	4.34	3.70	0.00	18.50	**<0.001**

N: Number; std: standard deviation; min: minimum; max: maximum. *p*-Values correspond to the comparison of volume, density and longest diameter between the two time points in the bevacizumab group, erlotinib/docetaxel group and in the entire cohort. Bold values show significance.

Table V. *Comparative statistics for tumor metrics between baseline and third cycle for the entire cohort and by treatment group for patients with available data at both time points.*

	Baseline						Third cycle						
	N	Mean	Std	Median	Min	Max	N	Mean	Std	Median	Min	Max	*p*-Value
Volume (max)													
Bevacizumab	16	65.52	98.38	23.18	2.12	378.00	16	30.97	48.22	8.42	0.00	148.00	**0.011**
Erlotinib	8	55.59	77.81	8.29	3.20	213.00	8	21.12	33.45	6.33	0.00	94.72	0.15
Total	24	62.21	90.43	12.89	2.12	378.00	24	27.69	43.35	7.13	0.00	148.00	**0.001**
Volume (sum)													
Bevacizumab	16	72.07	98.16	35.13	2.12	383.74	16	32.59	47.93	8.42	0.00	148.00	**0.005**
Erlotinib	8	57.30	76.57	11.62	4.97	213.00	8	22.18	32.90	9.08	0.00	94.72	0.078
Total	24	67.15	90.10	17.20	2.12	383.74	24	29.12	43.04	9.07	0.00	148.00	**<0.001**
Density													
Bevacizumab	16	47.63	33.94	53.55	–32.30	107.00	16	23.22	30.00	20.50	–20.10	73.60	**0.006**
Erlotinib	8	–34.67	155.69	35.58	–387.90	55.30	8	–25.89	140.56	34.60	–351.90	59.10	0.31
Total	24	20.20	98.48	48.65	–387.90	107.00	24	6.85	84.61	22.40	–351.90	73.60	0.076
Longest diameter													
Bevacizumab	16	6.70	3.64	6.30	2.40	16.80	16	4.81	3.19	3.90	0.50	12.00	**0.015**
Erlotinib	13	6.84	5.50	4.10	3.00	22.50	13	4.99	3.45	4.32	0.00	14.10	0.15
Total	29	6.76	4.48	5.78	2.40	22.50	29	4.89	3.25	4.32	0.00	14.10	**0.001**

N: Number; std: standard deviation; min: minimum; max: maximum. *p*-Values correspond to the comparison of volume, density and longest diameter between the two time points in the bevacizumab group, erlotinib/docetaxel group and in the entire cohort. Bold values show significance.

while significance was not reached in the subgroup of patients treated with erlotinib (p=0.13).

The spider plots showing the percentage change from baseline over time for the longest diameter, volume (max), volume (sum) and density are presented in Figures 2-5, respectively. Figure 6 shows the waterfall plot of the percentage change from baseline to best response for the longest diameter.

Table VI. *Comparative statistics for tumor metrics between baseline and best response for the entire cohort and by treatment group for patients with available data at both time points.*

	Baseline						Best response						
	N	Mean	Std	Median	Min	Max	N	Mean	Std	Median	Min	Max	*p*-Value
Volume (max)													
Bevacizumab	25	56.99	86.98	20.84	2.12	378.00	25	14.72	25.62	6.27	0.00	113.00	**<0.001**
Erlotinib	18	57.08	62.38	34.74	3.20	213.00	18	43.72	60.24	17.97	0.00	199.00	0.20
Total	43	57.02	76.80	28.19	2.12	378.00	43	26.86	45.32	7.62	0.00	199.00	**<0.001**
Volume (sum)													
Bevacizumab	25	64.36	88.36	30.01	2.12	383.74	25	15.85	25.80	6.30	0.00	113.00	**<0.001**
Erlotinib	18	74.70	94.30	43.70	3.20	379.81	18	63.30	97.21	28.33	0.00	368.76	0.27
Total	43	68.69	89.93	32.77	2.12	383.74	43	35.71	69.04	9.03	0.00	368.76	**<0.001**
Density													
Bevacizumab	25	15.83	93.15	50.90	–313.50	107.00	25	–27.67	102.41	0.00	–338.00	90.20	**0.001**
Erlotinib	18	1.57	105.62	31.00	–387.90	55.30	18	–10.04	133.70	28.60	–509.40	59.10	0.23
Total	43	9.86	97.59	35.50	–387.90	107.00	43	–20.29	115.35	13.20	–509.40	90.20	**<0.001**
Longest diameter													
Bevacizumab	25	6.54	3.65	5.90	2.00	16.80	25	3.75	2.44	3.20	0.50	8.81	**<0.001**
Erlotinib	33	8.71	6.34	7.47	1.50	26.27	33	7.70	6.20	6.28	0.00	30.04	0.085
Total	58	7.77	5.42	6.55	1.50	26.27	58	6.00	5.29	3.80	0.00	30.04	**<0.001**

N: Number; std: standard deviation; min: minimum; max: maximum. *p*-Values correspond to the comparison of volume, density and longest diameter between the two time points in the bevacizumab group, erlotinib/docetaxel group and in the entire cohort. Bold values show significance.

Table VII. *Comparative statistics for tumor metrics between first cycle and best response for the entire cohort and by treatment group for patients with available data at both time points.*

	First cycle						Best response						
	N	Mean	Std	Median	Min	Max	N	Mean	Std	Median	Min	Max	*p*-value
Volume (max)													
Bevacizumab	25	23.64	40.10	7.62	1.12	171.00	25	14.72	25.62	6.27	0.00	113.00	**<0.001**
Erlotinib	18	47.71	60.49	21.34	1.22	199.00	18	43.72	60.24	17.97	0.00	199.00	**0.031**
Total	43	33.72	50.44	8.95	1.12	199.00	43	26.86	45.32	7.62	0.00	199.00	**<0.001**
Volume (sum)													
Bevacizumab	25	25.90	40.28	8.53	1.12	171.00	25	15.85	25.80	6.30	0.00	113.00	**<0.001**
Erlotinib	18	68.10	97.00	32.72	2.27	368.76	18	63.30	97.21	28.33	0.00	368.76	**0.016**
Total	43	43.57	71.96	12.98	1.12	368.76	43	35.71	69.04	9.03	0.00	368.76	**<0.001**
Density													
Bevacizumab	25	–0.26	85.66	11.70	–253.90	90.20	25	–27.67	102.41	0.00	–338.00	90.20	**<0.001**
Erlotinib	18	–6.83	126.66	30.25	–474.70	66.60	18	–10.04	133.70	28.60	–509.40	59.10	0.13
Total	43	–3.01	103.43	21.10	–474.70	90.20	43	–20.29	115.35	13.20	–509.40	90.20	**<0.001**
Longest diameter													
Bevacizumab	25	4.75	2.31	4.00	1.60	8.81	25	3.75	2.44	3.20	0.50	8.81	**<0.001**
Erlotinib	33	8.08	6.04	7.40	1.10	30.04	33	7.70	6.20	6.28	0.00	30.04	**0.002**
Total	58	6.64	5.05	4.95	1.10	30.04	58	6.00	5.29	3.80	0.00	30.04	**<0.001**

N: Number; std: standard deviation; min: minimum; max: maximum. *p*-Values correspond to the comparison of volume, density and longest diameter between the two time points in the bevacizumab group, erlotinib/docetaxel group and in the entire cohort. Bold values show significance.

Discussion

In this hypothesis-generating trial, we evaluated the effects of the anti-VEGF monoclonal antibody bevacizumab on tumor metrics (longest diameter, volume and density), indirectly compared to the combination of the chemotherapeutic agent docetaxel and the EGFR-TKI erlotinib. We hypothesized that the distinct mechanism of action of bevacizumab, leading to

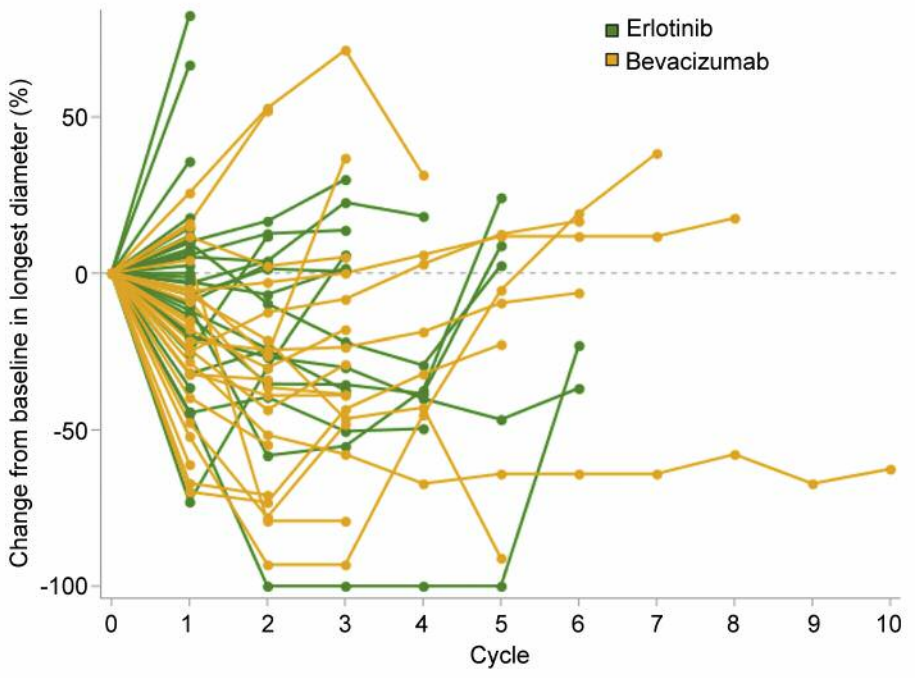

Figure 2. *Spider plot showing the change from baseline in longest diameter over time.*

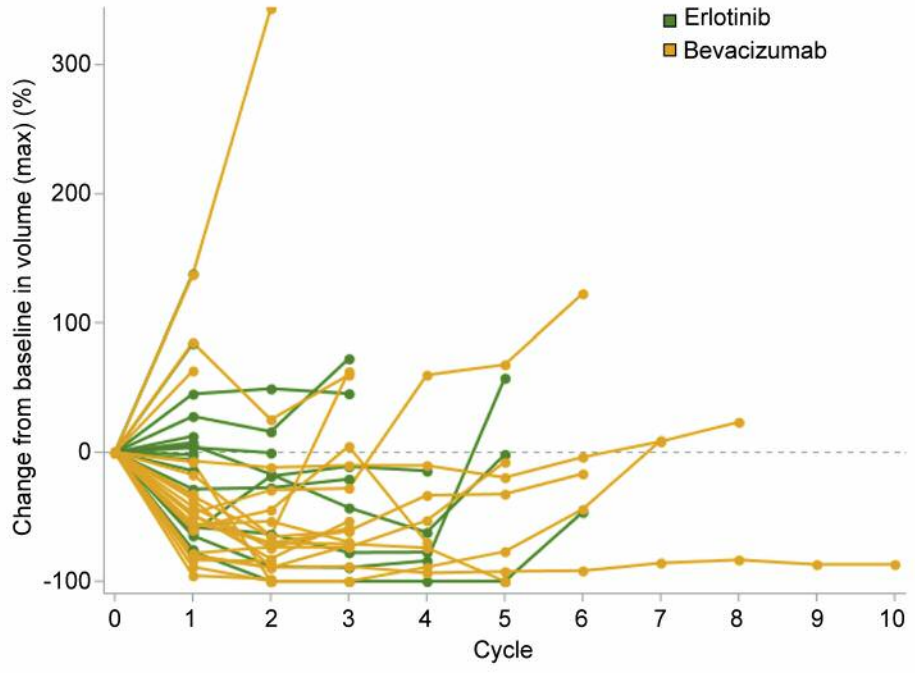

Figure 3. *Spider plot showing the change from baseline in volume (max) over time.*

tumor shrinkage due to internal ischemic necrosis, would affect different tumor metrics, compared to docetaxel and erlotinib, which are known to act more in a cytostatic and not cytotoxic way. We found that patients who received bevacizumab had improved tumor metrics at best response compared to patients treated with erlotinib, as well as in the evaluation between baseline and the first, second and third treatment course. Moreover, there was a striking difference

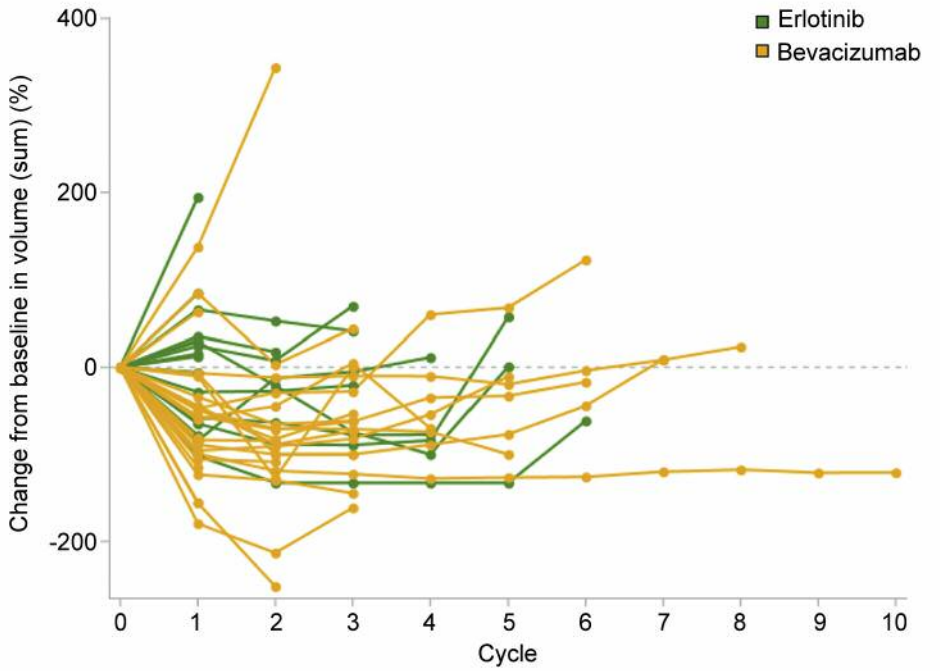

Figure 4. *Spider plot showing the change from baseline in volume (sum) over time.*

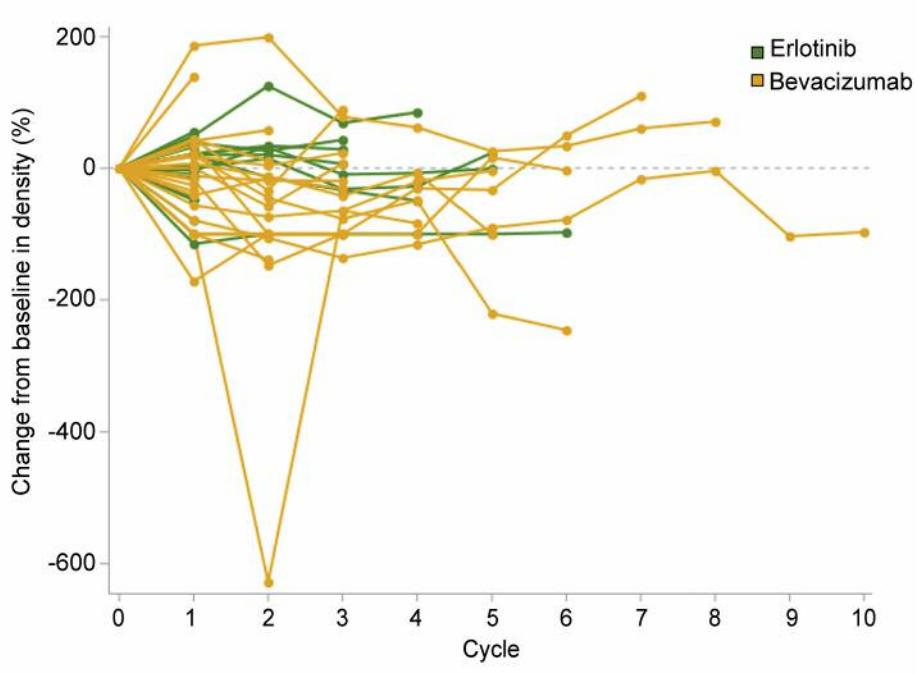

Figure 5. *Spider plot showing the change from baseline in density over time.*

between baseline tumor parameters and metrics at best response in favor of bevacizumab, although these metrics were also improved for the erlotinib arm between the first cycle and the best response. To our knowledge, this is the first trial evaluating tumor metrics under bevacizumab treatment, with indirect comparison to other treatment modalities.

Our results are in accordance with the presumed mechanism of action of bevacizumab: A growing body of

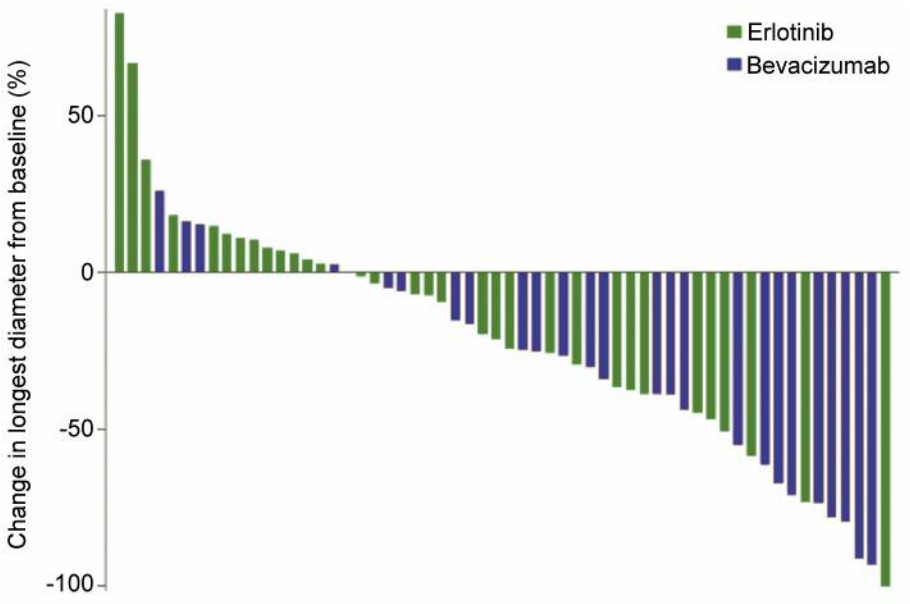

Figure 6. *Waterfall plot showing the change in the longest diameter from baseline to best response.*

evidence indicates that VEGF blockers have distinct mechanisms of action in the advanced macro- *versus* early micro-metastatic disease setting, a notion that is often poorly understood by many clinicians and only partially addressed in the clinical trial design. Bevacizumab's efficacy in metastatic disease is at least in part related to its synergy with cytotoxic agents (12). Several mechanisms have been proposed: First, since VEGF is a "survival factor" for endothelial cells, VEGF deprivation sensitizes tumor endothelial cells to pro-apoptotic effects of chemotherapy (13, 14). Second, chemotherapy also suppresses the mobilization of circulating cells that can contribute to tumor angiogenesis, including bone marrow-derived cells (BMDCs), circulating progenitor cells (CPCs), Tie-2-expressing monocytes, Gr1+CD11b+ myeloid cells, macrophages and dendritic cells (15). Since VEGF recruits these cells, combining VEGF inhibition with cytotoxic chemotherapy may have an additive suppressive effect. Third, another mechanism that could explain this synergy relates to findings that VEGF blockade promotes (at least during a transitory window) normalization of the leaky, tortuous and dilated tumor vasculature, thereby enabling improved delivery and efficacy of cytotoxic chemotherapy (16). Therefore, bevacizumab is expected, alone or in combination with chemotherapy to increase tumor necrosis and thus to reduce tumor viability and density in imaging modalities.

Similarly as for macrometastatic cancer, bevacizumab might also synergize with chemotherapy in the micrometastatic environment through the above described mechanisms. Anti-VEGF treatment may prevent "awakening" and expansive growth of dormant tumor cells in premetastatic niches by blocking the formation of new vessels. By producing VEGF and other pro-angiogenic molecules, residual tumor cells can induce an "angiogenic switch" in avascular micrometastases that is necessary to convert them into macrometastases (17). Micrometastases might be more sensitive to VEGF depletion than large metastases, where VEGF is only one of the multiple factors perpetuating tumor angiogenesis (18). Finally, anti-VEGF treatment could block tumor dissemination and inhibit early growth of micrometastatic lesions: Indeed, by increasing vascular permeability and reducing vessel coverage with pericytes, VEGF could promote tumor cell intra- and extravasation (19).

Being a proof-of-concept study, our trial harbors several caveats: First, due to the prospective nature the DOPERLO study, a number of patients showed low compliance with the scheduled re-evaluation CT and MRI scans, thus reducing the number of patients who were eligible for full evaluation. This fact forced us to include more patients receiving bevacizumab plus platinum-based chemotherapy that did not participate in a clinical protocol. Still, the number of patients for the final analysis is relatively low. Second, the comparison between the two treatment arms (bevacizumab and erlotinib) is indirect, since this was not a randomized clinical trial between these two treatment arms and no formal comparison can be performed. Third, the combination of docetaxel with erlotinib renders the comparison between the two targeted agents (bevacizumab as an anti-VEGF and erlotinib as an anti-EGFR) difficult, since the effect on tumor metrics in this arm is the synergistic result of both the

chemotherapeutic agent docetaxel and erlotinib. However, the prospective-retrospective nature of the trial and the strict pre-specified criteria for comparison of tumor metrics between the two study groups, render the results of this study at least hypothesis-generating.

In conclusion, we found that among patients with advanced NSCLC, treatment with bevacizumab results in a tumor response pattern which is characteristic of internal necrosis and shrinkage, compatible with the drug's mechanism of action. Bevacizumab substantially improved tumor metrics, including size, volume and density, between baseline and each cycle of treatment, as well as between baseline and best response, compared indirectly with a patient population receiving erlotinib plus docetaxel. These results require validation within the context of a prospective randomized clinical trial, in order to confirm the distinct mechanism of action of bevacizumab in patients with advanced NSCLC.

Conflicts of Interest

Epaminontas Samantas: Advisory Board of Merck, MSD, Asta-Zeneca, Roche, Amgen and Genesis. Paris A. Kosmidis: Honoraria: Novartis, MSD, Pfizer. Travel: Pfizer, MSD, Genesis. George Fountzilas: Advisory Board of Pfizer, Sanofi and Roche. Honoraria from Astra-Zeneca.

Authors' Contributions

Giannis Mountzios: Writing-original draft preparation. Xanthippi Mavropoulou: conceptualization, writing-original draft preparation. Georgia-Angeliki Koliou: formal analysis, writing-original draft preparation. Helena Linardou: resources. Epaminontas Samantas: resources. Paris A. Kosmidis: resources. George Fountzilas: resources. Aphrodite Charitandi: resources. Anna Kalogera-Fountzila: conceptualization, supervision, writing-original draft preparation. Writing-review and editing: All authors.

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