ANTIANGIOGENIC EFFECT OF TAMOXIFEN COMBINED WITH EPIRUBICIN IN BREAST CANCER PATIENTS.

Teresa Mele^{1,2}, Daniele Generali¹, Stephen Fox⁶, Maria Pia Brizzi², Alessandra Bersiga³, Manuela Milani¹, Giovanni Allevi¹, Simone Bonardi¹, Sergio Aguggini¹, Marco Volante⁴, Luigi Dogliotti², Alberto Bottini¹, Adrian Harris⁵, Alfredo Berruti².

- 1. Breast Unit, Azienda Ospedaliera Istituti Ospitalieri di Cremona, Viale Concordia 1, 26100 Cremona, Italy**
- 2. Oncologia Medica, Dipartimento di Scienze Cliniche e Biologiche Università di Torino, Azienda Ospedaliera Universitaria San Luigi di Orbassano, Regione Gonzole 10, 10043 Orbassano, Italy**
- 3. Dipartimento Anatomia Patologica Azienda Ospedaliera Istituti Ospitalieri di Cremona, Viale Concordia 1, 26100 Cremona, Italy**
- 4. Dipartimento Anatomia Patologica Università di Torino, Azienda Ospedaliera San Luigi di Orbassano, Regione Gonzole 10, 10043 Orbassano, Italy**
- 5. Weatherall Molecular Oncology Laboratories, Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford Headley Way, OX3 9DS, UK**
- 6. Peter Mac Callum Cancer Centre, St Andrews Place, East Melbourne, Victoria, 3002 Australia**

Correspondence to:

Teresa Mele

Oncologia Medica

Azienda Ospedaliero Universitaria

San Luigi Gonzaga

Regione Gonzole 10

10043 ORBASSANO

Italy

Abstract

Background: Vascular endothelial growth factor A (VEGF-A) and vascular endothelial growth factor receptor 2 (VEGFR2) are key factors mediating neo-vascularization. They are often coexpressed in breast cancer. Sex steroids may stimulate angiogenesis via the estrogen receptor (ER) pathway. We investigated the effects of the addition of tamoxifen to epirubicin versus epirubicin alone on VEGF and VEGFR2 expression in breast cancer patients.

Patients and methods: The expression of VEGF and VEGFR2 was assessed on tissue microarray by immunohistochemistry at baseline conditions and after treatments in 191 patients with T2-4 N0-1 breast cancer enrolled in a randomized trial comparing four cycles of single agent epirubicin versus epirubicin plus tamoxifen as primary systemic treatment.

Results: Epirubicin alone failed to induce changes in VEGF expression ($p=0.54$), while the addition of tamoxifen to epirubicin resulted in a significant reduction in VEGF expression ($p<0.001$). As a consequence baseline VEGF had a negative prognostic role in patients who received epirubicin alone but not in patients receiving epirubicin plus tamoxifen (interaction test $p<0.05$). VEGFR2 expression increased at residual tumor histology in both treatment arms, with a lesser extent in patients receiving tamoxifen plus epirubicin. Decrease in VEGFR2 expression was significantly associated with response rate ($p=0.02$).

Conclusion: The addition of tamoxifen to epirubicin resulted in a suppression of a key angiogenic pathway. These data suggest a potential synergism of these two drugs.

INTRODUCTION

Chemotherapy and endocrine therapy are the standard treatments of breast cancer with positive hormonal receptors and their association is known to be more efficacious than either treatment alone in terms of disease free survival (DFS) and overall survival (OS) [1] [2].

Chemotherapy followed by endocrine therapy is now recognized as the optimal method to combine the two treatment strategies. A multicenter prospective American trial comparing FEC with concomitant Tamoxifen *versus* sequential Tamoxifen administration, demonstrated that concomitant administration is detrimental in terms of DFS (INT 100 trial [3]).

Whilst acknowledging that chemotherapy and endocrine therapy have different mechanisms of action, pharmacological targets and toxicity patterns, it is still possible that concomitant administration could be helpful since tamoxifen has effects besides inhibition of proliferation. Numerous animal models of breast cancer have previously demonstrated a pro- effect of estrogens and an anti-angiogenic effect of tamoxifen *in vivo* [4]; [5]; [6]; [7]; [8]; [9]. Estradiol increases extracellular levels of vascular endothelial growth factor (VEGF) while tamoxifen inhibits the secretion of VEGF in breast cancer *in vivo* [8]. These data suggest that VEGF expression in breast is at least in part modulated by estrogen receptor, and VEGF gene is a target of for estrogen receptor [10].

VEGF-A, a key mediator of tumour angiogenesis, is known to exert its angiogenic effects via two tyrosine kinase receptors, VEGFR-1 (flt-1) and VEGFR-2 (flk-1/KDR) [11];[12];[13];[14];[15];[16]. These receptors regulate physiological as well as pathological angiogenesis. VEGFR2 signalling after its phosphorylation plays a crucial role in VEGF-A signalling mediating cell proliferation, survival, migration, and actin reorganization through a variety of signalling cascades including MEK/ERK and PI3-kinase/AKT [11][17]. To our

knowledge it is unknown whether the concomitant administration of tamoxifen with cytotoxic therapy might result in a greater anti-angiogenic activity.

Primary systemic antineoplastic therapy is an excellent method to explore the effects of treatment on tumor biology and represents the ideal model to assess drug interactions within tumoral microenvironment. In the present study, we evaluated VEGF and VEGFR2 tissue expression before and after treatment in a series of breast cancer patients enrolled in a prospective randomized trial comparing single agent epirubicin versus epirubicin plus tamoxifen as primary systemic therapies [18]. The primary aim of the study was to evaluate the effect of study treatments on these two markers, secondary aims were: a) to correlate angiogenic markers with clinical and biological features, b) to correlate the changes of angiogenic markers with tumor response, c) to evaluate the prognostic role of VEGF and VEGFR2 assessed either at baseline or at post chemotherapy residual histology.

PATIENTS AND METHODS

Patients

Patients with T2-4 N0-1 breast cancer were recruited to a randomized trial comparing single agent epirubicin (EPI arm) versus epirubicin plus tamoxifen (EPI-TAM arm) as the primary systemic treatment [18]. They were required to have an Eastern Cooperative Oncology Group performance status < 2 , adequate bone marrow reserve (WBC count $> 3.5 \times 10^9/L$, platelets $> 100 \times 10^9/L$, and haemoglobin $> 10 \text{ g/dl}$), hepatic function (AST, ALT, bilirubin, and alkaline phosphatase levels < 1.25 the upper limit of normal value) and renal function (serum creatinine < 1.25 the upper limit of normal value). Patients were accrued from January 1997 to December 2001. The study was approved by the Institutional Investigations Committee. All patients gave written informed consent to the

diagnostic procedures, the proposed treatment, and the biological evaluations. Two-hundred and eleven patients were enrolled, 105 were randomized to receive epirubicin alone, and 106 were randomized to receive epirubicin plus tamoxifen. On first presentation, an incision biopsy was done on each patient and a small tissue sample (0.5-0.8 cm) was removed. Chemotherapy was started within 2 days of diagnosis. Patients in the EPI arm received 60 mg/m² of epirubicin (Farmorubicina, Pharmacia, Milan, Italy) by slow i.v. push on days 1 and 2; whereas patients on the EPI-TAM arm received 60 mg/m² of epirubicin by slow i.v. push on days 1 and 2 and 30 mg of tamoxifen (Kessar, Pharmacia) daily. Epirubicin injections were repeated every 21 days for three or four cycles before definitive surgery, whereas tamoxifen was given continuously until definitive surgery. All patients postoperatively received four cycles of the CMF regimen [i.v. cyclophosphamide (600 mg/m²), i.v. methotrexate (40 mg/m²), and i.v. 5-fluorouracil (600 mg/m²) on days 1 and 8, every 28 days]. Patients with estrogen receptor (ER) positive primary tumor in both treatment arms received tamoxifen (20 mg, i.e., lower than the primary dose) starting after surgery, up to progression or for a maximum of 5 years. The median follow up of patients was 53 months (range 13-95).

Treatment evaluation

Each month the size of the primary tumor and the size of the axillary lymph nodes, when appreciable, were measured by the same clinician using a caliper. Response was assessed before definitive surgery by the clinical measurement of the changes in the product of the two largest diameters recorded in two sequential evaluations. According to WHO Criteria, tumor progression was defined as an increase of at least 25% in tumor size; stable disease as an increase of less than 25% or a reduction less than 50%; partial response as tumor shrinkage greater than 50%; and complete response as the complete disappearance of any clinical sign of disease. Pathological complete response was defined

as the complete absence of neoplastic cell either in the breast or in the axillary lymph nodes.

Surgery was performed after primary chemotherapy and clinical reassessment (quadrantectomy or modified radical mastectomy, together with axillary node dissection, when indicated). If subjected to quadrantectomy, the patients underwent irradiation of residual breast.

Histopathologic and immunohistochemistry.

Tumour grade was evaluated using the Nottingham prognostic index [19]. Immunohistochemical evaluation was done on paraffin embedded tumor samples obtained at diagnosis and at definitive surgery. Bcl-2, p53, ER, PgR, HER2 and Ki67 staining were done at the Pathology Unit of Azienda Ospedaliera Istituti Ospitalieri of Cremona, Italy as described elsewhere [20]. All staining was scored by counting the number of positively stained cells and expressed as a percentage of the total tumor cells (at least 1000) counted across several representative fields of the section using a standard light microscope equipped with a 10 x 10 square graticule. Reproducibility of counting was assessed by a second investigator rescoring 10 slides. The relative intensity of ER and PgR staining was assessed in a semiquantitative fashion, incorporating both the intensity and distribution of specific staining. A value (HSCORE) was derived from the sum of the percentages of positive-stained epithelial cells multiplied by the weighted intensity of staining. Specimens were deemed receptor positive if the HSCORE was greater than 100 [20].

Immunohistochemistry for angiogenesis markers (HIF1 α (hypoxia inducible factor 1- α) and Ca IX (carbonic anhydrase IX), VEGF, VEGFR2) was performed on 5- μ sections of tissue microarray containing two 1-mm diameter cores taken from selected morphologically

representative tumor regions from the incisional biopsy and from tumor remaining at definitive surgery. Quality control was assessed on each block by hematoxylin and eosin staining. The Envision HRP kit (Dako; Cambridgeshire, United Kingdom) system was used for subsequent visualization [21],[22].

The first step consisted on an antigen-retrieval procedure, by heating a tissue section in a citrate buffer. The primary antibodies were: VEGF (VGI) (Oxford University) dilution 1 : 4, overnight incubation at room temperature, KDR (34a) (Oxford University) dilution 1 : 2, overnight incubation at room temperature. All sections had a negative control slide (no primary antibody) of an adjacent section to preclude nonspecific staining. Positive controls included breast carcinomas known to exhibit high levels of each marker. A single pathologist, blinded to patient outcome and to the origin of the samples, used a semi-quantitative method. Intensity was semi-quantitatively assessed: 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining) for VEGF, VEGFR2 (*Figure 2*).

The immunohistochemical analysis of the angiogenesis markers was performed at the Weatherall Institute of Molecular Medicine of John Radcliffe Hospital of Oxford, UK.

Statistical methods

For statistical computations VEGF and VEGFR2 expression were considered either as discrete variables or dichotomized as follows: low levels, score 0,1, high levels, score 2,3. Comparison of categorized variables was done by χ^2 or χ^2 for trend test when indicated. Mann-Whitney U test or Wilcoxon rank sum test for non parametric data were used, when indicated, to compare continuous variables. Spearman test for non parametric data was employed to analyze the correlations between variables. Disease free survival (DFS) and overall survival (OS) curves were estimated using the Kaplan-Meier method and compared using the log rank test.

Cox proportional hazard models were used to estimate hazard ratios (HR) for the role of VEGF and VEGF2R expression in predicting disease progression or death. Cox models and logistic regression models were used to assess the presence of heterogeneity in the effect of VEGF and VEGF2R expression on disease progression and disease response, respectively, in patient subgroups defined by treatment randomization, by including in the model the appropriate treatment/covariate interaction terms. This procedure is a test of the homogeneity of the hazard ratios associated with VEGF and VEGF2R between strata defined by treatment arm.

Missing data were dealt with by excluding patients from particular analyses if their files did not contain data on the required variables. All P values reported are the result of two-sided tests. P values of less than 0.05 were considered to indicate statistical significance. Statistical computation was done by SPSS for Windows software (version 16.0).

RESULTS

Patient characteristics.

191 out of 211 (90.52%) patients prospectively enrolled in the trial were evaluable for VEGF, and VEGFR2 expression. Patient's characteristics and expression levels of each parameter are shown in *Table 1*. 91 patients (47.6%) were randomized to the EPI arm and 100 patients (52.4%) were randomized to the EPI-TAM arm respectively. As outlined in the Consort diagram (*Figure 1*), VEGF was evaluated at baseline excision in 160 patients (83,7%) and VEGFR2 in 171 (89,5%), respectively. The corresponding evaluation at residual histology was 143 (74,8%) for VEGF and 137 (71,7 %) for VEGFR2.

Relationship between VEGF, and VEGFR2 expression and clinico-pathological variables.

In a univariate analysis, VEGF at baseline significantly correlated with HIF1- α ($p < 0.0001$), whereas it failed to show any relationship with T-N status, grading, hormonal receptor status, p53, her-2, bcl2, Ki67 and CA IX (all $p > 0.05$). (*Table 2*). Baseline VEGFR2 failed to show any significant relationship with the clinical and biological parameters considered (all $p > 0.05$). A significant but weak relationship was found between VEGF and VEGFR2 expression (Spearman R 0.55, $p < 0.01$).

VEGF and VEGFR2 expression and prediction of response to treatment.

Among the 191/192 patients with evaluable histological samples, 35 (18.3%) had a complete clinical response, 112 (58.6%) had a partial clinical response and 43 (22.5%) had no response. VEGF and VEGFR2 expression showed no association with overall clinical response and clinical complete response in all cases and or in each treatment arms (data not shown).

Effect of treatment on VEGF and VEGFR2 expression.

The effect of epirubicin plus/minus tamoxifen on neoangiogenesis marker expression was assessed in patients with matched tumor samples before and after treatment.

VEGF expression significantly decreased after treatment in overall patients ($p < 0.02$) (data not shown). Stratifying patients according to treatment arm, VEGF expression significantly decreased in the EPI-TAM arm ($p < 0.01$) whereas it did not significantly change in the EPI arm ($p = 0.54$) (*Table 3*). As a consequence of these changes, while baseline VEGF expression did not differ between the two arms (chi square for trend $p = 0.83$), VEGF at residual histology was significantly higher in EPI patients as opposed to EPI-TAM patients (chi square for trend $p = 0.035$).

VEGFR2 expression, conversely, significantly increased after treatment in all patients ($p < 0.002$) (data not shown), and in patients the EPI arm and EPI-TAM arm ($p < 0.02$ and $p = 0.03$ in, respectively) (*Table 3*). Baseline VEGFR2 expression did not differ between the two arms (chi square for trend $p = 0.115$), whereas VEGFR2 expression at residual histology was significantly higher in patients randomized in the EPI arm as opposed to those randomized in the EPI-TAM arm ($p = 0.045$).

A similar pattern of VEGF and VEGFR2 expression before and after treatments was also observed in patients with estrogen receptor positive tumors only. Unfortunately the number of patients with ER negative tumors is too few to assess whether in this subset there is a different behaviour of angiogenic markers or not.

Relationship between changes in VEGF and VEGFR2 expression and clinical response.

VEGF expression in overall patients decreased in 44 patients (37.3%) while no change or increase was observed in 74 patients out of 118 (62.7%).

As shown in *Table 4*, no significant difference was observed in the distribution of VEGF decrease according to disease response, in all patients. Similar results were observed in both treatment arms.

VEGFR2 expression in all patients decreased in 27 out of 121 patients (22.3%) whilst there was no change or an increase in 94 (77.7%). The proportion of patients showing decreased VEGFR2 expression was greater in responding patients as opposed to non-responders ($p = 0.02$). This was statistically significant in the EPI-TAM arm ($p = 0.05$) and not in the EPI arm ($p = 0.26$) (*Table 4*), but no difference between the 2 arms was observed at the interaction test ($p = 0.54$).

VEGF and VEGFR2 expression and disease outcome.

VEGF expression at baseline was significantly associated with a shorter disease free survival ($p=0.047$), whereas VEGFR2 showed no association ($p=0.65$) (*Figure 3a and 3b*). Conversely, high VEGFR2 expression ($p=0.032$) but not high VEGF ($P=0.66$) at post chemotherapy residual histology, was significantly predictive of poor disease free survival (*Figures 3c and 3d*).

Dividing patients according to the treatment arms, the negative prognostic role of VEGF expression at baseline conditions was evident in patients randomized in the EPI arm but not in those randomized in the EPI-TAM arm (interaction test, $p<0.05$) (*Figure 4*). No interaction between treatment arms was observed for VEGFR2 at baseline and VEGF and VEGFR assessed at post chemotherapy residual histology.

Both VEGF and VEGFR2, either at baseline condition or at residual tumor histology, did not show any relationship with overall survival (data not shown).

Discussion

The present study aimed to investigate prospectively the effects of tamoxifen administered in association with epirubicin on angiogenic markers in a randomized trial of primary systemic therapy. Although the relationship between VEGF and tamoxifen has previously been explored in various other clinical settings, the effects of chemotherapy plus tamoxifen on angiogenesis and a comparison of these effects with chemotherapy alone have not been studied. Matched tumor samples from the neoadjuvant prospective trial of epirubicin plus minus tamoxifen has allowed an unparalleled opportunity to analyse changes in tissue expression of VEGF and VEGFR2.

These results clearly demonstrate that epirubicin administered at the conventional maximum dose of 120 mg/m² every 21 days has no anti-angiogenic effect since there was

no change in VEGF expression after treatment. However, the addition of tamoxifen to epirubicin resulted in a significant anti-angiogenic activity with a reduction in VEGF expression. Furthermore, VEGFR2 expression at post chemotherapy residual histology, were significantly lower in patients randomized to receive epirubicin plus tamoxifen compared with patients who received epirubicin alone. The contrasting changes in VEGF and VEGFR2 expression before and after treatment could constitute, a feed back loop with loss of VEGF ligand, relating in part to the increased VEGFR2 expression at residual tumor histology.

VEGF and VEGFR2 assessed at baseline were not predictive of subsequent clinical response and failed to demonstrate a predictive role of angiogenesis markers for tumor response to chemotherapy as already described [23][24][25]. Treatment-induced-decrease in tissue VEGF expression also did not correlate with tumor response either in overall cases or stratifying patients according to treatment arm. However, a decrease in VEGFR2 was more frequently associated with disease response than no change or increase. In this trial the addition of tamoxifen to epirubicin resulted in a greater antitumor activity than epirubicin alone [18], and it is possible that these results at least in part are due to a greater anti-angiogenic activity of the combination therapy.

VEGF expression has been repeatedly correlated with poor outcome of breast cancer patients. Our data confirm previously published papers [26] [27] [28] [29] [30] [31] [32] since high expression of VEGF was predictive of shorter DFS. VEGF expression at residual histology, however, was not associated with prognosis. Conversely, VEGFR2 expression at post treatment residual histology was significantly associated with short DFS but did not have any prognostic effect when assessed at baseline. The different behavior of these two markers before and after treatment (i.e a decreasing trend for VEGF and

increasing trend for VEGFR) might account for the different prognostic significance of VEGF and VEGFR2 observed at baseline and post treatment.

Interestingly, dividing patients according to treatment arm the negative prognostic value of elevated VEGF expression at baseline was evident in the EPI patients but not in the EPI-TAM patients. These data suggest the reduction in VEGF induced by tamoxifen may have modified tumour growth in those randomized to the EPI-TAM arm. Conversely no interaction with the treatment arm was observed as far VEGFR2 expression either at baseline or after treatment. It is reported that tamoxifen has both an estrogen and an estrogen-independent effect on angiogenesis. Tamoxifen inhibits angiogenesis in a chick egg chorioallantoic membrane model. This inhibition is not altered by the presence of excess estrogens, suggesting that the mechanism is independent of tamoxifen's effect on the estrogen receptor [5]. This interesting issue could not be explored in our series, since, due to the low number of patients with ER negative tumors, we were unable to evaluate the anti-angiogenic effect of tamoxifen in this subset.

Although a trend with overall survival was observed, due to the low number of events observed in this trial, both markers failed to be significantly associated with overall survival.

In conclusion, the addition of tamoxifen to epirubicin resulted in an anti-angiogenic activity and supports, at least in part, the greater activity of the combination observed in the clinical trial [18]. This result seems to be opposite to the INT100 data showing a negative interaction of tamoxifen with chemotherapy. The different chemotherapy employed in the two studies, FEC vs single agent epirubicin, the greater epirubicin dose employed in our study and the possible interference of TAM in the metabolism of cyclophosphamide in the INT100 study could have accounted for these discrepancies. Nevertheless, this study provides further support for a link between VEGF and the

estrogen receptor pathways suggesting that further exploration of the interaction between chemotherapy and endocrine therapy administered concomitantly is warranted. It will be of interest to evaluate whether aromatase inhibitors have similar effects.

Legends to Figures

Figure 1: Consort diagram

Figure 2: Immunohistochemistry of VEGF: strong staining (2a), moderate/weak staining (2b) or negative (2c), and VEGFR2: strong staining (2d), moderate/weak staining (2e) or negative (2f)

Figure 3: Disease free survival according to VEGF expression at baseline (3a) and residual tumor histology (3b), and VEGFR2 expression at baseline (3c) and residual tumor histology (3d)

Figure 4: Prognostic role for DFS of VEGF and VEGFR2 expression either at baseline or at post chemotherapy histology, dividing patients according to treatment arm. P values refer to an interaction test.

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Figure 1. Consort diagram

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Author(s): Teresa Mele

Author's signature: Teresa Mele

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Table 1 Patient characteristics and biological markers at baseline

Patients	91	100
	Epi	Epi + Tam
Clinical Characteristics		

T2	71 (78,0%)	77 (77%)
T3-T4	20 (21,9%)	23 (23%)
N0	51 (56,0%)	58 (58%)
N1	40 (43,9%)	42 (42%)
Ca IX +	20 (21,9%)	21 (21%)
Hormonal Status		
ER +	72 (79,1%)	78 (78%)
	19 (20,9%)	21 (21%)
Pgr+	40 (43,9%)	52 (52%)
	51 (56,0%)	47 (47%)
missing		1
Biological Characteristics		
G2	25 (27,47%)	24 (24%)
G3	64 (70,33%)	74 (74%)
Ki67 < 10	24 (26,37%)	26 (26%)
Ki67 11-29	50 (54,94%)	58 (58%)
Ki67 >30	17 (18,68%)	16 (16%)
Her2+	20 (21,9%)	30 (30%)
p53+	45 (49,45%)	50 (50%)
Bcl2	65 (71,43%)	73 (73%)
Angionenesis		
HIF1 = 0	14 (15,4%)	19 (19%)
HIF1 >= 1	70 (76,9%)	68 (68%)
VEGF = 0	14 (15,4%)	18 (18%)
VEGF = 1	21 (23,08%)	18 (18%)
VEGF = 2	18 (19,78%)	19 (19%)
VEGF = 3	28 (30,77%)	24 (24%)
VEGFR2 = 0	3 (3,29%)	10 (10%)
VEGFR2 = 1	27 (29,67%)	30 (30%)
VEGFR2 = 2	18 (19,78%)	22 (22%)
VEGFR2 = 3	35 (38,46%)	26 (26%)

Table 2. Distribution of prognostic variables and immuno-histochemical features according to VEGF and VEGFR2 expression

VEGF	0	1	2	3	p
T ₁₋₂	26/32(81.25%)	32/39(82.05%)	27/37(72.97%)	39/52(75.00%)	0.358
T ₃₋₄	6/32(18.75%)	7/39(17.95%)	10/37(27.03%)	13/52(25.00%)	
N ₀	20/32 (62.50%)	25/39(64.10%)	15/37(40.54%)	29/52(55.77%)	0.282
N ₁	12/32(37.50%)	14/39(35.90%)	22/37(59.46%)	23/52(44.23%)	
G2	7/32(21.88%)	16/39(41.03%)	5/36(13.89%)	14/50(28.00%)	0.771
G3	25/32 (78.13%)	23/39(58.97%)	31/36(86.11%)	36/50(72.00%)	
ER -	4/32 (12.50%)	8/39 (20.51%)	6/37 (16.22%)	12/51(23.53%)	0.296
ER +	28/32(87.50%)	31/39(79.49%)	31/37(83.78%)	39/51(76.47%)	
PgR -	15/32(46.88%)	23/39(58.97%)	15/37(40.54%)	27/51(52.94%)	0.981
PgR +	17/32(53.13%)	16/39(41.03%)	22/37(59.46%)	24/51(47.06%)	
p53	20/32(62.50%)	18/39(46.15%)	18/36(50.00%)	23/52(44.23%)	0.176
her-2	6/32(18.75%)	10/39(25.64%)	9/37(24.32%)	16/52(30.77%)	0.256
bcl2	24/31(77.42%)	29/39(74.36%)	32/37(86.49%)	34/52(65.38%)	0.304
HIF1- α	20/30(66.67%)	27/39(69.23%)	33/37(89.19%)	50/52(96.15%)	0.00006
CA IX	6/28 (21.43%)	7/38 (18.42%)	10/36(27.78%)	15/50(30.00%)	0.239
Topo2A	1/21(4.76%)	3/23(13.04%)	16/27(59.26%)	27/46(58.70%)	0.000001
Ki67	21.53	22.05	24.62	19.44	0.64
95 % IC	(16.12 – 26.94)	(15.40 - 28.70)	(17.45 - 31.79)	(14.89 - 23.99)	
VEGFR2	0	1	2	3	p
T ₁₋₂	11/13 (84.62%)	45/57 (78.95%)	30/40 (75%)	44/61 (72.13%)	0.257
T ₃₋₄	2/13 (15.38%)	12/57 (21.05%)	10/40 (25%)	17/61 (27.87%)	
N ₀	7/13 (53.85%)	34/57 (59.65%)	23/40 (57.5%)	34/61 (55.74%)	0.828
N ₁	6/13 (46.15%)	23/57 (40.35%)	17/40 (42.5%)	27/61 (44.26%)	
G2	5/13 (38.46%)	11/53 (20.75%)	10/40 (25%)	17/61 (27.87%)	0.91
G3	8/13 (61.54%)	42/53 (79.25%)	30/40 (75%)	44/61 (72.13%)	
ER -	2/13 (15.38%)	13/57 (22.81%)	8/40 (20%)	10/60 (16.67%)	0.619
ER +	11/13 (84.62%)	44/57 (77.19%)	32/40 (80%)	50/60 (83.33%)	
PgR -	5/13 (38.46%)	31/57 (54.39%)	24/40 (60%)	27/60 (45%)	0.729
PgR +	8/13 (61.54%)	26/57 (45.61%)	16/40 (40%)	33/60 (55%)	
p53	7/13 (53.85%)	36/57 (63.16%)	14/39 (35.9%)	28/61 (45.9%)	0.089
her-2	3/13 (23.08%)	15/57 (26.32%)	10/40 (25%)	17/61 (27.87%)	0.754
bcl2	10/13 (76.92%)	40/56 (71.43%)	30/40 (75%)	46/61 (75.41%)	0.782
HIF1- α	7/11 (63.64%)	44/54 (81.48%)	35/39 (89.74%)	51/60 (85%)	0.171
CA IX	4/12 (33.33%)	12/53 (22.64%)	14/39 (35.9%)	10/58 (17.24%)	0.327
Topo2A	1/8 (12.5%)	6/32 (18.75%)	14/30(46.67%)	27/54 (50%)	0.002
Ki67	25	22.82	21.55	21.72	0.29
95 % IC	(8.19 – 41.80)	(18.34 – 27.30)	(14.04 - 29.05)	(17.34 - 26.10)	

Table 3. Effect of treatment on VEGF and VEGFR2 expression

EPI				EPI-TAM		
	Baseline	Post-treatment	p *	Baseline	Post-treatment	p *

VEGF	n = 63	n = 63	0.54	n = 55	n = 55	<0.01
0	11 (17.46%)	5 (7.94%)		13 (23.64%)	12 (21.82%)	
1	14 (22.22%)	22 (34.92%)		8 (14.54%)	23 (41.82%)	
2	14 (22.22%)	21 (33.33%)		13 (23.64%)	9 (16.36%)	
3	24 (38.10%)	15 (23.81%)		21 (38.18%)	11 (20.0%)	
VEGFR2	n = 62	n = 62	<0.02	n = 59	n = 59	0.03
0	3 (4.84%)	2 (3.23%)		7 (11.87%)	5 (8.47%)	
1	17 (27.42%)	5 (8.06%)		17 (28.81%)	7 (11.87%)	
2	15 (24.19%)	19 (30.65%)		17 (28.81%)	23 (38.98%)	
3	27 (43.55%)	36 (58.06%)		18 (30.51%)	24 (40.68%)	

* Wilcoxon rank sum test

Table 4. Relationship between changes in VEGF and VEGFR2 expression and clinical response.

	NR	PR	CR	p
VEGF decrease				
Overall	11/29 (37.93%)	26/76 (34.21%)	7/13 (53.85%)	0.521
EPI arm	7/20 (35.0%)	12/37 (32.43%)	3/6 (50.0%)	0.699
EPI-TAM arm	4/9 (44.44%)	14/39 (35.90%)	4/7 (57.14%)	0.685
VEGFR2 decrease				
Overall	2/30 (6.67%)	20/76 (26.32%)	5/15 (33.33%)	0.020
EPI arm*	2/21 (9.52%)	9/36 (25.0%)	1/5 (20.0%)	0.260
EPI-TAM arm*	0/9 (0%)	11/40 (27.50%)	4/10 (40.0%)	0.05

*Interaction test p=0.54