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Report

Tumor-specific VEGF-A and VEGFR2 in postmenopausal breast cancer patients with long-term follow-up. Implication of a link between VEGF pathway and tamoxifen response

Lisa Rydén[1], Maria Stendahl[1], Håkan Jonsson[2], Stefan Emdin[2], Nils O Bengtsson[2], and Göran Landberg[1]
[1]*Division of Pathology, Department of Laboratory Medicine, Lund University, Malmö University Hospital, Malmö;* [2]*Departments of Oncology and Surgery, Umeå University, Umeå, Sweden*

Key words: adjuvant tamoxifen treatment, breast cancer, treatment prediction, VEGF-A, VEGFR2

Summary

Vascular endothelial growth factor (VEGF-A) is considered a prognostic indicator for clinical outcome in breast cancer. Conflicting results nevertheless exist and there is a need for larger studies including untreated patients in order to clarify the importance of tumor-specific VEGF-A regarding prognosis as well as potential links to predictive treatment information. VEGF-A and its receptor, vascular endothelial growth receptor 2 (VEGFR2), were therefore analyzed by immunohistochemistry in postmenopausal breast cancers enrolled in a clinical trial where patients were randomized to adjuvant tamoxifen treatment ($n = 124$) for 2 years or no treatment ($n = 127$) with a median follow-up of 18 years. The tumors were arranged in a tumor tissue microarray system enabling parallell analysis of the angiogenic factors and hormone receptor status. Tumor-specific expression of VEGFR2 correlated strongly with expression of VEGF-A and progesterone receptor (PR) negativity, whereas VEGF-A was not associated with hormone receptor status. Among patients with estrogen receptor (ER) positive (fraction > 10%) tumors, there was a statistically significant tamoxifen response in VEGF-A negative tumors at both 10-year and 18-year disease-free survival (DFS), contrasting to VEGF-A positive tumors who had no beneficial effect of tamoxifen. A treatment-interaction variable indicated a marked difference in tamoxifen response depending on VEGFA-status in terms of DFS at 10 and 18 years of follow-up, $p = 0.046$ and $p = 0.039$, respectively. VEGFR2 status did not yield significant predicitve information for tamoxifen response in patients with ER fraction > 10%, whereas in patients with ER fraction > 90% both VEGF-A and VEGFR2 status were associated with tamoxifen treatment effect.

Introduction

Adjuvant tamoxifen treatment for at least 2 years increases disease-free survival (DFS) and overall survival in hormone responsive breast cancer irrespective of age [1].

In some patients, however, the endocrine therapy fails and the field of research exploring tamoxifen resistance is today trying to elucidate the mechanisms behind treatment failure. Growth factor receptors with intracellular tyrosine kinase activity have been shown to activate estrogen receptor (ER) transcription in a non-ligand-dependent way through activation of intracellular signaling pathways [2–4]. This opens up opportunities to block the cross-talk between growth factors and the ER at several levels [5]. For members of the epidermal growth factor family, i.e., EGFR and erbB2/HER-2, monoclonal antibodies directed towards the membrane-bound part of the receptor as well as tyrosine kinase inhibitors have been developed and are currently being tested in clinical trials. *In vitro* data suggests that combination therapy with the monoclonal antibody trastuzumab directed towards HER-2 together with tamoxifen suppresses tumor growth in a synergistic way [6].

Increasing cytosolic level of vascular endothelial growth factor (VEGF-A) in breast cancer has been associated with clinical aggressiveness and relapse of breast cancer [7, 8]. VEGF-A binds two receptors, vascular endothelial growth receptor 1 and 2 (VEGFR1 and VEGFR2), with tyrosine kinase activity, where VEGFR2 is the most important for proliferative activity [9]. The receptors are not specific for endothelial cells and have been localized on several epithelial tumor cells, among them breast cancer, supporting autocrine and paracrine roles for VEGF-A besides angiogenic stimulation [10–12]. Cytosolic VEGF-A is a strong prognostic factor in both early and advanced hormone receptor positive breast cancer disease treated with adjuvant tamoxifen [7, 8, 13, 14]. VEGF-A has therefore been suggested as a marker of response to adjuvant tamoxifen, although the biological mechanisms are not known. However, the association between VEGF-A and tamoxifen response has hitherto not been explored in any randomized trial of adjuvant tamoxifen.

The knowledge about adjuvant endocrine treatment in the elderly (over 65 years of age) is sparse due to a limited number of studies in this group of patients [15, 16]. The results from studies including older patients with breast cancer address the high rate of co-morbidity, which must be taken into account when overall survival is used as the primary end-point [17]. A recent publication of a randomized study, has demonstrated that 1 year of adjuvant tamoxifen in combination with prednisolone prolonged DFS in an elderly breast cancer population with long-term follow-up [16].

In this report, we have analyzed VEGF-A and its receptor VEGFR2 by immunohistochemistry in a tumor tissue microarray system of tumors from postmenopausal patients randomized to tamoxifen treatment for 2 years or no treatment. VEGF-A and VEGFR2 were semiquantitatively scored negative or positive according to cytoplasmatic staining intensity and related to disease-free overall survival at 10-year analysis and from the last follow-up. VEGF-A and VEGR2 were thus related to predictive information of tamoxifen response in randomized patients with ER-positive tumors and to prognostic information in the control arm which only had loco-regional treatment with a prolonged follow-up.

Material and methods

Patients

The patients included were enrolled in a clinical trial at Umeå University Hospital, Sweden, during 1980–1987 (Trial II), which was included in the Oxford meta-analysis [1]. The inclusion criteria were postmenopausal patients (>55 years) with stage II (pT1, pN1, pM0, pT2, pN0, pM0, pT2, pN2, pM0) invasive breast cancer. For patients older than 70 years, stage II and stage III breast cancer patients were included. All patients received radical surgery in the form of modified radical mastectomy. Patients were randomized to control or tamoxifen treatment (40 mg/day) for 2 years by the Regional Oncological Center and oral informed consent was registered on admission to the Department of Oncology. The study was approved by the Ethics Committee at the Umeå University 1980 as well as 2003 (the latter pertaining to modification regarding molecular markers used in tissue microarray). The median age was 66.5 years (55–75 years) at inclusion. Hormone receptor status was not determined at time of randomization. The median follow-up time was 18 years (range 15–22 years) and the follow-up period was extended to April 2000 for survival.

Clincopathological data in relation to treatment arm is provided in Table 1.

Table 1. Patient and tumor characteristics according to treatment arm

Variable	Control arm $N = 127$	Tamoxifen arm $N = 124$
Age		
Years, median (range)	66 (54–74)	66 (54–74)
Tumor size, (mm)		
Median (range)	25 (8–76)	25 (3–55)
T1	52 (41%)	46 (37%)
T2	71 (56%)	76 (62%)
T3	4 (3%)	1 (1%)
Unknown	0	1
Node status		
N0	81 (64%)	82 (67%)
N1+	45 (36%)	41 (34%)
Unknown	1	1
ER fraction		
0–10%	28 (29%)	20 (23%)
11–90%	12 (13%)	14 (16%)
> 90%	56 (58%)	53 (61%)
Unknown	31	37
PR fraction		
0–10%	50 (56%)	49 (58%)
11–90%	22 (24%)	19 (22%)
>90%	18 (20%)	17 (20%)
Unknown	37	39
VEGF-A		
VEGF negative	12 (11%)	16 (16%)
VEGF positive	97 (89%)	86 (84%)
Unknown	18	22
VEGFR2		
VEGFR2 negative	33 (31%)	38 (37%)
VEGFR2 positive	73 (69%)	64 (63%)
Not known	21	22

Abbreviations: (1) original study N0 = node negative, N1+ = node positive, ER = estrogen receptor, fraction of stained nuclei, PR = progesterone receptor, fraction of stained nuclei,VEGF-A = vascular endothelial growth factor A, VEGFR2 = vascular endothelial growth factor receptor 2.

Tumor tissue microarray construction

Paraffin blocks were available for 224 patients. The flowchart of the trial is shown in Figure 1. Clearly-defined areas of tumor samples were indicated on a slide with a fresh tissue section from the paraffin block. Two or more biopsies, 0.6 mm in size, were taken from each donor paraffin block corresponding to the marked area. Each section was mounted in a recipient paraffin block using a tissue array machine (Beecher Instruments, MD, USA).

The order of the tumors was documented in a spreadsheet in order to link ER, progesterone receptor (PR), VEGF-A and VEGFR2/KDR staining results for each unique tissue with the original donor tumor samples. The tissue array was monitored after completion by a hematoxylin stain and in case of lack of visible tumor cells a duplicate biopsy was processed in a second round.

Immunohistochemistry

Sections, 3–4 μm, of the paraffin embedded tissue arrays were dried, deparaffinized, rehydrated and microwave

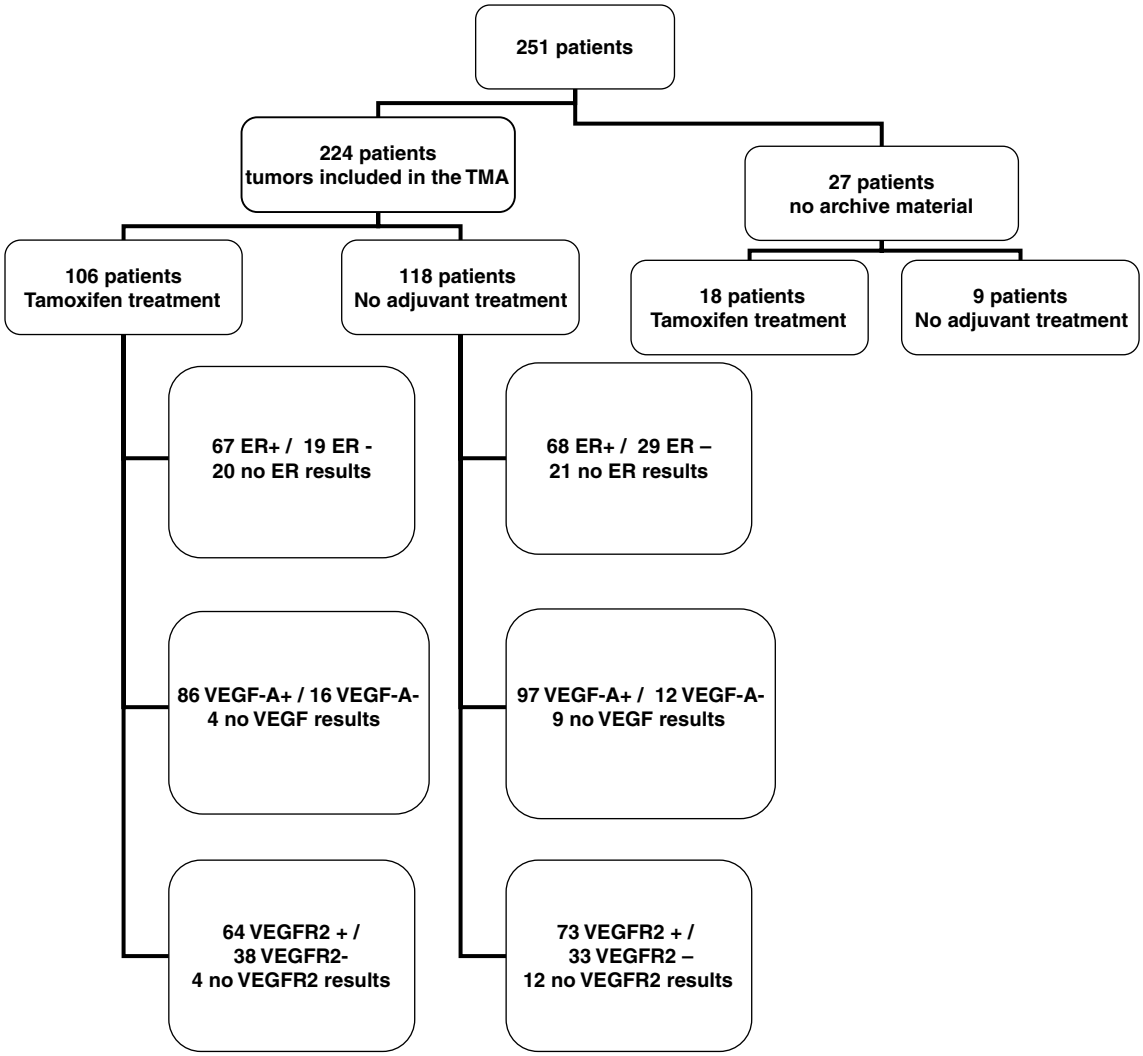

Figure 1. Flow-chart of the trial.

treated for 5 + 5 min in a citrate buffer (pH 6.0) before being processed in an automatic immunohistochemistry staining machine according to standard procedures (TechMate500, Dako, Denmark) using a polyclonal VEGF (A-20) antibody diluted 1:400 recognizing VEGF-A (Santa Cruz, Ca, USA) and a monoclonal VEGFR2/KDR antibody diluted 1:1000 (Santa Cruz, Ca, USA). For VEGF-A, normal human kidneys were used as positive controls and for VEGFR2 human aortic endothelium served as positive controls.

ER and PR were determined using the Ventana Benchmark system (Ventana Medical Systems Inc., AZ, USA) with prediluted antibodies (Anti-ER Clone 6F11 and Anti-PgR Clone 16).

Two biopsies from each tumor were examined to ensure reproducibility in the analysis. If neither of the examined biopsies was satisfactory regarding quality of the cells or staining, a second set of biopsies was examined in a separate tumor array. The tumor array was examined by two investigators to whom the clinical data were blinded and all divergent results were re-examined followed by a mutual conclusive decision. The VEGF and VEGFR2 cytoplasmatic staining intensity was evaluated semiquantitatively using a classification from 0 to 3, with 0 representing lack of staining, 1 = low staining intensity, 2 = intermediate staining intensity and 3 = intense staining intensity. The fraction of positively stained cells was determined as well (0 = lack of staining, $1 \leq 1\%$ cells staining, 2 = 1–10% cells staining, 3 = 10–50% cells staining, 4 = 50–90% cells staining and $5 \geq 90\%$ cells staining). The cytoplasmatic staining intensity correlated strongly with the cytoplasmatic staining fraction for both antibodies (VEGF-A: $r = 0.82$, VEGFR2: $r = 0.72$). Staining intensity was used in further analysis and for survival analysis, VEGF-A and VEGFR2 were categorized into absence of staining and presence of staining of any intensity, denoted negative and positive, in order to achieve sufficient numbers in each group.

ER and PR were determined by estimating the fraction of positively stained nuclei using the same protocol as for VEGF-A and VEGFR2 (0–5), where 0–2 (<10% fraction of stained nuclei) were classified as negative and 3–5 (≥ 10%) as positive.

Statistical analysis

Chi-square test was used to analyze the association between VEGF-A and VEGFR2 and clinicopathological parameters. Pearson correlation was used to explore the correlation between staining intensity of VEGF-A (and VEGFR2) and fraction of stained cells of VEGF-A (and VEGFR2). DFS was used as end-point in this study and considered loco-regional recurrences, distant recurrences, and death. Patients were censored after any primary event. Due to long follow-up, 10-year survival was additionally used, i.e., patients having their primary event more than 10 years were censored at 10 years. Survival was calculated by the Kaplan-Meier method and differences between groups were tested by log rank test. Cox proportional hazards model was also used for estimation of relative hazards adjusted or not for potential prognostic factors. The model was used to estimate the interaction effect between treatment and VEGF-A or VEGFR2 measuring a possible difference in treatment effect for different VEGF-A status and VEGFR2 status and an interaction variable was constructed (tamoxifen treatment $(+/-) \times$ VEGF-A or VEGFR2 $(+/-)$).

All reported *p*-values are two-sided and where the *p*-value was less then 0.05, it has been considered statistically significant.

All calculations were performed in SPSS version 11.0 (SPSS inc., Ill., USA).

Results

Distribution of VEGF-A and VEGFR2

Relevant array biopsies stained for VEGF-A were obtained for 208 tumors and 203 for VEGFR2. The relationship of VEGF-A and VEGFR2 to clinicopathological parameters is shown in Table 2, demonstrating a significant association between VEGF-A and VEGFR2 and for VEGFR2 to PR negativity.

Effect of tamoxifen on DFS

At 10-year follow-up, 115 primary events were recorded and at the last follow-up at a median of 18 years, 160 primary events were noted. The effect on DFS by tamoxifen treatment for all patients irrespective of hormone receptor content was non-significant, Figure 2a. The hazard ratios (HRs) for tamoxifen

Table 2. Relationship between clinicopathological parameters and VEGF-A and VEGFR2

Variable	VEGF-A−	VEGF-A+	*p*-value	VEGFR2−	VEGFR2+	*p*-value
Age						
<median	10	91	0.2	28	71	0.1
>median	18	92		43	66	
Node status						
N0	15	122	0.2	43	91	0.4
N1+	13	60		28	45	
Tumor size						
T1	10	69		22	53	
T2	18	109	0.9	47	81	0.3
T3		5		2	3	
ER status						
ER −	4	42	0.3	13	34	0.6
ER +	20	110		42	86	
PR status						
PR −	8	88	0.1	20	76	0.002
PR+	13	60		31	41	
VEGFR2						
−	24	47	<0.001			
+	3	132				

VEGF-A and VEGFR2 in relation to clinicopathological variables according to χ^2 test.
Abbreviations: N0 = node negative, N1+ = node positive, ER = estrogen receptor (ER− <10% fraction of stained nuclei, ER+ ≥ 10% fraction of stained nuclei), PR = progesterone receptor (PR− <10%fraction of stained nuclei, PR+≥ 10% fraction of stained nuclei), VEGF-A = vascular endothelial growth factor A, VEGFR2 = vascular endothelial growth factor receptor 2.

response in patients with ER positive tumors (fraction > 10% positive cells) and strongly ER positive tumors (fraction > 90% positive cells) are given in Table 4, indicating a notable response for patients with strongly ER positive tumors up to 10 years of DFS as illustrated in Figure 2b. The tamoxifen response in PR positive tumors was non-significant (HR (tamoxifen versus control): 1.0; (95% CI (confidence interval): 0.6–1.9), $p = 0.9$ for both 10-year and 18-year DFS).

VEGF-A, VEGFR2 and tamoxifen treatment

VEGF-A status

The tamoxifen response in terms of DFS in ER positive and VEGF-A negative or positive tumors is given in Figure 3a, illustrating a statistically significant effect in VEGF-A negative disease after 10 years of follow-up (HR 0.2; 95% CI: 0.04–0.9), which was not evident for VEGF-A positive tumors (HR 1.1; 95% CI: 0.6-2.0), Figure 3b. The HRs for tamoxifen response at 18-year DFS is given in Table 3. According to the Cox proportional hazards model including VEGF-A status, tamoxifen treatment and an interaction variable, the interaction variable was significant at both 10-year and 18-year DFS, $p = 0.046$ and $p = 0.039$, respectively. When adjusting the model for PR status (positive versus negative), node status (positive versus negative) and tumor size (T3 and T2 versus T1), the term of interaction was still significant, $p = 0.006$ at 10-year DFS and $p = 0.002$ at 18-year DFS, whereas PR status had no significant effect on outcome at any time of follow-up.

The HRs for tamoxifen reponse according to VEGF status in patients with strongly ER positive tumours (> 90%), is given in Table 3 for both 10-year DFS and 18-year DFS. According to the Cox proportional hazards model described above, the adjusted term of interaction at 10-year and 18-year DFS was 0.011 and 0.002, respectively.

VEGFR2 status

The tamoxifen response in ER positive patients for VEGFR2 negative and positive patients are illustrated in Figure 4a and b and the HRs are given in Table 3, indicating a difference in tamoxifen response depend-

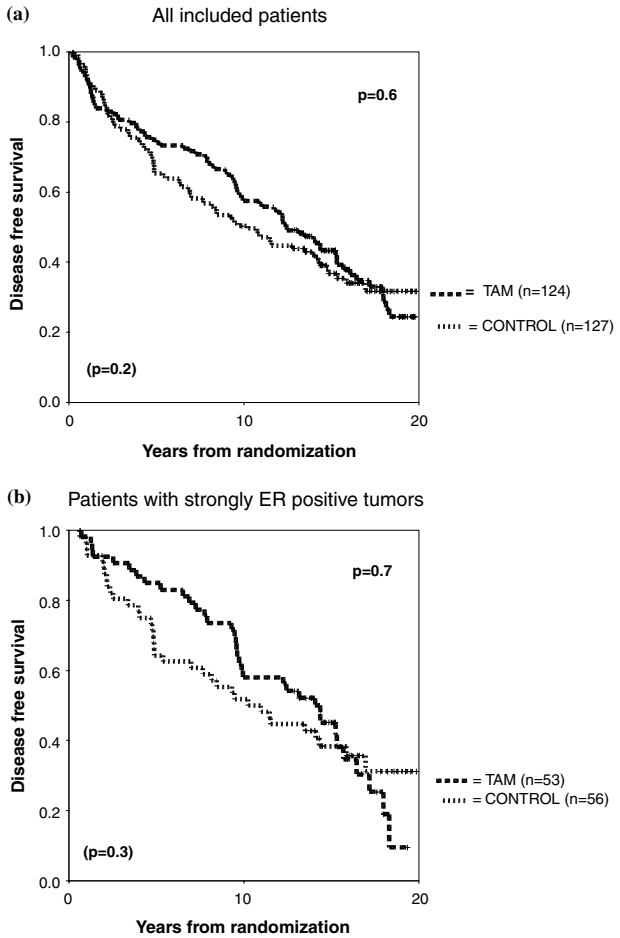

Figure 2. Kaplan–Meier estimate with for patients according to treatment arm, (a) of DFS for all included patients, $n = 251$. The p-value within parenthesis denotes10-year DFS (b) of DFS for patients with tumors with ER positive tumors (fraction > 90%), $n = 109$. The p-value within parenthesis denotes 10-year DFS. The log rank test was used to calculate the p-values.

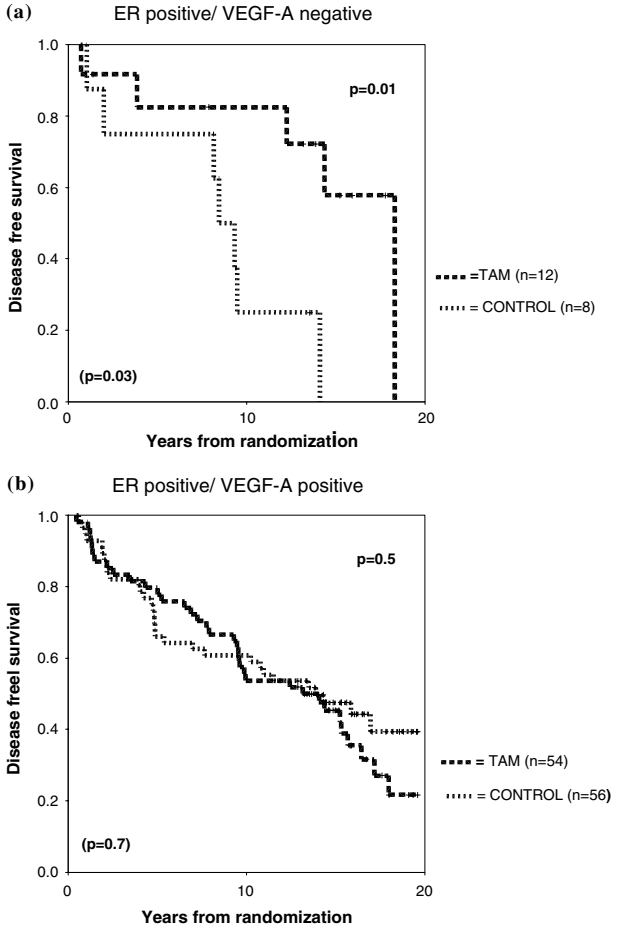

Figure 3. Kaplan–Meier estimate for patients according to treatment arm in patients with ER positive tumors (fraction > 10%) (a) of DFS for patients with VEGF-A negative tumors ($n = 20$). The p-value within parenthesis denotes 10-year DFS. (b) of DFS for patients with VEGF-A positive tumors ($n = 110$). The p-value within parenthesis denotes 10-year OS. The log rank test was used to calculate the p-values.

Table 3. Relative risks for 10-year DFS and 18-year DFS by tamoxifen treatment in relation to ER status, VEGF-A status and VEGFR2 status

Covariate category	10-year DFS			18-year DFS		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
ER+						
Control	1.0			1.0		
Tamoxifen	0.9	0.5–1.5	0.7	1.0	0.6–1.5	0.9
ER+ and VEGF-A−						
Control	1.0			1.0		
Tamoxifen	0.2	0.04–0.9	0.049	0.2	0.05–0.8	0.02
ER+ and VEGF-A+						
Control	1.0			1.0		
Tamoxifen	1.1	0.6–2.0	0.7	1.2	0.7–1.9	0.5
ER+ and VEGFR2−						
Control	1.0			1.0		
Tamoxifen	0.5	0.2–1.3	0.1	0.7	0.3–1.6	0.4
ER+ and VEGFR2+						
Control	1.0			1.0		
Tamoxifen	1.1	0.6–1.1	0.7	1.1	0.6–1.9	0.7
ER++						
Control	1.0			1.0		
Tamoxifen	0.7	0.4–1.3	0.3	0.9	0.6–1.5	0.8
ER++ and VEGF-A−						
Control	1.0			1.0		
Tamoxifen	0.2	0.05–1.1	0.07	0.2	0.06–0.9	0.04
ER++ and VEGF-A+						
Control	1.0			1.0		
Tamoxifen	1.0	0.5–1.7	0.6	1.1	0.6–1.9	0.8
ER++ and VEGFR2−						
Control	1.0			1.0		
Tamoxifen	0.3	0.1–0.9	0.03	0.5	0.2–1.2	0.09
ER++ and VEGFR2+						
Control	1.0			1.0		
Tamoxifen	1.0	0.5–2.1	0.9	1.2	0.6–2.2	0.6

Abbreviations: HR = hazard ratio, CI = confidence interval, ER+ = ER fraction ≥10% positive cells, ER++ = ER fraction ≥ 90% positive cells, VEGF-A = vascular endothelial growth factor A, VEGFR2 = vascular endothelial growth factor receptor 2.

ing on VEGFR2 status. However, when estimating the treatment effect at 10-year DFS for VEGFR status and tamoxifen treatment as above, neither the unadjusted, nor the adjusted term of interaction was statistically significant ($p = 0.15$ and $p = 0.19$).

For strongly ER positive and VEGFR2 negative tumors ($n = 34$), the effect of tamoxifen treatment, was statistically significant at 10 years in terms of DFS (HR: 0.3; 95% CI: 0.1–0.9), contrasting to a non-significant effect in VEGFR2 positive tumors ($n = 68$), (HR: 1.0; 95% CI: 0.5–2.1), Table 3. In a Cox proportional hazards model, the unadjusted interaction variable indicated a difference in tamoxifen response between patients with VEGFR2 negative and VEGFR2 positive tumors, although not strictly statistically significant ($p = 0.055$). In a second Cox model adjusting for PR status, nodal status and tumor size, the term of interaction was of the same magnitude, $p = 0.050$. PR status had no effect on clinical outcome in this model. At 18 year of follow-up, the adjusted term of interaction for VEGFR2 status and tamoxifen treatment was still significant, $p = 0.028$.

VEGF-A, VEGFR2 and prognostic information

VEGF-A positivity was associated with a more favourable clinical outcome in terms of DFS at 10 year of follow-up in multivariate analyses, Table 4, whereas VEGFR2 yielded no prognostic information according to Cox uni- and multivariate analyses as illustrated in Table 4. Node-status (positive versus negative) was the only factor with statistically significant association with prognosis in the presented analysis.

Discussion

This is one of the first reports from a randomized trial relating tamoxifen response to expression of VEGF-A and VEGFR2 in tumor cells. We noted a tamoxifen

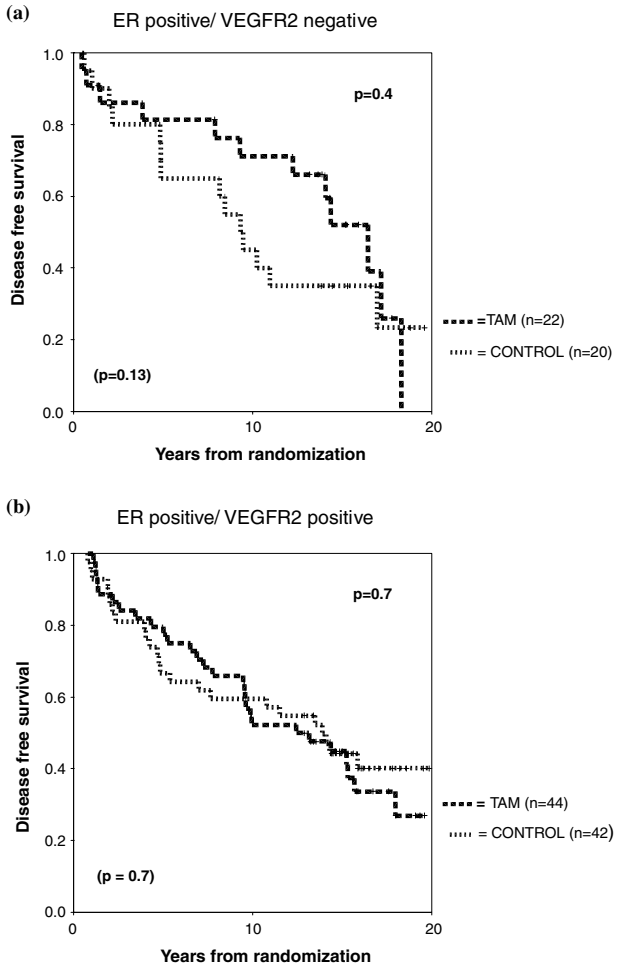

Figure 4. Kaplan–Meier estimate for patients according to treatment arm in patients with ER positive tumors (fraction $>10\%$) (a) of DFS for patients with VEGFR2 negative tumors ($n=42$). The p-value within parenthesis denotes 10-year DFS. (b) of DFS for patients with VEGFR2 positive tumors ($n=86$). The p-value within parenthesis denotes 10-year DFS. The log rank test was used to calculate the p-values.

response on DFS at 10 years of follow-up in ER-positive tumors for VEGF-A negative tumors in contrast to no effect by tamoxifen in VEGF-A positive tumors. In multivariate analysis for interaction between tamoxifen treatment and VEGF-A status, the term of interaction for VEGF-A was significant, whereas the difference in tamoxifen response depending on VEGFR2 status was non-significant. Regarding VEGFR2 status, there was no clear distinction between tamoxifen responders and non-responders in patients with ER positive tumors. However, in patients with strongly ER positive tumours, both VEGF-A and VEGFR2 status were predictors of tamoxifen response which was demonstrated using multivariate models for test of interaction.

The indication of a cross-talk between VEGF-A and ER is evident from experimental data showing that the gene coding for VEGF has functional estrogen response elements [18]. VEGF-A stimulates ER-independent proliferation in experimental models of breast cancer [19]. On the other hand, both estrogen and tamoxifen can stimulate VEGF expression, whereas tamoxifen reduces secretion of VEGF-A [20], although these observations cannot explain why high level of VEGF-A at time of surgery is associated with an impaired tamoxifen response. Overexpression of HER-2 in ER positive tumors reduces the tamoxifen effect on proliferation [21] and the mechanism is probably an interaction with the receptor tyrosine kinase pathway promoting estrogen-independent stimulation of ER and growth of tumor cells [22]. VEGF-A and VEGFRs are co-expressed in several epithelial tumors, including breast cancer, giving further evidence for an autocrine pathway for VEGF-A and its receptor [10–12]. VEGF-A has therefore been attributed a dual function not only by stimulating neoangiogenesis but also promoting tumor cell growth as indicated by increased proliferation in tumor cells after stimulation with VEGF-A [19]. VEGFR2's intracellular domain is a receptor tyrosine kinase pathway and the noted tamoxifen resistance in VEGFR2 expressing tumors can theoretically be explained by similar mechanisms as in HER-2 expressing tumors with tamoxifen resistance, but deserves confirmation in experimental studies.

High level of cytosolic VEGF-A has been associated with inferior outcome in several reports from non-randomized trials of tamoxifen-treated hormone-responsive patients indicating that VEGF-A can be a marker of response for endocrine therapy [7, 8, 13, 14]. However, the origin of VEGF-A with this method can not be pinpointed and a possible origin from tissues other than tumor cells have to be taken into account. Immunohistochemical quantification of tumorspecific VEGF-A in breast tumors has been introduced as an alternative method of determining levels of VEGF-A [23], but data related to clinical outcome are so far sparse and do not support the prognostic information yielded by cytosolic determinations of VEGF-A except for one report [22, 24, 25]. On the other hand, VEGF-A high tumors by IHC has been linked to a more favorable outcome in a study only including patients without adjuvant treatment [22]. In the present report of tumor-specific determination of VEGF-A by IHC, the clinical outcome in terms of DFS was better for patients with VEGF-A positive tumours, supporting the data by De Paola. One explanation to the contrasting results when VEGF-A is analyzed by IHC compared to ELISA based methods in cytosols, can be that cytosolic-based methods better reflect the amount of biologically available VEGF-A engaged in proangiogenic pathways. On the other hand, the IHC-based method yields better opportunities for understanding mechanisms on a tumor cell basis.

The benefit of tamoxifen treatment on prognosis and survival is well established for hormone-responsive patients (i.e., patients with ER positive and/or PR positive tumors) at all ages [1]. The tamoxifen effect in elderly patients is recognized, although it may be overlooked when using overall survival as an end-point because many deaths will not be caused by breast cancer. To overcome the confounding by intercurrent deaths, we

Table 4. 10-year DFS with Cox univariate and multivariate analysis for 127 untreated patients

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Node status						
N0	1.0					
N1+	2.7	1.7–4.3	< 0.001	3.9	1.9–7.9	< 0.001
Tumor size						
T1	1.0					
T2 + T3	1.5	1.0–2.4	0.06	1.7	0.8–3.4	0.2
ER status						
–	1.0					
+	0.6	0.4–1.0	0.08	1.0	0.4–2.3	0.99
PR status						
–	1.0					
+	0.7	0.4–1.2	0.20	0.4	0.2–1.1	0.09
VEGF-A						
–	1.0					
+	0.5	0.3–1.0	0.06	0.3	0.1–0.8	0.01
VEGFR2						
–	1.0					
+	0.8	0.5–1.4	0.50	0.9	0.4–1.9	0.7

Abbreviations: HR = hazard ratio, CI = confidence interval, N0 = node negative, N1+ = node positive, ER = estrogen receptor (ER– < 10% fraction of stained nuclei, ER+ ≥10% fraction of stained nuclei), PR = progesterone receptor (PR– < 10% fraction of stained nuclei, PR+ ≥10% fraction of stained nuclei), VEGF-A = vascular endothelial growth factor A, VEGFR2 = vascular endothelial growth factor receptor 2.

used DFS as end-point and analyzed both 10-year and 18-year follow-up data. In this relatively small study, there was no significant response by tamoxifen on DFS for all included patients. However, in patients with ER-positive tumors with more than 90% positive cells, there was a trend in favour of tamoxifen treatment up to 10 years of follow-up. When stratifying for VEGF-A and VEGFR2 status, we selected patients with ER fraction > 10% and ER fraction > 90% in separate analysis.

This is one of the first reports from a randomized trial linking the VEGF-pathway to antiestrogen treatment response highlighting a new treatment strategy targeting VEGF-A signaling in combination with endocrine therapy. Interestingly, VEGF-A was a predictor of tamoxifen response among ER positive patients with both low and high fraction of ER positive cells. The difference in tamoxifen response depending on VEGF-A status, was consistent at both 10 years and 18 years of follow-up. When using a multivariate model to test interaction between VEGF-A status and tamoxifen response, the interaction variable was significant both unadjusted and when adjusted for PR status, nodal status and tumor size. The result from this small study strongly supports data from non-randomized studies indicating that VEGF status is a predictor of response to tamoxifen in early breast cancer. In strongly ER positive tumors with a more notable effect by tamoxifen, VEGFR2 was an additional predictor of tamoxifen response. The signaling pathway for VEGFR2 can be blocked by drugs directed towards the membrane-bound part of the receptor by a monoclonal antibody as well as by tyrosine kinase inhibitors and both types of substances are developed as inhibitors of angiogenesis. The compounds developed for antiangiogenic therapy can theoretically serve as adjuncts in tamoxifenresistant tumors expressing VEGF-A and/or VEGFR2 and open up new opportunities for treatment of hormone-resistent breast cancer.

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Address for offprints and correspondence: Lisa Rydén, Division of Pathology, Department of Laboratory Medicine, Lund University, Malmö, University Hospital, SE – 205 02 Malmö, Sweden; *Fax:* +46-(0)-40-337063; *E-mail:* lisa.ryden@pat.mas. lu.se