In 212 postmenopausal women with node-positive oestrogen receptor-positive (ER_LBA) breast cancer subjected to radical surgery and adjuvant tamoxifen, the risk of 6-year relapse increased with increasing values of intratumoral vascular endothelial growth factor (VEGF) in patients whose tumours had a low/intermediate ER_LBA content compared to patients with high-ER_LBA tumours. These findings indicate that tumour progression, activated or sustained by high VEGF levels, may be counteracted in high-ER_LBA cancers by tamoxifen, which in contrast fails to contrast the metastatic potential in low-ER_LBA tumours.

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PATIENTS AND METHODS
Patients
The study included postmenopausal patients with primary resectable invasive breast cancer, histologically classified as N+, who underwent surgery at the Istituto Nazionale Tumori in Milan between March 1991 and December 1995 and received only adjuvant tamoxifen (20 mg day⁻¹) for at least 2 years (median duration time, 4 years) because of their positive ER status (ER tumour concentration higher than 10 fmol mg⁻¹ of protein). From a total of 859 N+ tumors, ER+ postmenopausal patients, consecutive with respect to steroid receptor determination at the time of diagnosis, 289 were selected on the basis of treatment, histology (pure or mixed ductal or lobular invasive tumors) and follow-up (i.e. a minimum potential of 6 years from the date of surgery to the date of last updating of patient records).

Of the 289 eligible patients, 212 (73%) were available for VEGF evaluation. Their median age was 64 years (range, 50–85); 88 patients (42%) were treated by mastectomy and 124 (58%) by breast-conserving surgery plus radiotherapy. All of them underwent complete axillary lymph node dissection (median number of examined nodes, 18). Most patients had one to three metastatic axillary lymph nodes (139, 66%). Small (≤ 2 cm) and large (> 2 cm) tumours were equally represented (93, 48% and 99, 52%, respectively). After surgery, the patients were followed at 6-month intervals during the first 5 years and at 12-month intervals thereafter; disease status was assessed by means of physical examination, chest X-ray, bone scan and abdominal sonography. Treatment failure was defined as the first documented evidence of
new disease manifestations in locoregional areas (seven cases), distant sites (40 cases), or in the contralateral breast (five cases). Relapse-free survival was calculated as the time elapsed from diagnosis to the date of first recurrence or to the last clinical examination for patients without documented disease manifestation. Median follow-up for the whole series was 68 months (interquartile range, 52–82 months).

Steroid receptor determination by LBA
Steroid receptor content was determined according to the EORTC recommendations and within national (Pifanelli et al, 1991) and international (EORTC Breast cancer co-operative group, 1980) quality control programmes by a double-labelling assay (Coradini et al, 2000), and expressed as fmol mg⁻¹ of protein. Tumours with an ER_LBA concentration higher than 10 fmol mg⁻¹ of protein were defined as ER +.

Vascular endothelial growth factor determination
The predominant VEGF isoform, VEGF₆₅ (henceforth referred to as VEGF), was measured by a quantitative enzyme immunoassay technique (Quantikine, human VEGF; R&D Systems, Minneapolis, MN, USA) as described elsewhere (Coradini et al, 2001). Concentrations were expressed as pg of VEGF protein per mg of total protein.

Statistical analysis
The overall association of VEGF level with patient age, tumour size, number of metastatic lymph nodes, ER_LBA and PgR_LBA content was evaluated by Spearman’s rank correlation coefficient. The effect of VEGF, ER_LBA and PgR_LBA content on RFS was investigated by multivariate analysis using a Cox regression model in which also the number of metastatic lymph nodes was included. All variables were considered on a continuous scale after logarithmic transformation. Null values for PgR_LBA content were arbitrarily set at 1, taking a sensitivity threshold value of 2 fmol mg⁻¹ of protein. According to a previous finding (Coradini et al, 2001), linear terms for log(ER_LBA), log(PgR_LBA) and log(VEGF) and the interaction between ER_LBA and VEGF were included in the model.

The proportional hazard assumption of the Cox model was evaluated and the effect of model terms was tested as previously reported (Coradini et al, 2001). The library written by Harrell et al (1996) was applied in some steps of the model building procedure and the SAS macro programme RELIMPCR designed by Heinze and Schemper (2001) was adopted for evaluation of the relative prognostic contribution of the covariates.

RESULTS
In this series of ER_LBA-positive tumours from postmenopausal patients, the VEGF content ranged from 7 to 2186 pg mg⁻¹ of protein with 52, 95 and 200 as the 25th, 50th and 75th percentiles, respectively; the PgR_LBA concentration ranged from 1 to 2563 fmol mg⁻¹ of protein with 40, 111 and 355 as the 25th, 50th and 75th percentiles, respectively. Vascular endothelial growth factor content did not show any significant correlation with any of the other variables considered; estimated correlation coefficients were all in the range ±0.08.

In the multivariate model, the number of metastatic lymph nodes, VEGF, ER_LBA and the interaction between ER_LBA and VEGF were significantly related to prognosis (Table 1), whereas no significant contribution was observed for PgR_LBA. As for ER_LBA–VEGF and the ER_LBA–VEGF interaction, the results were similar to those previously obtained in patients with N– breast cancer who did not receive systemic treatment (Coradini et al, 2001). This finding further supports the role of ER_LBA in modulating the prognostic effect of VEGF and the negative interaction term indicates a decrease in the unfavourable effect of VEGF for increasing values of ER_LBA. To provide a description of the combined effect of VEGF and ER_LBA, the relative hazard (RH) for increasing VEGF concentrations was plotted (Figure 1) for selected values of ER_LBA (70 and 220 fmol mg⁻¹ of protein) that were approximately equal to the first and third quartiles of the distribution. The solid line corresponds to an ER_LBA = 70 fmol mg⁻¹ of protein, whereas the dashed line corresponds to an ER_LBA = 220 fmol mg⁻¹ of protein. Dotted lines indicate 95% pointwise confidence limits (upper: UCL; lower: LCL).

Figure 1
Plots of the logarithm of the relative hazard of disease recurrence as a function of VEGF level for different ER_LBA values (fixed approximately at the first and third quartiles of the distribution). The solid line corresponds to an ER_LBA = 70 fmol mg⁻¹ of protein, whereas the dashed line corresponds to an ER_LBA = 220 fmol mg⁻¹ of protein. Dotted lines indicate 95% pointwise confidence limits (upper: UCL; lower: LCL).

DISCUSSION
According to the recently revised treatment guidelines for early breast cancer (Goldhirsch et al, 2001), postmenopausal women...
with ER+ primary breast cancer should receive adjuvant hormone therapy with tamoxifen. However, a recent clinical study (Linderholm et al, 2000) provided evidence that also the intratumoral VEGF content could predict outcome following adjuvant endocrine treatment: patients with ER+ tumours, but a high VEGF expression, had a significantly shorter RFS and overall survival. In agreement with these findings, our results showed that, due to the presence of a negative interaction between ER_LBA and VEGF, already observed in a series of N-+ cancers (Coradini et al, 2001), patients whose tumours had a low/intermediate ER_LBA content exhibited an increased risk of disease recurrence with increasing values of intratumoral VEGF. Conversely, the VEGF level did not show prognostic effect in patients whose tumours had a high ER_LBA content. These findings suggest that in tumours characterised by a high ER_LBA concentration and therefore more likely to be hormonally regulated, tumour progression, activated or sustained by VEGF, may be counteracted by the protective effect of endocrine therapy. Conversely, when a patient has a high level of VEGF and a low ER_LBA concentration, tamoxifen could fail to contrast the tumour’s metastatic potential and more tailored adjuvant treatment would be required including, for example, an antiangiogenic agent.

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REFERENCES
Coradini D, Boracchi P, Daidone MG, Pellizzaro C, Miodini P, Ammatuna M, Tomasic G, Bignanoli E (2001) Contribution of vascular endothelial growth factor to the Nottingham prognostic index in node-negative breast cancer. Br J Cancer 85: 795–797
Coradini D, Daidone MG, Boracchi P, Bignanoli E, Oriana S, Bresciai G, Pellizzaro C, Tomasic G, Di Fronzo G Marubini E (2000) Time-dependent relevance of steroid receptors in breast cancer. J Clin Oncol 18: 2702–2709
EORTC Breast Cancer Co-operative Group (1980) Revision of the standards for the assessment of hormone receptors in human breast cancer. Report of the second EORTC Workshop, held on 16–17 March 1979, in the Netherlands Cancer Institute. Eur J Cancer 16: 1513–1515
Gasparini G, Toi M, Gion M, Verderio P, Dittadi R, Hanatani M, Matsubara S, Vinante O, Bonoldi E, Boracchi P, Gatti C, Suzuki H, Tominaga T (1997) Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 89: 139–147
Gasparini G, Toi M, Miceli R, Vermeulen PB, Dittadi R, Bignanoli E, Morabito A, Fanelli M, Gatti C, Suzuki H, Tominaga T, Dirix L.Y, Gion M (1999) Clinical relevance of vascular endothelial growth factor and thymidine phosphorylase in patients with node-positive breast cancer treated with either adjuvant chemotherapy or hormone therapy. Cancer J Sci Am 5: 101–119
Goldhirsch A, Glick JH, Gelber RD, Coates AS, Senn H-J (2001) Meeting highlights: International Consensus Panel on the treatment of primary breast cancer. J Clin Oncol 19: 3817–3827
Grambsch P, Therneau T (1994) Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 81: 515–526
Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86: 353–364
Harrell Jr FE, Lee KL, Mark DB (1996) Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 15: 361–387
Heinzle G, Schemper M (2001) RELIMPCR, and RELIMPLR SAS-macros for the analysis of relative importance of prognostic factors in Cox and logistic regression. Vienna: University of Vienna. Department of Medical Computer Sciences, Section of Clinical Biometrics, Technical Report 3/2001
Hyder SM, Huang JC, Nawaz Z, Boettger-Tong H, Makela S, Chiappetta C, Stancel GM (2000a) Regulation of vascular endothelial growth factor expression by estrogens and progestins. Environ Health Perspect 108: 785–790
Hyder SM, Nawaz Z, Chiappetta C, Stancel GM (2000b) Identification of functional response elements in the gene coding for the potent angiogenic factor vascular endothelial growth factor. Cancer Res 60: 3183–3190
Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B, Heriksson R (2000) A multiinstitutional comparison of vascular endothelial growth factor content with recurrences, survival and first relapse site in primary node-positive breast carcinoma after adjuvant treatment. J Clin Oncol 18: 1423–1431
Nakamura J, Lu Q, Aberdeen G, Albrecht E, Brodie A (1999) The effect of estrogen on aromatase and vascular endothelial growth factor messenger ribonucleic acid in the normal nonhuman primate mammary gland. J Clin Endocrinol Metab 84: 1432–1437
Piffaneli A, Giovannini G, Pelizzola D, De Bortoli M, Catozzi L, Giganti M (1991) Steroid receptor assays: an Italian quality assessment program. Ann Ist Super Sanita` 27: 523–529
Ruohola JK, Valve EM, Karkkainen MJ, Joukov V, Alitalo K, Harkonen PL (1999) Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. Mol Cell Endocrinol 25: 29–40
Vogel PM, Georgiade NG, Fetter BF, Vogel FS, Mc Carty Jr KS (1981) The correlation of histologic changes in human breast with the menstrual cycle. Am J Pathol 104: 23–34