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Increased regulatory T-cell numbers distinguish high-risk breast cancer patients and those at risk of late relapse

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Purpose: To assess the clinical significance of tumour-infiltrating FOXP3+ regulatory T-cells (TR) in breast cancer patients with long-term follow-up.

Methods: FOXP3+ TR were detected by immunohistochemistry with our new FOXP3 monoclonal antibody, 236A/E7. Numbers of FOXP3+ lymphocytes in tissue microarray cores from pure ductal carcinoma in situ (DCIS) (n = 62), invasive breast cancer (n = 237) or from comparable areas of normal terminal duct lobular breast tissue from patients without cancer (n = 10) were determined. A median cut-off of 15 defined patients with high numbers of TR.

Summary of results: TR numbers were significantly higher in DCIS and invasive breast carcinomas when compared to normal breast, with invasive tumours having significantly higher numbers than DCIS (p = 0.001). High numbers of FOXP3+ TR identified patients with DCIS at increased risk of relapse (p = 0.04) and patients with invasive tumours having both shorter relapse-free (p = 0.004) and overall survival (p = 0.007). High TR numbers were present in high-grade tumours (p=<0.001), in patients with lymph node involvement (p = 0.01) and in estrogen receptor alpha (ER) negative tumours (p = 0.001). Importantly, quantification of FOXP3+ TR identified a group at high-risk of relapse, within the so-called good prognostic group of ER+ patients (p = 0.005) and these patients have a prognosis as poor as those that lack ER expression. Multivariate analyses, in ER+ patients, demonstrated that greater TR numbers independently conferred a significantly higher hazard ratio than that of tumour grade and nodal status for relapse-free and overall survival respectively. Unlike conventional clinicopathological factors, high numbers of FOXP3+ TR identified patients at risk of late relapse after five years disease-free survival.

Conclusion: These findings indicate that quantification of FOXP3+ TR in breast tumours is valuable for assessing disease prognosis and progression and represents a novel marker for identifying late relapse patients who may benefit from aromatase therapy after five years of tamoxifen treatment. Furthermore, tumour vaccination approaches in combination with targeting TR cells, are just entering clinical trials and our data strongly suggest that such therapy would be beneficial for a significant proportion of breast cancer patients.

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A new independent validation of the 70-gene signature in node-negative breast cancer

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Introduction: Recently we have identified a 70-gene expression profile ("signature") using microarray analysis, which was a more powerful prognostic factor for distant metastases than current clinicopathological features in breast cancer patients (van 't Veer *et al.*, Nature 2002; Van de Vijver *et al.*, New Engl J Med 2002). This 70-gene signature may improve the selection of patients eligible for adjuvant systemic therapy. To obtain a more reliable estimate of the prognostic value of the 70-gene profile, independent validation is indispensable. Therefore, we assessed the prognostic value of the 70-gene signature in a series of lymph node negative breast cancer patients.

Methods: Using the 70-gene signature, we classified tumors from 123 consecutive patients younger than 55 years with node-negative breast cancer (pT1–2, treated 1996–1999) as having a "good" or a "poor prognosis signature" based on the 70-gene expression profile. Pathological data were centrally reviewed. Using univariable and multivariable statistical analysis, hazard ratios were estimated to compare event rates in "poor" versus the "good prognosis signature" groups for the endpoints metastasis as first event, disease-free survival and overall survival.

Results: Of the 123 patients with node-negative breast cancer, 36% received adjuvant systemic treatment (13% chemotherapy, 11% endocrine and 12% both). At a median follow-up of 5.6 years (0.1–8.6), 30 events had occurred among which 16 metastases as first event. According to the 70-gene signature, 48% had a "poor" and 52% had a "good prognosis signature". Estimated cumulative incidence of metastasis as first event at five years is 23.0% (SE 5.8%) for "poor" versus 1.6% (SE 1.6%) for "good prognosis signature" tumors (estimated hazard ratio (HR) 5.9, 95% CI 1.8–20, P = 0.0017). When adjusted for tumor size, histological grade, ER-status, adjuvant chemotherapy and hormonal treatment, the HR was 4.5 (95%CI 1.3–15, P = 0.018). Patients with a "poor prognosis signature" had a 5-year overall survival of 82.1% (SE 5.1%) against 96.8% (SE 2.2%) for the "good prognosis signature" group (HR 3.4, 95% CI 1.2–9.5, P = 0.021). Patients with a "poor prognosis signature" had a 5-year disease free survival of 69.8% (SE 6.1%) against 92.0% (SE 3.4%) for "good prognosis signature" group (HR 2.8, 95% CI 1.4–5.9, P = 0.0057).

Conclusion: This study provides validation of the 70-gene signature as an independent prognostic factor in node-negative breast cancer.

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The level of progesterone receptor positivity is coupled to tamoxifen response in premenopausal breast cancer patients

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For several years tamoxifen has been the drug of choice in adjuvant endocrine therapy for steroid-hormone receptor positive breast cancer. However, in spite of the fact that many oestrogen receptor (ER) and/or progesterone receptor (PR) positive tumors initially respond to tamoxifen some may be de novo resistant and some acquire resistance over time. Until now, no reliable biomarkers for identifying subgroups at risk have been identified although many have been proposed, aided by the development of high throughput molecular techninques. This is indeed promising for the future but for the present, the use of existing predictive factors should be optimized. ER and PR are the most important predictive factors used today to tailor adjuvant therapies but they are routinely reported as positive or negative rather than quantified. Using the immunohistochemical assay which has gradually replaced the biochemical, tumors with >10% positive nuclei are considered positive. We believe that by using this cut-off, part of the predictive power of the hormone receptors is lost.

In the present study, we quantified the immunohistochemical expression of ER and PR in a tissue microarray (TMA) with tumors from 500 premenopausal breast cancer patients included in a randomized tamoxifen trial with a mean follow-up of more than 13 years. By subdividing receptor positivity into four subgroups, we demonstrate that only tumors with >75% PR positive nuclei respond to tamoxifen treatment, with an improved recurrence-free as well as overall survival, irrespective of ER status. Strikingly, for tumors with a PR fraction lower than 75%, tamoxifen treatment did not improve recurrence free survival compared to controls and the overall survival was shorter than for controls, although not statistically significant. The PR works downstream of ER and is dependent on its activation. The connection between ligand-dependent and independent activation of the ER and its effect on the PR is therefore subject for further studies.