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# Clinical significance of the nuclear receptor co-regulator DC-SCRIPT in breast cancer: an independent retrospective validation study

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**Introduction:** In this study we aimed to validate the prognostic value of *DC-SCRIPT* mRNA expression in a large independent breast cancer cohort. In addition, since DC-SCRIPT is a transcriptional co-regulator of nuclear receptors, we explored its prognostic value in relation to estrogen-receptor-α (*ESR1*) and -β (*ESR2*) and evaluated its predictive value for response to tamoxifen treatment.

**Methods:** *DC-SCRIPT* mRNA levels were measured by real-time PCR in 1,505 primary invasive breast cancers and associated with outcome (disease-free survival (DFS), metastasis-free survival (MFS) and overall survival (OS)) using univariate and multivariable Cox regression analysis. Logistic and Cox regression were used to associate *DC-SCRIPT* levels with clinical benefit and progression-free survival (PFS) for 296 patients treated with first-line systemic tamoxifen for advanced disease.

**Results:** In univariate and multivariable analysis higher *DC-SCRIPT* levels were associated with a favorable outcome for both the entire cohort and patients with lymph node-negative (LNN) disease that did not receive adjuvant therapy (DFS, MFS and OS; all, $P<0.001$). This association was most pronounced in small (pT1) tumors, in *ESR1*-positive tumors and in tumors with low *ESR2* expression. For first-line endocrine therapy for advanced disease no predictive association was seen with clinical benefit or PFS.

**Conclusions:** This study provides a higher level of evidence that *DC-SCRIPT* is indeed an independent, pure prognostic, factor for primary breast cancer and shows that *DC-SCRIPT* mRNA expression is most informative for either *ESR1*-positive and/or *ESR2*-low pT1 tumors.

## Introduction

Estrogens influence the aggressiveness of breast cancer through their cognate nuclear receptors. In particular the estrogen receptor alpha (ERα, *ESR1*) -present in tumor cells of about 70-75% of all breast tumors- is considered crucial due to its proliferation-inducing actions and for that reason is an important target for therapy. Next to *ESR1* a second ER exists, ERß (*ESR2*). *ESR2* counteracts the activity of *ESR1* in many systems [1, 2] and is also expressed in the majority of breast cancers. Apart from breast epithelial tumor cells, *ESR2* is also expressed in adjacent infiltrating lymphocytes, fibroblasts and endothelial cells, all of which are known to influence tumor growth [3]. However, its precise role in breast cancer progression is less well defined.

DC-SCRIPT (*ZNF366*) is a recently identified nuclear receptor co-regulator first identified in immune cells [4-6]. Nuclear receptor coregulators are proteins that can activate or repress the transcriptional activity of nuclear receptors. DC-SCRIPT is in this respect a unique co-regulator as we have shown that it enhances the activities of the nuclear retinoic acid receptor (RAR) and peroxisome proliferator-activated receptor (PPAR) heterodimers, RARα/RXRα and PPARγ/RXRα, but represses the activities of *ESR1* and progesterone receptor (*PGR*) [7]. We also showed that DC-SCRIPT was an independent prognostic factor, particularly for hormone receptor-positive breast cancer. This led us to postulate that the anti-proliferative effect of DC-SCRIPT in breast cancer cells could be mediated by simultaneous modulation of the activity of multiple nuclear receptors.

To provide a higher level of evidence for DC-SCRIPT mRNA expression as a prognostic marker, we now report on the expression and significance of *DC-SCRIPT* expression in a retrospective validation study on 1,505 breast cancer patients with known *ESR1, ESR2* and *PGR* expression levels. The primary objective of this study was to confirm the relationship between DC-SCRIPT mRNA levels measured in primary breast cancers and tumor aggressiveness in a much larger and independent breast cancer cohort. The main clinical endpoints for assessing the prognostic value of *DC-SCRIPT* expression were DFS, MFS and OS in lymph node-negative (LNN) patients who had not received adjuvant systemic therapy, which allowed us to determine tumor aggressiveness during the natural course of the disease.

As DC-SCRIPT modulates ER receptor activity, we also analyzed the prognostic value of *DC-SCRIPT* separately in tumors stratified by *ESR1* and *ESR2* expression. Since several co-regulators of nuclear receptors also modulate response to therapy [8, 9], we also assessed, as secondary aim of this study, the predictive value of *DC-SCRIPT* using clinical benefit and PFS after first-line tamoxifen for advanced disease as the main endpoints.

## Materials and methods

### Patients

The protocol to study biological markers associated with disease outcome was approved by the medical ethics committee of the Erasmus Medical Center Rotterdam, the Netherlands (MEC 02.953). This retrospective study used 1,505 M0 and an additional 32 M1 blind-coded freshly frozen primary tumor tissues of female patients with primary operable breast cancer from 1978 through 2000. The study was performed in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands [10], consent was not required, and, wherever possible, has been reported in line with the Reporting Recommendations for Tumor Marker Prognostic Studies guidelines [11]. The primary breast tumors were from patients with detailed clinical follow-up as previously described [12-14]. ER protein status was determined by routine ligand-binding assays or enzyme immunoassays [15] and *ESR1*, *ESR2* and *PGR* mRNA status by real-time reverse transcriptase PCR [14, 16, 17]. Follow-up, tumor staging, and response to therapy was defined by standard International Union Against Cancer (Geneva, Switzerland) classification criteria [18] and applied previously by Foekens et al [19]. All 1,537 patients underwent breast conserving lumpectomy (44%) or modified mastectomy (56%). Of the 1,505 patients included for the evaluation of tumor aggressiveness, 462 lymph node-positive patients (31%) were treated with adjuvant systemic therapy, 207 patients received hormonal, 233 chemo- and 22 combination therapies. Disease recurrence occurred in 836 patients and 703 developed a distant metastasis. The median follow-up time of patients alive was 90 months (range 4 to 260 months).

Eight hundred thirty seven patients had no involved nodes and did not receive systemic adjuvant therapy..Of these 837 LNN patients, 383 had a disease relapse, 300 developed a distant metastasis and 273 had died during follow-up.

Of the 703 patients who developed a distant metastasis, 296 ER-positive patients, including the additional 32 M1 patients, received hormonal therapy as first line therapy for advanced disease. Clinical benefit of first-line tamoxifen treatment was observed in 185 patients. Median follow-up time for treatment of advanced disease was 38 (4 to 120) months. Two-hundred nineteen patients had died at the end of the

follow-up. None of these patients had received prior adjuvant hormonal therapy while 19% received prior adjuvant chemotherapy.

More detailed descriptions of the patients and their therapy are given in the Supplementary materials and methods (Additional file 1). Patient and tumor characteristics combined with *DC-SCRIPT* mRNA expression and clinical outcome are listed in Table 1.

**RNA isolation and Quantitative RT-PCR**

Tissue processing, RNA isolation, cDNA synthesis and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) were performed as previously described [16]. Real-time quantitative PCR reactions were performed in a 25 µL reaction volume in a Mx3000P$^{TM}$ Real-Time PCR System (Agilent, Amsterdam, The Netherlands). In addition to a SYBR-based assay to detect a 129 bp DC-SCRIPT transcript covering exon 4 to 5 (forward primer: 5'-AAAGTCAAGCATGGAGTCATG-3'; reverse primer: 5'-GCTTCTGAGAGAGGTCAAAG-3'), a commercially available Taqman Gene Expression Assay from Applied Biosystems (Nieuwerkerk aan den IJssel, the Netherlands) covering exon 3 to 4 and generating a 62 bp product and was used (Hs00403536_m1, RefSeq NM_152625.1). DC-SCRIPT levels were readily detected with both assays and data generated with these assays correlated significantly (Spearman's Rho=0.87; $P$<0.0001). We therefore performed our analyses on the real-time RT-PCR data generated with the Taqman assay which is generally considered to be more specific. Intron-spanning primer sequences for the three reference genes, i.e., hydroxymethylbilane synthase (*HMBS*), hypoxanthine-guanine phospho-ribosyltransferase (*HPRT1*) and ß-2-microglobulin (*B2M*) and for *ESR1*, *ESR2*, *PGR* and real time PCR conditions for these SYBR-based assays were as described previously [16, 17]. Forty rounds of amplification were performed and fluorescent signals of the Taqman probe or SYBR green signal were used to generate Cycle threshold (Ct) values from which mRNA expression levels were calculated. Ct-values of *HPRT1* and *B2M* were adjusted to the higher *HMBS* Ct-values. Next, the expression levels of DC-SCRIPT were normalized against the average expression levels of the thus same ranged three reference genes as follows: mRNA target = $2^{(\text{mean Ct reference genes} - \text{mean Ct Target})}$[16].

**Tissue Processing**

Primary tumor tissue was processed as described previously [16]. To assess the amount of invasive tumor cell nuclei relative to the amount of surrounding stromal cells, 5 µm sections were cut for hematoxylin and eosin staining, before, in between, and after cutting the sections for RNA isolation. Only specimen with at least 30% invasive tumor cell nuclei were included in this study.

**Data analysis and statistics**

The relationship between *DC-SCRIPT* with patient and tumor characteristics were investigated with the use of nonparametric methods (Spearman rank correlations for continuous variables and Wilcoxon rank-sum for dichotomized or Kruskal-Wallis test for ordered variables). To reduce skewness *DC-SCRIPT* levels were transformed using the Box-Cox transformation. *DC-SCRIPT* levels were dichotomized using the previously identified 66.7% high versus 33.3% low cut off for *DC-SCRIPT* [7]. To test for an association with tumor aggressiveness and the time to progression during first-line therapy, Cox regression analysis was applied on the Box-Cox-transformed and dichotomized *DC-SCRIPT* mRNA levels.The hazard ratio (HR) and its 95% confidence interval (95% CI) was computed to correlate the expression levels with DFS, MFS, OS and PFS, respectively. In multivariable analysis, Cox proportional-hazards models for DFS, MFS, OS and PFS were applied to test *DC-SCRIPT* levels added to models with traditional factors. The proportional hazards assumptions were checked using Schoenfeld residuals. The analyses were stratified if necessary. The models for DFS, MFS and OS for LNN patients who had not received adjuvant systemic therapy included age, menopausal status, tumor size, grade, *ESR1* and *PGR* mRNA levels. Survival curves were generated using the method of Kaplan and Meier. The log-rank test was used to test for differences between survival curves. Logistic regression was used for the association of *DC-SCRIPT* with clinical benefit. Computations were performed with the STATA statistical package, release 11.0 (STATA Corp., College Station, TX) and SPSS 15.0. All P-values are two-sided and $P<0.05$ was considered statistically significant.

## Results

### Associations of *DC-SCRIPT* with clinicopathological factors and histological and intrinsic breast cancer subtypes

In analogy with our previous study, DC-SCRIPT mRNA expression was readily detected by quantitative RT-PCR in 5 normal breast tissues taken adjacent from tumor tissue and 5 prophylactic breast tissues (median [inter-quartile]: 0.063 [0.015] and 0.054 [0.035], respectively), while median levels were over 8-fold lower ($P<0.05$) in 1,505 invasive breast tumors (0.0069 [0.0074]). Table 1 shows the median expression levels and inter-quartile range of *DC-SCRIPT* transcripts and relation with patient and tumor characteristics for these 1,505 patients who were evaluable for prognosis. *DC-SCRIPT* levels were positively associated with tumor grade and *ESR1, PGR* and *ESR2* steroid hormone receptor expression level and negatively with invasive epithelial tumor cell content and tumor size. In addition *ESR2* was higher expressed in tumors with a higher percentage of stromal cells (786 tumors with 30 to 70% invasive epithelial cells) and *ESR1* was higher expressed in tumors with a high percentage of invasive epithelial cells (719 tumors with at least 70% invasive epithelial cells), $P<0.001$ (data not shown). High levels of *DC-SCRIPT* were found in breast tumors with a DCIS component or infiltrating lobular carcinoma compared with infiltrating ductal carcinomas (both $P<0.01$). Of 308 LNN tumors intrinsic subtyping data were available [20]. In these tumors, basal-like tumors had the lowest levels and normal-like breast tumors expressed significantly higher levels of *DC-SCRIPT* compared with the other intrinsic subtypes ($P<0.001$, Figure S1 in Additional file 2). Furthermore, luminal A tumors expressed higher levels of *DC-SCRIPT* and *ESR2* but lower levels of *ESR1* compared with luminal B tumors (median levels in luminal A versus luminal B: 0.0078 and 0.056 for *DC-SCRIPT*, $P=0.003$, 0.0095 and 0.0023 for *ESR2*, $P<0.001$ and 6.1 and 13.6 for *ESR1*, $P<0.001$). This may at least partly be explained by the fact that in this cohort of 308 LNN tumors the luminal B tumors contained a higher percentage of invasive epithelial cells [mean ± SD: 77± 9% for the n=64 luminal B tumors versus 67±12% for the n=71 luminal A tumors].

**DC-SCRIPT and tumor aggressiveness in univariate and multivariable analysis**

In the analyses including all 1,505 M0 patients, increasing levels of DC-SCRIPT mRNA were significantly associated with a favorable DFS, MFS and OS (HR 0.78, 0.74 and 0.77, respectively, all $P<0.001$). To test for a relation between DC-SCRIPT mRNA levels and tumor aggressiveness, i.e. the natural course of the disease without the confounding effect of systemic adjuvant therapy, we restricted our next analyses for MFS to those 837 patients with LNN disease who had not received (neo)adjuvant systemic therapy. The significant relationships of *DC-SCRIPT* as a continuous variable in these univariate analyses justified the use of the previously identified cut-point of 33.3% of the patients with low levels and 66.7% patients with high levels of DC-SCRIPT mRNA in their primary tumors [7]. In univariate analysis, high levels of *DC-SCRIPT* were significantly associated with a favorable prognosis HR 0.55, $P<0.001$, Table 2). When added to a multivariable base model for LNN disease - which included the traditional prognostic factors age, menopausal status, grade, *PGR* - stratified by *ESR1* and tumor size to meet the proportional hazards assumption, the association of *DC-SCRIPT* with MFS remained highly significant (HR 0.60, $P<0.001$,Table 2). Adding *ESR2* to the model did not significantly affect the prognostic value of *DC-SCRIPT* in these analyses (Table 2).

Because the proportional hazards assumptions were violated by *ESR1* and tumor size and because DC-SCRIPT is a transcriptional co-regulator of nuclear receptors - including the for breast cancer biologically relevant steroid hormone receptors - we next explored its prognostic value as continuous variable in subgroups of tumors stratified by steroid hormone receptor status and tumor size (Table 3 and Figure 1). Subdividing the 837 primary LNN tumors in *ESR1*-positive and negative [14] showed that increasing levels of *DC-SCRIPT* were in univariate and multivariable analysis only associated with good prognosis for the patients with *ESR1*-positive tumors. Subdividing these LNN tumors at the median level of *ESR2* in high and low revealed that, in contrast to *ESR1*, increasing levels of *DC-SCRIPT* were in both uni- and multivariable analysis only associated with good prognosis for patients with primary tumors with low levels of *ESR2*. With respect to tumor size, in uni- and multivariable analysis increasing levels of *DC-SCRIPT* were only associated with good prognosis for pT1 tumors and not for larger tumors. These and additional exploratory Cox univariate analyses are summarized in Table

3. The prognostic value of *DC-SCRIPT* are visualized in Kaplan-Meier curves (Figure 1) as dichotomized variable in these biologically relevant LNN *ESR1* negative (Figure 1a) and positive (Figure 1b) and LNN *ESR2* high (Figure 1d) and low (Figure 1e) subsets in combination with patients with pT1 primary tumors (Figure 1c and 1f).

**DC-SCRIPT and response to first-line endocrine therapy**

*DC-SCRIPT* expression levels were evaluated in 296 hormone-naïve ER-positive primary breast tumors from patients whose relapse was treated with first-line tamoxifen monotherapy. These patients had not received (neo)adjuvant endocrine systemic treatment. In univariate analyses no statistically significant associations were observed between *DC-SCRIPT* as transformed continuous variable and PFS or clinical benefit after start of first line treatment with tamoxifen (HR=1.08 [0.99-1.18], *P*=0.07 and OR=0.88 [0.74-1.05], *P*=0.16, respectively).

**Discussion**

DC-SCRIPT has previously been identified as a key modulator of nuclear receptor activity that has prognostic value in breast cancer [7]. The clinical conclusions about DC-SCRIPT mRNA expression as a prognostic marker in breast cancer were based on nonrandomized retrospective analyses in 3 small breast cancer cohorts from Nijmegen and still required independent validation. In this study, we provide a higher level of evidence as we confirm that mRNA expression values of DC-SCRIPT predict outcome in an independent retrospective cohort of 1,505 primary breast cancers from Rotterdam. In addition, we confirm that DC-SCRIPT mRNA expression is a pure prognostic marker as it predicts - independently of current clinical prognostic markers such as age, menopausal status, grade, T-size and receptor status - the occurrence of distant metastasis in patients who did not receive any adjuvant systemic treatment. Because we used mRNA extracted from tumor tissue using a different mRNA isolation method (RNA-B versus column-based), an independent real-time PCR assay to detect *DC-SCRIPT*, a different type of machine to amplify the transcript and other personnel from another institute, we consider *DC-SCRIPT* a

robust prognostic marker for early breast cancer patients. The patients described in this retrospective study entered the clinic during 1978–2000, at which time disease was diagnosed later and adjuvant therapy was not as wide-spread as nowadays. But the latter was at the same time the strength of our cohort for the evaluation of a prognostic marker. The data that emerged from this study thus validates the hypothesis that DC-SCRIPT is associated with good prognosis in early disease, and supports the idea that DC-SCRIPT acts as a tumor suppressor in breast cancer progression [7].

Because of the size of this cohort and based on the biological function of DC-SCRIPT as a nuclear receptor co-regulator we were able to include additional subgroup analyses to extend our insights into the clinical behavior and relevance of measuring *DC-SCRIPT* in primary breast cancers. High levels of *DC-SCRIPT* mRNA in primary tumors of breast cancer patients were significantly related with tumor characteristics that are associated with good prognosis such as DCIS, infiltrating lobular carcinoma, breast tumors of the normal-like and luminal A subtype, and small (pT1), well-differentiated, steroid hormone receptor positive tumors. While *ESR1* is mainly localized in tumors with at least 70% invasive epithelial cells, ($P$<0.001), we showed for both *ESR2* and *DC-SCRIPT* a positive correlation with tumors with <70% invasive epithelial cells, ($P$<0.001). As normal epithelial cells present in tumors with <70% invasive epithelial cells express the highest levels of DC-SCRIPT, they could be responsible for this correlation. Furthermore, infiltrating leukocytes in the stroma might have contributed in part to the detected signal [4, 5]. Alternatively, or in addition, stromal cells may have played a role in the induction of DC-SCRIPT in the epithelial tumor cells. In analogy *ESR2* is - apart from breast cancer epithelial tumor cells - also expressed in adjacent infiltrating lymphocytes, fibroblasts and endothelial cells [3].

Interestingly, in tumors that express relatively high *ESR2* mRNA levels and which in general are tumors with a higher stromal content, DC-SCRIPT expression has little or no prognostic value. Thus, while in early *ESR1* positive breast cancer DC-SCRIPT inhibits progression of breast cancer, this effect appears to be neutralized in tumors high in *ESR2*. Indeed, *ESR2* has been reported to be dominant over *ESR1* and able to counteract the proliferation-inducing activities of *ESR1* [1, 2]. Unraveling the precise role of DC-SCRIPT in the complex genomic and non-genomic interplay between *ESR1*, *ESR2* and their

isoforms [21-23] might turn out to be rewarding for elucidating the “Yin-Yang” role of these factors in breast cancer.

As DC-SCRIPT can inhibit ERα and PgR activity, a second aim of the study was to address whether DC-SCRIPT affects the response to endocrine therapy. In our previous study we had already explored the value of DC-SCRIPT mRNA expression to predict outcome in a cohort of breast cancer patients who received adjuvant tamoxifen [7]. However, in the adjuvant setting - that for ethical reasons nowadays only includes non-randomized patients among treated and untreated arms - one cannot discriminate between tumor aggressiveness and response to treatment [24]. The current retrospective study included hormone-naïve patients (i.e. not having received any (neo)adjuvant endocrine treatment) who received first-line tamoxifen treatment for their advanced disease and was therefore better suited to study a putative relation of *DC-SCRIPT* and response to therapy. Despite the positive association of *DC-SCRIPT* with *ESR1*, *DC-SCRIPT* levels were unable to identify patients with *ESR1* positive primary tumors at high or low risk to progress if treated with tamoxifen. Thus, although DC-SCRIPT can modulate the activity of *ESR1*, it does not affect the response to endocrine therapy with tamoxifen in advanced breast cancer. The early loss of DC-SCRIPT during cancer progression might explain this absence of a response in the metastatic disease setting.

**Conclusions**

This independent retrospective qRT-PCR study validates that high levels of *DC-SCRIPT* are associated with reduced tumor aggressiveness. The association is particularly strong for small tumors with high *ESR1* and/or low *ESR2* mRNA levels. Finally, despite the fact that DC-SCRIPT negatively regulates *ESR1* and *PGR* activity, but in line with DC-SCRIPT as an early marker for disease, *DC-SCRIPT* levels measured in the primary tumors are not associated with response to first-line endocrine treatment for advanced disease.

**Abbreviations**

B2M, Beta-2-Microglobulin; bp, base product; cDNA, copy DeoxyriboNucleic Acid; CI, Confidence Interval; CMF, Cyclophosphamide Methotrexate Fluorouracil; Ct, Cycle threshold; Ct, Cycle threshold; DCIS, Ductal Carcinoma In Situ; DC-SCRIPT, Dendritic Cell-specific transSCRIPT; DFS, Disease Free Survival; ER; Estrogen Receptor; ERBB2+, HER2neu positive; ESR, Estrogen Receptor; FAC, 5-Fluorouracil Adriamycin Cyclophosphamide; FEC, 5-Fluorouracil Epirubicin Cyclophosphamide; HMBS, HydroxyMethylBilane Synthase; HPRT1, Hypoxanthine guanine PhosphoribosylTransferase 1; HR, Hazard Ratio; IDC, Infiltrating Ductal Carcinoma; ILC, Infiltrating Lobular Carcinoma; LNN, lymph node-negative; M0, no metastasis; M1, with metastasis; MFS, Metastasis Free Survival; mRNA, messenger RiboNucleic Acid; OR, Odds Ratio; OS, Overall Survival; PCR, Polymerase Chain Reaction; PFS, Progression Free Survival; PGR, Progesterone Receptor; PPAR, Peroxisome Proliferator-Activated Receptor; pT1, small tumor without lymphatic/vascular invasion; qPCR, quantitative Polymerase Chain Reaction; RAR, Retinoic Acid Receptor; RT-PCR, Reverse Transcriptase Polymerase Chain Reaction; SYBR, N',N'-dimethyl-N-[4-[(E)-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-phenylquinolin-1-ium-2-yl]-N-propylpropane-1,3-diamine; T-size, Tumor-size; ZNF366, ZiNc Finger protein 366.

**Competing interests**

The authors declare that they have no competing interests

**Authors' contributions**

AS participated in the study design, collected laboratory data on the patients, performed laboratory work and statistical analyses and wrote the manuscript; MA participated in the study design, performed laboratory work and provided critical revision of the manuscript; ML collected laboratory data on the patients, performed the clinical statistical analyses and provided critical revision of the manuscript; PS provided critical revision of the manuscript and participated in the study design; VdW and AvG performed the laboratory work and provided critical revision of the manuscript; JF and JM participated in the study design, provided the study material and clinical information and provided critical revision of the

manuscript; GA participated in the study design and provided critical revision of the manuscript. All authors read and approved the final manuscript.

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**References**


1. Strom A, Hartman J, Foster JS, Kietz S, Wimalasena J, Gustafsson JA: **Estrogen receptor beta inhibits 17beta-estradiol-stimulated proliferation of the breast cancer cell line T47D.** *Proc Natl Acad Sci U S A* 2004, **101:**1566-1571.

2. Liu MM, Albanese C, Anderson CM, Hilty K, Webb P, Uht RM, Price RH, Jr., Pestell RG, Kushner PJ: **Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression.** *J Biol Chem* 2002, **277:**24353-24360.

3. Hartman J, Strom A, Gustafsson JA: **Estrogen receptor beta in breast cancer--diagnostic and therapeutic implications.** *Steroids* 2009, **74:**635-641.

4. Triantis V, Moulin V, Looman MW, Hartgers FC, Janssen RA, Adema GJ: **Molecular characterization of the murine homologue of the DC-derived protein DC-SCRIPT.** *J Leukoc Biol* 2006, **79:**1083-1091.

5. Triantis V, Trancikova DE, Looman MW, Hartgers FC, Janssen RA, Adema GJ: **Identification and characterization of DC-SCRIPT, a novel dendritic cell-expressed member of the zinc finger family of transcriptional regulators.** *J Immunol* 2006, **176:**1081-1089.

6. Lopez-Garcia J, Periyasamy M, Thomas RS, Christian M, Leao M, Jat P, Kindle KB, Heery DM, Parker MG, Buluwela L, Kamalati T, Ali S: **ZNF366 is an estrogen receptor corepressor that acts through CtBP and histone deacetylases.** *Nucleic Acids Res* 2006, **34:**6126-6136.

7. Ansems M, Hontelez S, Looman MW, Karthaus N, Bult P, Bonenkamp JJ, Jansen JH, Sweep FC, Span PN, Adema GJ: **DC-SCRIPT: nuclear receptor modulation and prognostic significance in primary breast cancer.** *J Natl Cancer Inst*, **102:**54-68.

8. Ramadoss P, Marcus C, Perdew GH: **Role of the aryl hydrocarbon receptor in drug metabolism.** *Expert Opin Drug Metab Toxicol* 2005, **1:**9-21.

9. Osborne CK, Schiff R, Fuqua SA, Shou J: **Estrogen receptor: current understanding of its activation and modulation.** *Clin Cancer Res* 2001, **7:**4338s-4342s; discussion 4411s-4412s.

10. **Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands.** [http://www.federa.org/?s=1&m=99].

11. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM: **REporting recommendations for tumor MARKer prognostic studies (REMARK).** *Breast Cancer Res Treat* 2006, **100:**229-235.

12. Jansen MP, Sieuwerts AM, Look MP, Ritstier K, Meijer-van Gelder ME, van Staveren IL, Klijn JG, Foekens JA, Berns EM: **HOXB13-to-IL17BR expression ratio is related with tumor aggressiveness and response to tamoxifen of recurrent breast cancer: a retrospective study.** *J Clin Oncol* 2007, **25:**662-668.

13. Sieuwerts AM, Look MP, Meijer-van Gelder ME, Timmermans M, Trapman AM, Garcia RR, Arnold M, Goedheer AJ, de Weerd V, Portengen H, Klijn JG, Foekens JA: **Which cyclin E prevails as prognostic marker for breast cancer? Results from a retrospective study involving 635 lymph node-negative breast cancer patients.** *Clin Cancer Res* 2006, **12:**3319-3328.

14. Sieuwerts AM, Usher PA, Meijer-van Gelder ME, Timmermans M, Martens JW, Brunner N, Klijn JG, Offenberg H, Foekens JA: **Concentrations of TIMP1 mRNA splice variants and TIMP-1 protein are differentially associated with prognosis in primary breast cancer.** *Clin Chem* 2007, **53:**1280-1288.

15. Foekens JA, Portengen H, van Putten WL, Peters HA, Krijnen HL, Alexieva-Figusch J, Klijn JG: **Prognostic value of estrogen and progesterone receptors measured by enzyme immunoassays in human breast tumor cytosols.** *Cancer Res* 1989, **49:**5823-5828.

16. Sieuwerts AM, Meijer-van Gelder ME, Timmermans M, Trapman AM, Garcia RR, Arnold M, Goedheer AJ, Portengen H, Klijn JG, Foekens JA: **How ADAM-9 and ADAM-11 differentially from estrogen receptor predict response to tamoxifen treatment in patients with recurrent breast cancer: a retrospective study.** *Clin Cancer Res* 2005, **11:**7311-7321.

17. Dorssers LC, van Agthoven T, Brinkman A, Veldscholte J, Smid M, Dechering KJ: **Breast cancer oestrogen independence mediated by BCAR1 or BCAR3 genes is transmitted through mechanisms distinct from the oestrogen receptor signalling pathway or the epidermal growth factor receptor signalling pathway.** *Breast Cancer Res* 2005, **7:**R82-92.

18. Hayward JL, Carbone PP, Heuson JC, Kumaoka S, Segaloff A, Rubens RD: **Assessment of response to therapy in advanced breast cancer: a project of the Programme on Clinical Oncology of the International Union Against Cancer, Geneva, Switzerland.** *Cancer* 1977, **39:**1289-1294.

19. Foekens JA, Peters HA, Grebenchtchikov N, Look MP, Meijer-van Gelder ME, Geurts-Moespot A, van der Kwast TH, Sweep CG, Klijn JG: **High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer.** *Cancer Res* 2001, **61:**5407-5414.

20. Smid M, Wang Y, Zhang Y, Sieuwerts AM, Yu J, Klijn JG, Foekens JA, Martens JW: **Subtypes of breast cancer show preferential site of relapse.** *Cancer Res* 2008, **68:**3108-3114.

21. Lee LM, Cao J, Deng H, Chen P, Gatalica Z, Wang ZY: **ER-alpha36, a novel variant of ER-alpha, is expressed in ER-positive and -negative human breast carcinomas.** *Anticancer Res* 2008, **28:**479-483.

22. Chen JQ, Russo PA, Cooke C, Russo IH, Russo J: **ERbeta shifts from mitochondria to nucleus during estrogen-induced neoplastic transformation of human breast epithelial cells and is involved in estrogen-induced synthesis of mitochondrial respiratory chain proteins.** *Biochim Biophys Acta* 2007, **1773:**1732-1746.

23. Shaaban AM, Green AR, Karthik S, Alizadeh Y, Hughes TA, Harkins L, Ellis IO, Robertson JF, Paish EC, Saunders PT, Groome NP, Speirs V: **Nuclear and cytoplasmic expression of ERbeta1, ERbeta2, and ERbeta5 identifies distinct prognostic outcome for breast cancer patients.** *Clin Cancer Res* 2008, **14:**5228-5235.

24. Jansen MP, Foekens JA, Klijn JG, Berns EM: **Re: Limits of predictive models using microarray data for breast cancer clinical treatment outcome.** *J Natl Cancer Inst* 2005, **97:**1851-1852; author reply 1852-1853.

25. Foekens JA, Look MP, Bolt-de Vries J, Meijer-van Gelder ME, van Putten WL, Klijn JG: **Cathepsin-D in primary breast cancer: prognostic evaluation involving 2810 patients.** *Br J Cancer* 1999, **79:**300-307.

26. van Agthoven T, Sieuwerts AM, Meijer-van Gelder ME, Look MP, Smid M, Veldscholte J, Sleijfer S, Foekens JA, Dorssers LC: **Relevance of breast cancer antiestrogen resistance genes in human breast cancer progression and tamoxifen resistance.** *J Clin Oncol* 2009, **27:**542-549.

27. Meijer-van Gelder ME, Look MP, Peters HA, Schmitt M, Brunner N, Harbeck N, Klijn JG, Foekens JA: **Urokinase-type plasminogen activator system in breast cancer: association with tamoxifen therapy in recurrent disease.** *Cancer Res* 2004, **64:**4563-4568.

28. Martens JW, Nimmrich I, Koenig T, Look MP, Harbeck N, Model F, Kluth A, Bolt-de Vries J, Sieuwerts AM, Portengen H, Meijer-Van Gelder ME, Piepenbrock C, Olek A, Höfler H, Kiechle M, Klijn JG, Schmitt M, Maier S, Foekens JA: **Association of DNA methylation of phosphoserine aminotransferase with response to endocrine therapy in patients with recurrent breast cancer.** *Cancer Res* 2005, **65:**4101-4117.

**Table 1: Associations of *DC-SCRIPT* with clinicopathological and biological factors**

| Characteristic | No of patients | %* | *DC-SCRIPT*** [reference normalized] ($x10^2$) | |
|---|---|---|---|---|
| **All patients** | **1.505** | ***100%*** | **0,69** | ***0,73*** |
| **Age (years)** | | | | |
| ≤40 | 192 | *13%* | 0,69 | *0,72* |
| 41-55 | 561 | *37%* | 0,70 | *0,74* |
| 56-70 | 498 | *33%* | 0,70 | *0,77* |
| >70 | 254 | *17%* | 0,64 | *0,64* |
| | | | *P = 0.15*‡ | |
| **Menopausal status** | | | | |
| premenopausal | 637 | *42%* | 0,72 | *0,74* |
| postmenopausal | 868 | *58%* | 0,66 | *0,70* |
| | | | *P* = 0.06§ | |
| **Grade** | | | | |
| poor | 818 | *54%* | 0,64 | *0,74* |
| unknown | 452 | *30%* | 0,71 | *0,68* |
| moderate and good | 235 | *16%* | 0,80 | *0,70* |
| | | | ***P* = 0.001**‖ | |
| **Tumor size** | | | | |
| pT1, ≤2 cm | 517 | *34%* | 0,81 | *0,84* |
| >2 cm | 988 | *66%* | 0,63 | *0,64* |
| | | | ***P* <0.001**§ | |
| **Lymph nodes involved**† | | | | |
| no | 837 | *56%* | 0,69 | *0,73* |
| yes | 668 | *44%* | 0,68 | *0,75* |
| | | | *P* =0.64§ | |
| ***ESR*1 mRNA status**† | | | | |
| positive, ≥0.2 | 1176 | *78%* | 0,71 | *0,73* |
| negative, <0.2 | 329 | *22%* | 0,61 | *0,66* |
| | | | ***P = 0.004***‡ | |
| ***PgR* mRNA status**† | | | | |
| positive, ≥ 0.1 | 949 | *63%* | 0,72 | *0,74* |
| negative, <0.1 | 556 | *37%* | 0,61 | *0,66* |
| | | | ***P < 0.001***‡ | |
| ***ESR2* mRNA status**† | | | | |
| dichotomized high, ≥ 0.005 | 741 | *49%* | 0,89 | *0,95* |
| dichotomized low, <0.005 | 742 | *49%* | 0,54 | *0,49* |
| | | | ***P < 0.001***‡ | |
| **Invasive tumor cell content**x | | | | |
| ≥ 70% | 719 | *48%* | 0,57 | *0,51* |
| < 70% | 786 | *52%* | 0,85 | *0,91* |
| | | | ***P* <0.001**§ | |
| **Histological type** | | | | |
| DCIS + IDC | 194 | *13%* | 0,82 | *0,89* |
| ILC: infiltrating lobular | 135 | *9%* | 0,81 | *0,94* |
| IDC: infiltrating ductal | 810 | *54%* | 0,66 | *0,69* |
| mucinous | 40 | *3%* | 0,56 | *0,65* |
| medullary | 31 | *2%* | 0,69 | *1,18* |
| | | | ***P = 0.012***‖ | |
| **Intrinsic breast cancer subtype**≠ | **308** | | | |
| normal-like | 22 | *7%* | 1,43 | *1,19* |
| ERBB2+ | 63 | *20%* | 0,75 | *0,68* |
| luminal A | 76 | *25%* | 0,78 | *0,89* |
| luminal B | 65 | *21%* | 0,56 | *0,36* |
| basal | 82 | *27%* | 0,48 | *0,48* |
| | | | ***P* <0.001**‖ | |

* Due to missing cases numbers to not always add up to 100%.

** Median level and p50 inter-quartile after normalization on the reference gene set.

† With qPCR cut point for positive versus negative *ESR1* and *PGR*, 0.2 and 0.1, respectively, and for *ESR2* at the median level of 0.005 (mRNA levels relative to reference gene set).

x Dichotomized at the median level of 70% invasive tumor cells (TC).

≠ Intrinsic breast cancer subtypes assigned from Affymetrix microarray by hierarchical clustering in 308 patients with LNN disease that did not recieve systemic adjuvant treatment.

‡ *P* for Spearman rank correlation test.

§ *P* for Mann-Whitney U test.

| *P* for Kruskal-Wallis test, including a Wilcoxon-type test for trend when appropriate.

**Table 2: Univariate and multivariable analysis for MFS as a function of *DC-SCRIPT* in LNN disease**

| Factor | No. | Univariate analysis HR | [95% CI] | | P | Multivariable analysis* HR | [95% CI] | | P |
|---|---|---|---|---|---|---|---|---|---|
| | 837 | | | | | | | | |
| **Age (years)** | | | | | | | | | |
| ≤40 | 114 | 1 | | | | 1 | | | |
| 41-55 | 295 | 0,88 | 0,63 | 1,22 | | 0,95 | 0,67 | 1,35 | |
| 56-70 | 270 | 0,72 | 0,51 | 1,02 | | 0,69 | 0,40 | 1,20 | |
| >70 | 158 | 0,53 | 0,35 | 0,81 | **<0.01** | 0,49 | 0,27 | 0,90 | 0,077 |
| **Menopausal status** | | | | | | | | | |
| premenopausal | 350 | 1 | | | | 1 | | | |
| postmenopausal | 487 | 0,78 | 0,62 | 0,97 | **0,028** | 1,08 | 0,70 | 1,66 | 0,731 |
| **Grade** | | | | | | | | | |
| poor | 422 | 1 | | | | 1 | | | |
| unknown | 262 | 1,02 | 0,79 | 1,30 | | 1,12 | 0,87 | 1,44 | |
| moderate and good | 153 | 0,49 | 0,34 | 0,71 | **<0.001** | 0,54 | 0,37 | 0,78 | **<0.001** |
| ***PGR* mRNA status**[≠] | | | | | | | | | |
| negative, <0.1 | 312 | 1 | | | | 1 | | | |
| positive, ≥ 0.1 | 525 | 0,68 | 0,54 | 0,85 | **0,001** | 0,71 | 0,53 | 0,95 | **0,022** |
| **Tumor size** | | | | | | | | | |
| ≤2 cm | 378 | 1 | | | | | | | |
| >2 cm + unknown | 459 | 1,26 | 1,00 | 1,59 | **0,047** | ***Analyses stratified by tumor size*** | | | |
| ***ESR1* mRNA status**[†] | | | | | | ***to meet the proportional*** | | | |
| negative, <0.2 | 199 | 1 | | | | | | | |
| positive, ≥0.2 | 638 | 0,77 | 0,59 | 0,99 | **0,040** | ***hazards assumption*** | | | |
| **Factor analyzed** | | | | | | **Additions to the base model** | | | |
| ***DC-SCRIPT*** | | | | | | | | | |
| continuous | 837 | 0,77 | 0,67 | 0,88 | **<0.001** | 0,80 | 0,70 | 0,92 | **0,001** |
| 33.3% low | 277 | 1 | | | | 1 | | | |
| 66.7% high | 560 | 0,55 | 0,43 | 0,69 | **<0.001** | 0,60 | 0,47 | 0,76 | **<0.001** |
| ***ESR2* mRNA status**[≠] | | | | | | | | | |
| continuous | 820 | 0,88 | 0,79 | 0,99 | **0,034** | 0,86 | 0,76 | 0,96 | **0,011** |
| dichotomized low, <0.005 | 410 | 1 | | | | 1,00 | | | |
| dichotomized high, ≥ 0.005 | 410 | 0,80 | 0,63 | 1,00 | 0,052 | 0,75 | 0,59 | 0,94 | **0,014** |
| ***DC-SCRIPT* and *ESR2* combined** | | | | | | | | | |
| both low | 183 | 1 | | | | 1 | | | |
| *DC-SCRIPT* low *ESR2* high | 91 | 0,74 | 0,51 | 1,08 | | 0,71 | 0,49 | 1,04 | |
| *DC-SCRIPT* high *ESR2* low | 227 | 0,49 | 0,36 | 0,67 | | 0,55 | 0,40 | 0,76 | |
| both high | 319 | 0,50 | 0,38 | 0,67 | **<0.001** | 0,52 | 0,39 | 0,69 | **<0.001** |

* Multivariable analyses were conducted in two blocks. First, a model including all established clinicopathological factors was fitted.

The Cox proportional hazards assumptions were checked and the analyses were stratified by tumor-size and *ESR1* to meet the PH assumption.

In a second block, the contributions of *DC-SCRIPT* and *ESR2* (as continuous or dichotomized variables) were investigated.

**Table 3: DFS, MFS and OS as a function of continuous *DC-SCRIPT* in lymph node negative disease**

| Association with continuous *DC-SCRIPT* | | Disease-free survival | | | | Metastasis-free survival | | | | Overall survival | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cohort | No. | HR | [95% CI] | | *P* | HR | [95% CI] | | *P* | HR | [95% CI] | | *P* |
| **LNN** | **837** | 0,82 | 0,73 | 0,93 | **0,001** | 0,77 | 0,67 | 0,88 | **<0.001** | 0,82 | 0,72 | 0,94 | **0,005** |
| *ESR1* mRNA negative | 199 | 0,94 | 0,76 | 1,17 | 0,59 | 0,93 | 0,73 | 1,18 | 0,53 | 0,88 | 0,70 | 1,11 | 0,30 |
| *ESR1* mRNA positive | 638 | 0,79 | 0,68 | 0,90 | **0,001** | 0,72 | 0,62 | 0,85 | **<0.001** | 0,81 | 0,69 | 0,96 | **0,014** |
| *PGR* mRNA negative | 312 | 0,88 | 0,74 | 1,06 | 0,19 | 0,84 | 0,69 | 1,03 | 0,10 | 0,88 | 0,72 | 1,08 | 0,22 |
| *PGR* mRNA positive | 525 | 0,81 | 0,69 | 0,94 | **0,007** | 0,75 | 0,63 | 0,89 | **0,001** | 0,82 | 0,68 | 0,99 | **0,04** |
| *ESR2* mRNA low | 410 | 0,76 | 0,64 | 0,91 | **0,003** | 0,69 | 0,56 | 0,84 | **<0.001** | 0,73 | 0,64 | 0,97 | **0,026** |
| *ESR2* mRNA high | 410 | 0,93 | 0,78 | 1,11 | 0,43 | 0,89 | 0,73 | 1,09 | 0,26 | 0,92 | 0,75 | 1,13 | 0,41 |
| tumor size ≤2 cm (pT1)* | 378 | 0,74 | 0,61 | 0,89 | **0,001** | 0,67 | 0,54 | 0,83 | **0,000** | 0,73 | 0,59 | 0,91 | **0,005** |
| tumor size >2 cm* | 459 | 0,92 | 0,79 | 1,08 | 0,31 | 0,86 | 0,72 | 1,03 | 0,10 | 0,91 | 0,76 | 1,09 | 0,31 |
| *ESR1* mRNA positive, tumor size ≤2 cm | 306 | 0,69 | 0,56 | 0,85 | **0,001** | 0,61 | 0,48 | 0,78 | **<0.001** | 0,72 | 0,56 | 0,93 | **0,010** |
| *ESR1* mRNA positive, tumor size >2 cm | 332 | 0,91 | 0,75 | 1,10 | 0,34 | 0,84 | 0,68 | 1,05 | 0,13 | 0,90 | 0,72 | 1,14 | 0,39 |
| *ESR2* mRNA low, tumor size ≤2 cm | 175 | 0,57 | 0,43 | 0,76 | **<0.001** | 0,51 | 0,37 | 0,70 | **<0.001** | 0,60 | 0,44 | 0,83 | **0,002** |
| *ESR2* mRNA high, tumor size >2 cm | 218 | 0,98 | 0,78 | 1,23 | 0,84 | 0,91 | 0,71 | 1,18 | 0,49 | 0,93 | 0,74 | 1,21 | 0,58 |
| *ESR1* positive and *ESR2* low, tumor size ≤2 cm | 147 | 0,63 | 0,45 | 0,87 | **0,005** | 0,54 | 0,38 | 0,78 | **<0.001** | 0,63 | 0,43 | 0,92 | **0,017** |
| *ESR1* positive and *ESR2* low, tumor size >2 cm | 181 | 0,94 | 0,71 | 1,24 | 0,66 | 0,94 | 0,68 | 1,29 | 0,69 | 1,03 | 0,73 | 1,45 | 0,89 |
| *ESR1* positive and/or *ESR2* low, tumor size ≤2 cm | 334 | 0,65 | 0,53 | 0,79 | **<0.001** | 0,57 | 0,46 | 0,71 | **<0.001** | 0,67 | 0,53 | 0,84 | **0,001** |
| *ESR1* positive and/or *ESR2* low, tumor size >2 cm | 386 | 0,90 | 0,76 | 1,08 | 0,25 | 0,81 | 0,66 | 0,99 | **0,037** | 0,87 | 0,71 | 1,07 | 0,20 |

† With qPCR cut point for positive versus negative *ESR1* and *PGR*, 0.2 and 0.1, respectively, and for *ESR2* at the median level of 0.005 (mRNA levels relative to reference gene set).

* Interaction with continuous *DC-SCRIPT* ($P<0.05$).

## Figure legends

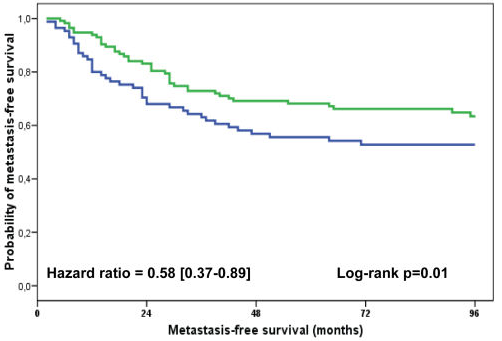
**Figure 1: Metastasis-free survival as a function of dichotomized *DC-SCRIPT*.** MFS as a function of dichotomized DC-SCRIPT in 837 LNN primary breast cancer patients after subdividing patients according high and low *ESR1* and *ESR2* in the primary tumor and tumor size. qPCR cut points for high versus low *DC-SCRIPT*, 66.7% versus 33.3% [7]; for positive versus negative *ESR1*, 0.2 [14]; and for *ESR2* low versus high, at the median level of 0.005 (mRNA levels relative to reference gene set). Patients at risk are indicated.

## Additional files

**Additional file 1: Supplementary materials and methods.** A word file containing additional Materials and methods [25-28].

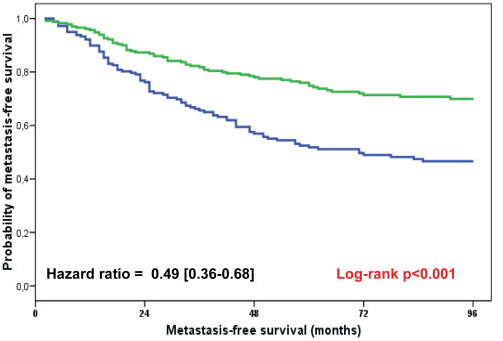
**Additional file 2: Figure S1 - *DC-SCRIPT* mRNA expression in breast cancer subtypes.** The box-plot shows the five statistics (lower whisker is 5% minimum, lower box part is 25$^{th}$ percentile, solid line in box presents the median, upper box part is 75$^{th}$ percentile and upper whisker is 95% maximum). Figure depicts *P* for Mann-Whitney U test to identify significantly different expression of *DC-SCRIPT* in between subtypes.

(a) *ESR1* negative primary breast tumors



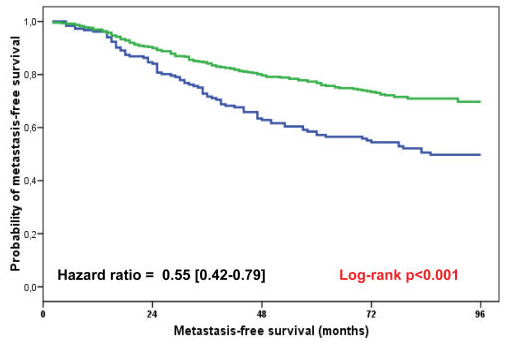

| Patients at risk | | | | | |
|---|---|---|---|---|---|
| low *DC-SCRIPT* | 88 | 58 | 47 | 36 | 22 |
| high *DC-SCRIPT* | 111 | 89 | 71 | 62 | 40 |

(d) *ESR2* low primary breast tumors



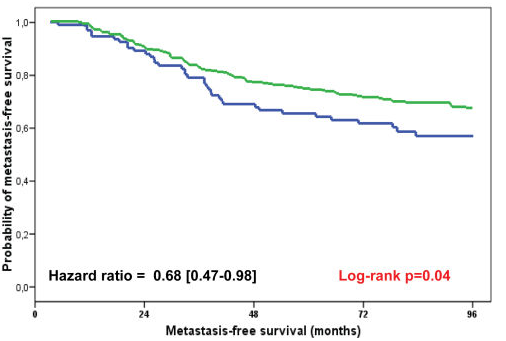

| | | | | | |
|---|---|---|---|---|---|
| low *DC-SCRIPT* | 173 | 137 | 96 | 68 | 41 |
| high *DC-SCRIPT* | 227 | 190 | 157 | 114 | 78 |

(b) *ESR1* positive primary breast tumors



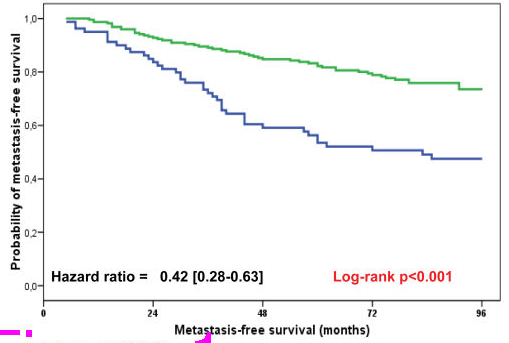

| Patients at risk | | | | | |
|---|---|---|---|---|---|
| low *DC-SCRIPT* | 189 | 156 | 109 | 78 | 46 |
| high *DC-SCRIPT* | 449 | 391 | 323 | 241 | 161 |

(e) *ESR2* high primary breast tumors



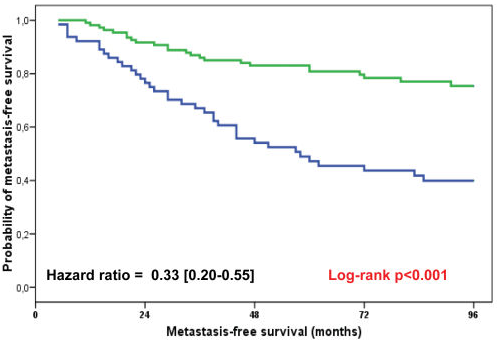

| | | | | | |
|---|---|---|---|---|---|
| low *DC-SCRIPT* | 91 | 75 | 58 | 44 | 26 |
| high *DC-SCRIPT* | 319 | 276 | 226 | 179 | 117 |

(c) *ESR1* positive pT1 primary breast tumors




| Patients at risk | | | | | |
|---|---|---|---|---|---|
| low *DC-SCRIPT* | 83 | 69 | 47 | 35 | 22 |
| high *DC-SCRIPT* | 224 | 202 | 174 | 136 | 86 |

(f) *ESR2* low pT1 primary breast tumors




| | | | | | |
|---|---|---|---|---|---|
| low *DC-SCRIPT* | 68 | 54 | 37 | 25 | 15 |
| high *DC-SCRIPT* | 107 | 96 | 82 | 63 | 41 |

Figure 1

**Additional files provided with this submission:**

Additional file 1: Online Only suppl materials and methods, additional file 1.doc, 33K
http://breast-cancer-research.com/imedia/1997644614489899/supp1.doc
Additional file 2: Online only figure 1, additional file 2.tif, 215K
http://breast-cancer-research.com/imedia/8717702774898990/supp2.tiff