Clinical and Molecular Characteristics of ER-Positive Breast Cancer Tumors Identified as Ultralow Risk by the 70-Gene Signature in a Randomized Clinical Trial

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Research

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Abstract

Background

Breast cancer tumors are heterogenous, including their metastatic potential and time to distant metastasis. Patients with estrogen receptor (ER)-positive tumors have a continuous long-term risk of fatal disease decades after diagnosis. Recently, ER-positive breast cancer patients classified as having ultralow risk tumors by the 70-gene expression signature (MammaPrint) were associated with a minimal risk of fatal disease. However, the underlying tumor characteristics of ultralow risk tumors are unknown.

Methods

Secondary analysis of the Stockholm tamoxifen randomized trial (STO-3, 1976-1990) enrolling postmenopausal lymph node-negative breast cancer patients. Immunohistochemistry of the clinically used breast cancer markers (n=727 patients) and gene expression profiling by Agilent microarrays (n=652 patients) were performed in 2014. Ultralow risk tumors, identified by the 70-gene signature, were compared to other ER-positive tumors (of low/high risk) and Luminal A and B PAM50 subtype tumors (ER-positive, low/high risk) by the clinical markers and multi-gene modules, representative of specific biological processes and pathways, using Fisher's exact test. Furthermore, differential gene expression analysis was performed contrasting ultralow risk tumors to other ER-positive tumors (of low/high risk) using t-statistics and false discovery rate (FDR).

Results

Ultralow risk tumors were significantly (P<0.05) more likely to be of smaller tumor size, lower tumor grade, progesterone receptor (PR)-positive, human epidermal growth factor receptor 2 (HER2)-negative and Ki-67-low. Moreover, several multi-gene modules were significantly differentially expressed in ultralow risk tumors, including AKT/mTOR, proliferation-marker AURKA, HER2/ERBB2, IGF1, PTEN-loss, PI3KCA-mutations, and immune response-modules IMMUNE1 and STAT1. Furthermore, ultralow risk tumors showed significantly (FDR<0.001) lower expression of genes involved in the immune response, histone modulation, PI3K/Akt/mTOR pathway, and higher expression of homeobox genes and genes involved in epithelial-to-mesenchymal transition, among others.

Conclusions

Ultralow risk tumors have significantly different tumor characteristics compared to other ER-positive tumors. Identifying clinical and biological characteristics of low-risk tumors is important to improve our understanding of non-fatal versus fatal breast cancer.

Trial registration

The trial center for the STO-3 trial was the Regional Cancer Center Stockholm-Gotland in Sweden. However, the start of the trial in 1976 was well before trial registration started in Sweden, therefore

information on trial number is not available.

Background

Women diagnosed with estrogen receptor (ER)-positive breast cancer have a continuous long-term risk for fatal breast cancer. Indeed, distant metastatic disease can occur anywhere between a few months to several decades after primary diagnosis (1–3). The introduction of mammographic screening enables detection of early breast cancer and has reduced the disease mortality, but can also lead to overdiagnosis of breast cancers that might never have come to clinical attention (4–6). Adding molecular risk prediction tools to standard clinical breast cancer markers may improve risk assessment, enable personalized treatment, and reduce overtreatment in patients with a low long-term risk of developing metastatic disease (7).

The US Food and Drug Administration-cleared MammaPrint 70-gene signature was originally designed to identify patients with high or low risk of early relapse. Clinical trials have validated that ER-positive patients of otherwise high clinical risk, but classified as low risk by the 70-gene signature, may not benefit from adjuvant chemotherapy (8), and this molecular risk prediction tool has now been implemented in several breast cancer treatment guidelines (9–11). It has also been shown that the additional information from the 70-gene signature improves clinicians' confidence in their treatment recommendations (12–14). Furthermore, we recently demonstrated that the "ultralow risk" threshold, as derived from the 70-gene signature, identifies patients with a very low long-term risk of fatal breast cancer. The 20-year breast cancer-specific survival rates for ER-positive patients randomized to adjuvant tamoxifen treatment vs not were 97% and 94%, respectively (7). Consequently, it is important to understand the underlying tumor characteristics of the ultralow risk tumors.

In this study, we have investigated the clinical and molecular characteristics of ultralow risk tumors in ER-positive and lymph-node negative patients from the Stockholm tamoxifen randomized trial (STO-3). Differences between ultralow risk tumors and other ER-positive tumors were assessed, including the clinically used breast cancer markers (i.e. tumor size, tumor grade, and immunohistochemistry [IHC] markers), as well as the expression of multi-gene modules representative of specific biological processes or pathways. Given that most ER-positive breast tumors are classified as luminal intrinsic subtype by the PAM50 classifier, ultralow risk tumors were compared to other ER-positive tumors including Luminal A and B PAM50 subtype tumors. Furthermore, an exploratory analysis of differentially expressed genes was conducted, to better understand the characteristics of ER-positive patients with very low risk of metastatic disease.

Materials And Methods

Study population

The Stockholm breast cancer study group conducted randomized trials 1976 to 1990 in lymph-node negative postmenopausal women with tumors less than or equal to 30 mm in diameter (28, 29). The Stockholm tamoxifen trial (STO-3) enrolled 1 780 patients that were randomized to 2 years of adjuvant tamoxifen (40 mg daily) vs no adjuvant treatment (28, 29). In 1983, patients who re-consented and were recurrence-free after 2 years of tamoxifen treatment were randomized to 3 additional years of tamoxifen. The STO-3 trial was approved by the ethics committee at Karolinska Institutet, and participants provided oral consent. At the time when the trial was approved and started only oral consent was accepted and practiced, since written consent was thought to disturb the trust between patient and doctor.

Molecular analysis was possible for 808 patients with available formalin-fixed paraffin-embedded (FFPE) tissue blocks from the primary breast cancer tumor (30), whereof 81 patients were excluded from analysis due to insufficient invasive tumor cells, see Fig. 1. Patients were well balanced to the original STO-3 trial cohort with regards to tumor characteristics (30). All patients included in STO-3 have detailed patient and clinical information.

ER, PR, HER2, and Ki-67 immunohistochemistry

Immunohistochemistry (IHC) for the clinically used markers was performed in 2014, including ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67. The annotation of whole-tumor sections (5 micrometers) from FFPE tumor blocks was conducted in a random order and at a single medical laboratory (University of California Davis Medical Center, UCDMC). Following standard recommended procedures, the slides were stained using DAKO Link48 Autostainer with antibodies for ER (SP1; Spring Bioscience M301), PR (PgR 636; DAKO IR068), HER2 (HercepTest; DAKO SK001), and Ki-67 (MIB-1; DAKO M7240). The percentage of cancer cells positive for ER, PR, HER2 and Ki-67 was scored by breast cancer pathologists (2). ER- and PR-positivity was defined by a threshold of 10% or greater, according to the Swedish National Guidelines (31), HER2-positivity as intensity 3 + by IHC, and Ki-67 was categorized as low (< 15%), medium (15–25%), and high (> 25%). Due to the small number of tumors with Ki-67 levels over 25%, medium and high were combined into one category.

Tumor grade

Tumor grade was retrospectively assessed by one pathologist according to the Nottingham Histologic Score system (Elston grade) (30).

Agilent microarray gene expression profiling

Agilent microarray profiling was performed in 2014. Gene expression data were independently generated using custom-designed arrays, Agilent Technologies (CA, USA), containing approximately 32.1K probes, representing approximately 21.5K unique genes from FFPE breast cancer tumor tissue. 652 of 727 breast cancer tumors passed the RNA quality check according to the diagnostic quality model and were used in the analysis, see Fig. 1.

70-gene signature risk classification

The 70-gene risk signature was performed according to standard protocols as previously described, including the use of 465 normalization genes and over 250 probes for hybridization and printing quality control (32, 33). Patient tumor samples were classified into “high risk”, “low but not ultralow risk” and “ultralow risk” using thresholds previously developed (7).

PAM50 intrinsic subtypes

Tumors were assigned to one of five molecular subtypes: Luminal A, Luminal B, HER2-enriched, Basal, or Normal-like, by the use of the PAM50 classification (34), see details in **Supplementary Methods**.

Analyzed groups

Only ER-positive patient tumors were included in this study. In all analyses, tumors classified as “ultralow risk” according to the 70-gene signature were compared to three other groups of tumors classified as either “low risk” or “high risk”: 1) all other ER-positive tumors 2) all other ER-positive tumors classified as Luminal A PAM50 subtype and 3) all other ER-positive patients classified as Luminal B PAM50 subtype.

Multi-gene modules

To investigate the molecular characteristics of ultralow risk tumors, multi-gene modules (proxy-signatures for activation of biological processes and pathways) (20, 25, 35) were analyzed, see details in **Supplementary Methods**. Entrez IDs were extracted for each gene using the R package biomaRt. Gene module scores were calculated for each multi-gene module using R package Genefu version 2.8.0 (36). The scores were categorized to tertiles based on all 652 STO-3 patients with gene expression data. The tertiles for each multi-gene module were then converted to two values: the most aggressive tertile (as previously determined) versus the non-aggressive two tertiles combined (20).

Statistical analysis

Fisher’s exact test was used to compare ultralow risk tumors to the other ER-positive tumors of low or high risk (all ER-positive tumors, Luminal A, and Luminal B) by the clinically used markers and multi-gene modules. A *P*-value less than 0.05 was considered statistically significant.

An exploratory analysis of differentially expressed genes was conducted using R package OCplus (37) with t-statistics and false discovery rate (FDR) cutoff of 0.001. Prior to this, probes from the gene expression microarray data were filtered out if the median expression value was among the bottom 5%, if the variance was among the 25% lowest, if containing any missing values, or if the probe was not annotated to any gene. Genes identified as differentially expressed in ultralow risk tumors were categorized by the MsigDB Hallmark gene sets (v6.2) (38) and Gene Ontology (GO) biological processes (C5 collection v6.2) (39, 40), see **Supplementary Table S1** and **Supplementary Methods**. A heatmap for the differentially expressed genes was produced using Z-scores of normalized gene expressions of all ER-positive tumors, ordered by the 70-gene signature classification, PAM50 subtype, tumor grade, and tumor size.

All data preparation and analysis were done using R version 3.5.2 and SAS software version 9.4. All statistical tests were two-sided.

Results

A total of 652 breast cancer tumors in the STO-3 trial were available for molecular analysis and were classified into three risk categories by the 70-gene signature; “high risk”, “low risk”, and “ultralow risk”, see Fig. 1. The 70-gene signature classified 15% (n = 98) of the patients in the STO-3 trial as ultralow risk. Given that all ultralow risk tumors were ER-positive, the analyses were focused on ER-positive breast cancer tumors only (n = 538). In Table 1 patient and tumor characteristics are presented for tumors classified as ultralow risk, other ER-positive tumors (of low or high risk), and Luminal A and Luminal B PAM50 subtype tumors (ER-positive, low or high risk).

Table 1. Patient and tumor characteristics of ER-positive patients in STO-3.

STO-3 trial	Ultralow Risk (n=98)	Low and High risk		
		ER-positive (n=440)	Luminal A (n=249)	Luminal B (n=122)
	No (%)	No (%)	No (%)	No (%)
Primary tumor characteristics				
Tumor size				
pT ≤ 20 mm	89 (90.8)	348 (80.2)	205 (83.7)	83 (68.0)
pT > 20 mm	9 (9.2)	86 (19.8)	40 (16.3)	39 (32.0)
Unknown	0 (-)	6 (-)	4 (-)	0 (-)
Tumor grade				
1	39 (39.8)	77 (17.8)	58 (23.5)	5 (4.2)
2	59 (60.2)	279 (64.6)	172 (69.6)	74 (62.2)
3	0 (0.0)	76 (17.6)	17 (6.9)	40 (33.6)
Unknown	0 (-)	8 (-)	2 (-)	3 (-)
PR status[a]				
Positive	82 (84.5)	285 (66.0)	174 (71.6)	74 (61.2)
Negative	15 (15.5)	147 (34.0)	69 (28.4)	47 (38.8)
Unknown	1 (-)	8 (-)	6 (-)	1 (-)
HER2 status[a]				
Positive	0 (0.0)	24 (5.5)	3 (1.2)	5 (4.1)
Negative	98 (100.0)	415 (94.5)	246 (98.8)	117 (95.9)
Unknown	0 (-)	1 (-)	0 (-)	0 (-)
Ki-67 status[a]				
Low	89 (95.7)	306 (73.0)	196 (82.7)	69 (58.5)
Medium	4 (4.3)	82 (19.6)	34 (14.3)	35 (29.7)
High	0 (0.0)	31 (7.4)	7 (3.0)	14 (11.9)

Unknown	5 (-)	21 (-)	12 (-)	4 (-)

The table shows the primary tumor characteristics including immunohistochemistry (IHC) markers for patients with ultralow risk tumors, ER-positive tumors (of low or high risk), and Luminal A and B tumor subtypes (ER-positive of low or high risk).

[a] PR-positivity was defined as ≥10%, HER2-positivity as intensity 3+, and Ki-67 was categorized as low (<15%), medium (15-25%) and high (>25%).

Clinical characteristics of ultralow risk breast cancer tumors

Figure 2 shows the distribution of the clinically used breast cancer markers for tumors classified as ultralow risk compared to other ER-positive tumors (of low or high risk), and Luminal A and Luminal B subtype tumors (ER-positive, low or high risk). The ultralow risk tumors were significantly (Fisher's exact test, $P < 0.05$) more likely to be of a smaller (pT ≤ 20 mm) tumor size, except for Luminal A tumors, and of lower tumor grade (none of the ultralow risk tumors were of grade 3), as compared to the other groups, see Fig. 2A and **Supplementary Table S2**. Furthermore, ultralow risk tumors differed from other ER-positive tumors in the immunohistochemistry (IHC) markers by being significantly more likely to be progesterone receptor (PR)-positive, human epidermal growth factor 2 (HER2)-negative and have a low expression of Ki-67 (< 15%), see Fig. 2B. Note, however, that all ultralow risk tumors were HER2-negative and that HER2-status did not differentiate ultralow risk tumors to tumors of Luminal A or B subtype.

The expression of multi-gene modules in ultralow risk breast cancer tumors

In order to further understand the molecular characteristics of ultralow risk tumors, the expression of multi-gene modules (proxy-signatures for activation of biological processes and pathways) in the ultralow risk tumors was compared to other ER-positive tumors (of low or high risk), and Luminal A and Luminal B subtype tumors (ER-positive, low or high risk). The multi-gene module expression scores were categorized to tertiles which were converted to low or high expression scores. The tertile (low or high expression scores) associated with clinical aggressiveness was compared to the two less aggressive tertiles combined. For example, the highest expression scores for the proliferation-marker AURKA, associated with higher proliferation and clinical aggressiveness, was compared to the two lower expression scores. For more details, see **Supplementary Methods**.

Eight multi-gene modules were significantly (Fisher's exact test, $P < 0.05$) different between ultralow risk tumors and the other three groups (other ER-positive tumors, and Luminal A and B subtype tumors), see Fig. 3. Lower expression of the AKT/mTOR-pathway, proliferation-marker AURKA, HER2/ERBB2-signaling, growth factor IGF1, PTEN-loss, and the immune response-modules IMMUNE1 and STAT1 was observed for the ultralow risk tumors, and higher expression was observed for the PIK3CA-mutation-associated module. Compared to all other ER-positive tumors, but not the Luminal A or B subtypes, ultralow risk

tumors showed significantly higher expression of the ESR1-module, see **Supplementary Table S2**. Furthermore, lower expression of the pathway-associated gene-modules MYC and E2F3 was observed in ultralow risk tumors compared to all other ER-positive tumors and Luminal B subtype tumors, but not the Luminal A subtype. Other multi-gene modules that specifically differentiated ultralow risk tumors from Luminal B subtype tumors included lower expression of CASP3, associated with apoptosis, and higher expression of MAPK, PLAU and STROMA1, associated with its pathway, tumor invasion/metastasis, and the stromal environment, respectively.

Differentially expressed genes in ultralow risk breast cancer tumors

Differential gene expression analysis was performed contrasting ultralow risk tumors to other ER-positive tumors of low or high risk using t-statistics and false discovery rate (FDR)-values. Overall, 706 genes were significantly (FDR < 0.001) differentially expressed in ultralow risk tumors as compared to ER-positive tumors of low or high risk, whereof 454 genes were down-regulated and 252 genes were up-regulated, see **Supplementary Table S3**.

Figure 4 shows a heatmap of a subset of the differentially expressed genes categorized by common cancer-related biological pathways and processes. Patients are ordered by their 70-gene signature risk-category, PAM50 subtype, tumor grade and tumor size. None of the ultralow risk tumors were of Basal or HER2-enriched PAM50 subtype. Ultralow risk tumors generally expressed lower levels of genes coding for histones, MYC-signaling, reactive oxygen species (ROS), cell cycle, the PI3K/Akt/mTOR pathway, DNA repair, immune response, and apoptosis, see Fig. 4 and **Supplementary Table S3**. Genes coding for KRAS-signaling, epithelial structure, homeobox proteins, and genes involved in epithelial-to-mesenchymal transition (EMT) were up-regulated in ultralow risk tumors. A majority of the genes involved in EMT were recognized as tumor-suppressor genes. Genes involved in different metabolic processes, protein secretion, estrogen response or P53 pathway were both up- and down-regulated in ultralow risk tumors. Furthermore, the gene expression patterns in the heatmap were clearly different between the ultralow risk and high risk tumors, whereas the tumors classified as low risk showed a more heterogenous gene expression pattern, see Fig. 4.

Discussion

We have previously shown that the ultralow risk threshold of the 70-gene signature identifies women at minimal risk of death from breast cancer (7). In this study, we identified clinical and molecular characteristics of primary breast cancers classified as ultralow risk from the STO-3 tamoxifen randomized trial. The ultralow risk tumors were significantly more likely to be of a smaller tumor size and of lower tumor grade as compared to other ER-positive tumors of low/high risk in this trial. Furthermore, ultralow risk tumors differed from the other ER-positive low/high risk tumors by being significantly more likely to be PR-positive, HER2-negative and having low expression of Ki-67. Moreover, ultralow risk tumors

exhibited substantially different expression of “hallmark” gene sets as well as other important cancer-related biological processes and pathways.

Distant metastatic recurrences in ER-positive breast cancer patients can occur months to decades after primary diagnosis (1–3), and patients with tumors classified as low risk by the 70-gene signature but of high clinical risk may not benefit from adjuvant chemotherapy (8). Consequently, it is important to identify distinct biological characteristics that predict patients with long-term recurrence risk to improve our understanding and distinguish our management of non-fatal versus fatal ER-positive breast cancers – something that has proven a great challenge. Recently, ultralow risk tumors as identified by the 70-gene signature have been associated with minimal long-term risk of fatal ER-positive breast cancer outcomes (7). Our findings suggest that ultralow risk tumors have differential tumor characteristics as compared to other ER-positive tumors of low or high risk, including Luminal A subtype tumors which are generally considered to be of low risk. Therefore, the 70-gene signature ultralow risk threshold may be helpful to minimize overtreatment and reassure patients in terms of their low long-term risk of recurrence, together with more traditionally used clinical markers (tumor size, tumor grade, lymph-node status, ER, PR, HER2, and Ki-67) (1), and other prognostic predictors such as urokinase plasminogen activator (uPA) levels and its inhibitor (15), intrinsic subtype classification by PAM50 and risk of recurrence (ROR) score (16), and ER-intratumor heterogeneity levels (2).

Ultralow risk tumors differ significantly from other ER-positive tumors of low or high risk in relation to the clinically used breast cancer markers. Ki-67 has prognostic value in breast cancer (17), and we show that ultralow risk tumors are significantly more likely to have low expression levels of Ki-67 and express lower levels of the proliferation-associated multi-gene module AURKA. Thus, we suggest that lower proliferation is a key clinical characteristic of ultralow risk tumors. Furthermore, ultralow risk tumors are more likely to be PR-positive, and even though it is unclear whether PR-status is an independent predictor of endocrine therapy benefit, it is known to be of prognostic value (18, 19). Interestingly, the expression of HER2/*ERBB2* by the multi-gene module significantly differed between the ultralow risk tumors as compared to the other ER-positive tumor groups. Since this difference was not observed by the HER2 IHC assay, this finding should be confirmed in further studies. Finally, higher expression of ER has been associated with better prognosis (20) and, even though this study included only ER-positive tumors, ultralow risk tumors exhibited higher expression of the ESR1-module in comparison to the other ER-positive tumors.

Ultralow risk tumors also differ significantly from other ER-positive tumors of low or high risk by the expression of genes relating to additional cancer-associated biological processes and pathways. In brief, the PI3K/Akt/mTOR pathway, which has a role in endocrine sensitivity and survival in breast cancer (21, 22), is down-regulated in ultralow risk tumors. The multi-gene module reflecting loss of the PI3K-regulator and tumor suppressor, PTEN, is less frequent in ultralow risk tumors, likewise, PIK3CA-mutations, which have been associated with more favorable outcome in ER-positive breast cancer (23), are more common in the ultralow risk tumors. In addition, in the gene expression analysis, genes involved in the PI3K/Akt/mTOR pathway were found to be down-regulated in ultralow risk tumors as compared to the

other ER-positive tumors. Moreover, transcriptionally important homeobox genes and tumor suppressor genes involved in epithelial-to-mesenchymal transition (EMT) were up-regulated in ultralow risk tumors, whereas genes coding for histones, important for cell cycle progression and transcriptional regulation, were down-regulated along with genes involved in the immune response. Interestingly, the difference in expression of genes coding for histones might reflect that epigenetic alterations and histone modifications play a defining role in the ultralow risk phenotype by modifying chromatin architecture and making its DNA more or less accessible to DNA binding proteins (24). As well, while down-regulation of immune response genes has been associated with metastatic risk and worse survival for patients with ER-negative and HER2-positive breast cancers (25, 26), our observed down-regulation of immune response genes in ER-positive ultralow risk vs. low/high risk tumors is a new and clinically provocative finding that deserves further validation.

This study on the biological and molecular characteristics of ultralow risk tumors was conducted using STO-3 tumor samples from postmenopausal patients with generally low recurrence risks given their lymph-node negative status and smaller (< 30 mm) tumors. The STO-3 trial was conducted mainly prior to the introduction of mammographic screening in Sweden; therefore, the majority of the breast tumors in STO-3 were clinically detected. With modern mammographic screening, the number of newly diagnosed low-risk breast tumors has substantially increased, thus, a better clinical understanding of low-risk tumors characteristics is needed. There are limitations to this study. As with most long-term follow-up studies, clinical recommendations for disease management and treatment have changed since the initiation of the trial. Molecular analysis was possible for approximately half of the tumors in STO-3; however, we have confirmed that patient and tumor characteristics were equally distributed and well balanced relative to the original STO-3 cohort. Further, when dealing with IHC assays, there is often some degree of subjective inaccuracy. However, the clinical IHC markers in STO-3 were recently re-assessed at a single medical laboratory by dedicated breast cancer pathologists harmonized with regard to their IHC scoring of these breast cancer markers (27). Despite our relatively small study size with only 98 ER-positive patients classified with ultralow risk tumors, we were able to identify significant and informative differences between the analyzed groups. Other clear strengths of this study include the recent performance and annotation of genome-wide gene expression analyses as well as the reliable complete long-term follow-up data available for all patients in the STO-3 trial from unique Swedish national and regional registries.

In conclusion, we have shown that ultralow risk tumors have distinct clinical and biological characteristics as compared to other ER-positive tumors of low or high risk, including Luminal A tumors that generally are considered as low risk disease. A better understanding of the characteristics of breast cancer tumors of very low risk may improve our prediction of fatal versus non-fatal disease.

Abbreviations

ER Estrogen receptor; FDR False discovery rate; HER2 Human epidermal growth factor receptor 2; IHC Immunohistochemistry; PR Progesterone receptor; STO-3 Stockholm tamoxifen randomized trial

Declarations

Ethics approval and consent to participate: The STO-3 trial was approved by the ethics committee at Karolinska Institutet, and participants provided oral consent. At the time when the trial was approved and started only oral consent was accepted and practiced, since written consent was thought to disturb the trust between patient and doctor.

Consent for publication: Not applicable.

Availability of data and materials: The data that support the findings of this study are available from the Swedish Breast Cancer Group (SweBCG) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon request and with permission of SweBCG. R-code to reproduce the results and figures are publicly available at https://github.com/annelieewa/Ultralow_risk_STO3.

Competing interests: Dr. van 't Veer is one of the inventors of the MammaPrint 70-gene risk signature (patent no. WO2002103320) and is a cofounder, stockholder, and part-time employee of Agendia. All remaining authors declare no potential conflicts of interest.

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Competing interest: Dr. van 't Veer is one of the inventors of the MammaPrint 70-gene risk signature (patent no. WO2002103320) and is a cofounder, stockholder, and part-time employee of Agendia. All remaining authors declare no potential conflicts of interest.

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Figures

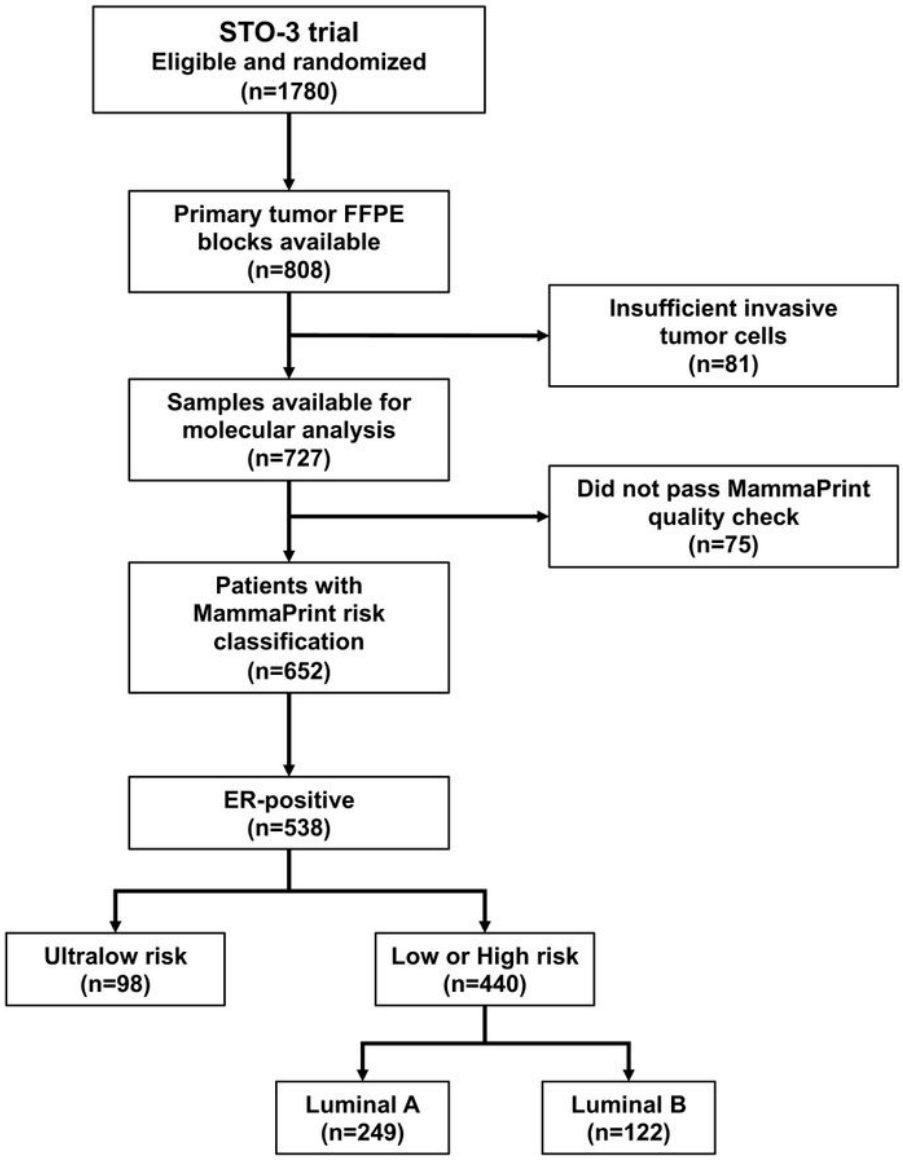

Figure 1

Consort diagram for the Stockholm tamoxifen randomized trial (STO-3).

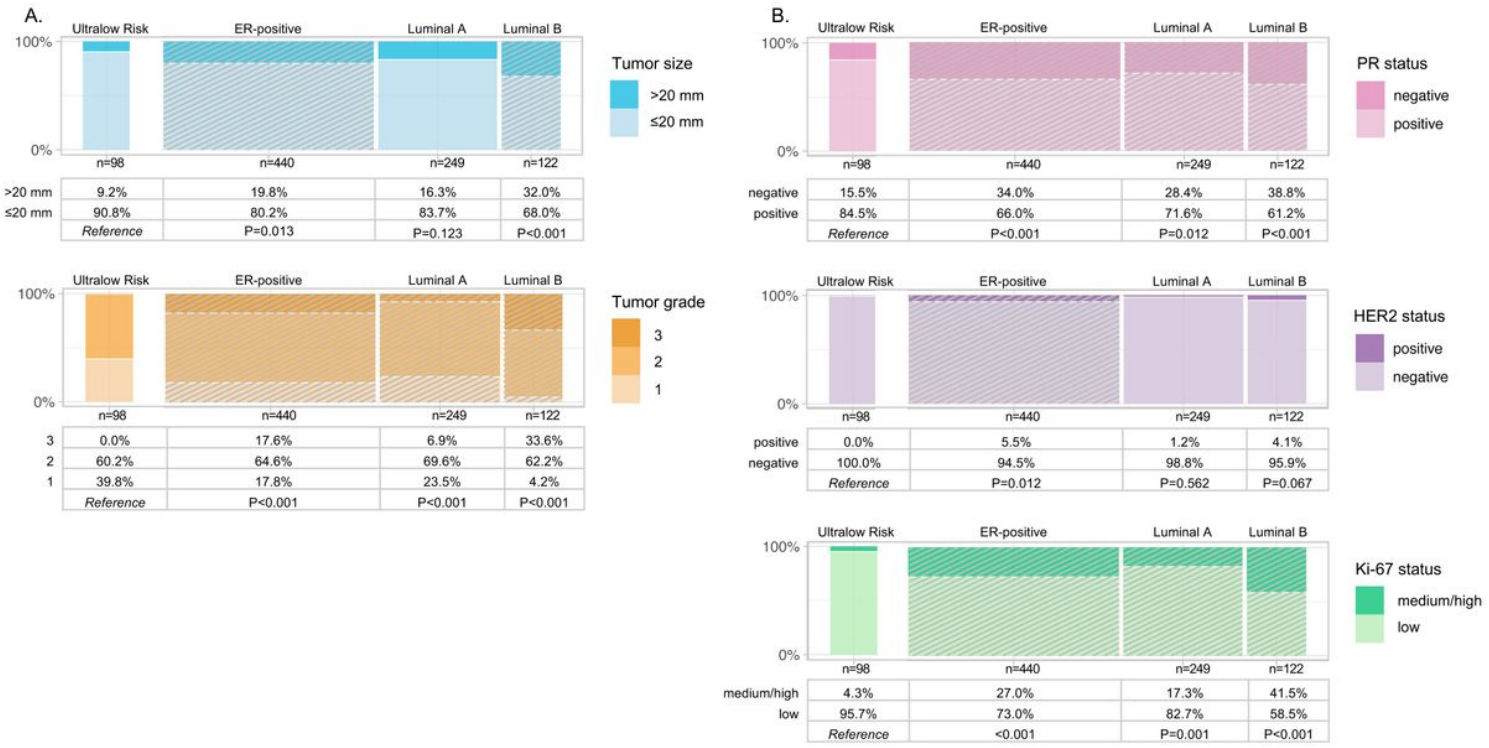

Figure 2

Clinical breast cancer markers in ultralow risk breast cancer tumors. The clinically used breast cancer markers including A) tumor size and tumor grade and B) immunohistochemistry (IHC) markersa for ultralow risk tumors compared to other ER-positive tumors (of low or high risk), and Luminal A and Luminal B tumors (ER-positive, low or high risk). The width of the categories reflects the number of patients in each group. Darker colors represent the more aggressive category and diagonal line shading indicates significant differences from the ultralow risk group by the Fisher's exact test (P<0.05). a PR-positivity was defined as ≥10%, HER2-positivity as intensity 3+, and Ki-67-low as <15%.

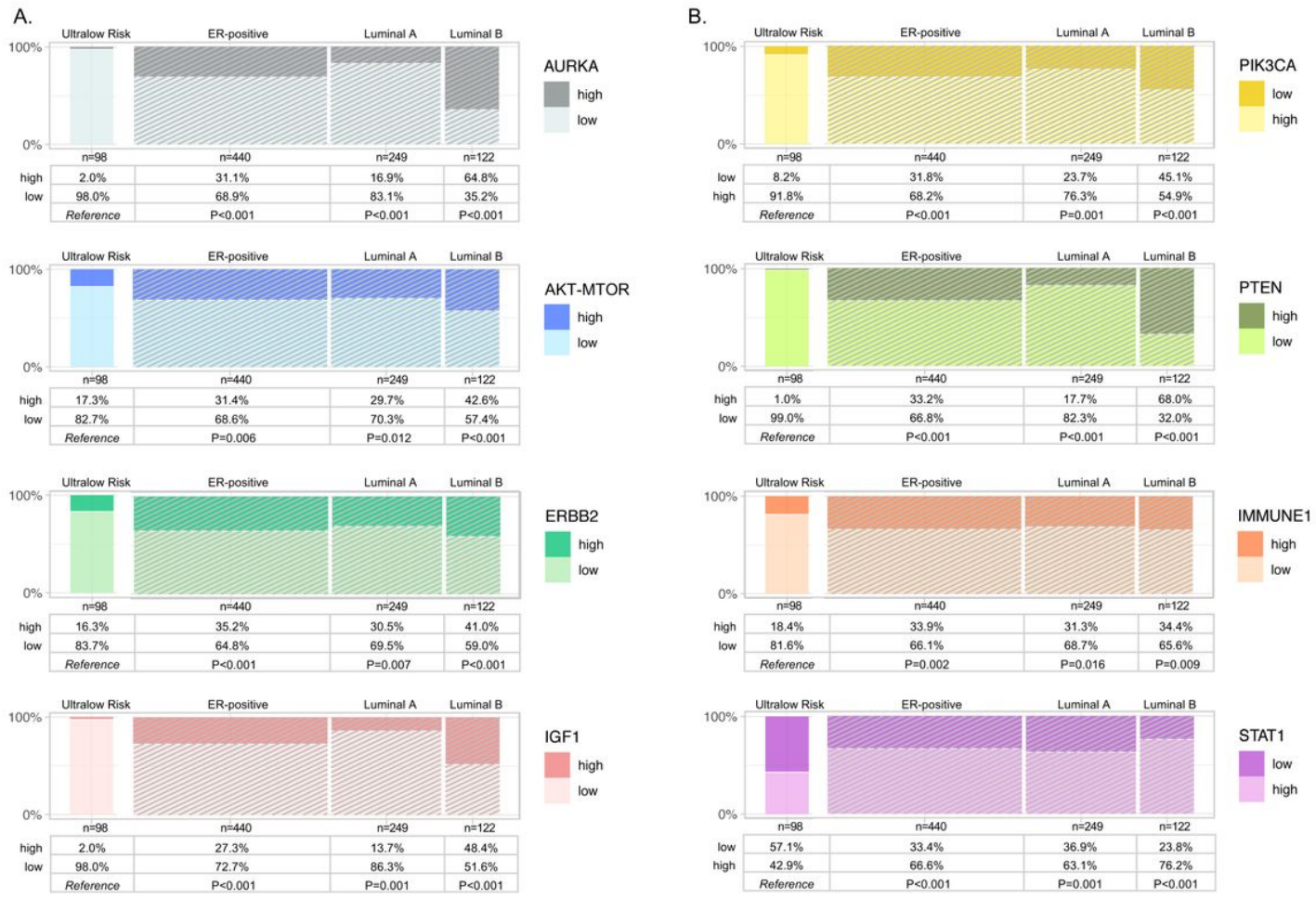

Figure 3

Multi-gene module expression in ultralow risk breast cancer tumors. The expression of multi-gene modules for ultralow risk tumors compared to other ER-positive tumors (of low or high risk), and Luminal A and Luminal B tumors (ER-positive, low or high risk). The modules included are: A) proliferation-marker AURKA, AKT/mTOR-pathway, HER2/ERBB2-signaling, growth factor IGF1, and B) PIK3CA-mutation-associated signature, PTEN-loss, and immune response modules IMMUNE1 and STAT1. The width of the categories reflects the number of patients in each group. Darker colors represent the more aggressive category and diagonal line shading indicates significant differences from ultralow risk population by Fisher's exact test (P<0.05).

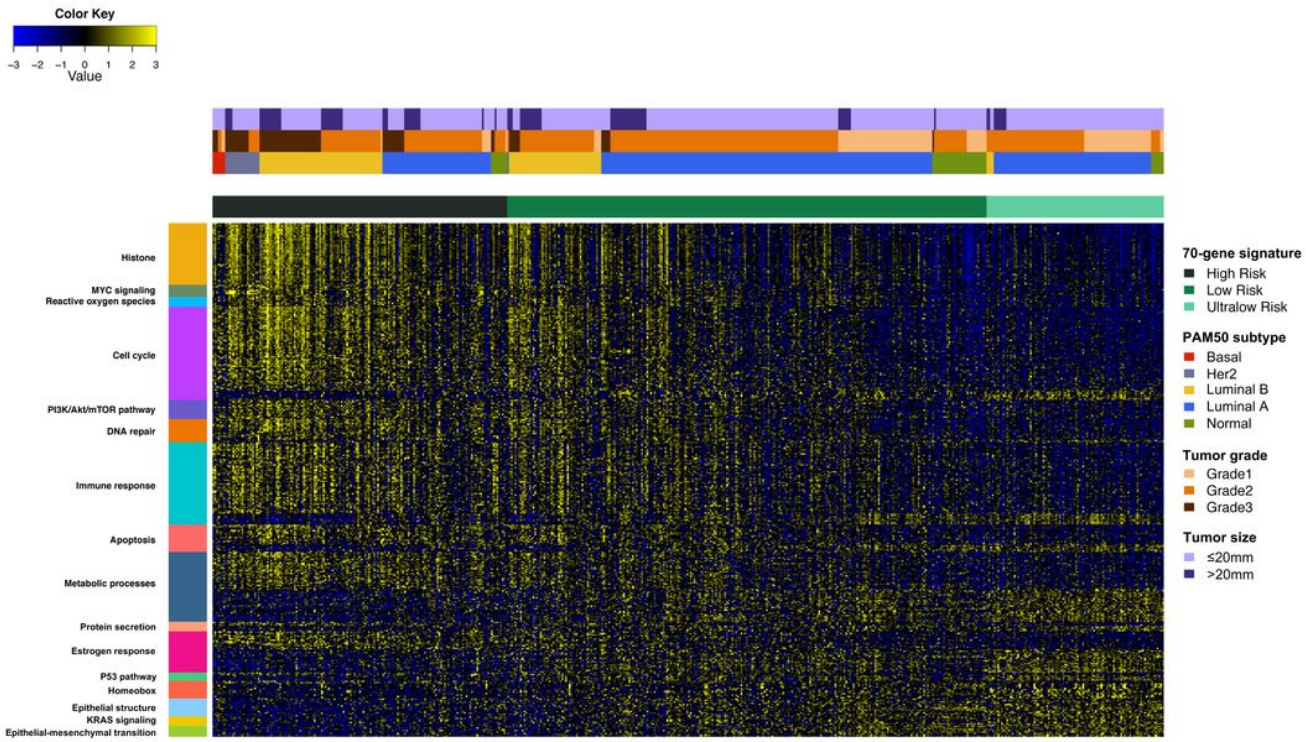

Figure 4

Differentially expressed genes in ultralow risk breast cancer tumors. Heatmap of genes differentially expressed between ultralow risk tumors and other ER-positive tumors (of low or high risk). Patients are ordered by the 70-gene signature classification, PAM50 subtype, tumor grade, and tumor size. Genes are categorized by their involvement in different biological processes or pathways (MsigDB Hallmark and Gene Ontology [GO] gene sets), and ordered by t-statistics between and within each gene category.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1SupplementaryMethods.pdf
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- Additionalfile3SupplementaryTableS2.pdf
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