Developing a new generation of breast cancer clinical gene expression tests

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Abstract

When treatment decisions are based purely on clinicopathological factors, many women with estrogen receptor-positive/human epidermal growth factor receptor 2-negative cancers are overtreated. Gene expression profiles are valuable clinical tools that stratify the recurrence risk to identify patients most likely to benefit from adjuvant systemic therapies. Building upon greater understanding of tumor biology and more rigorous approaches to validation (including independent studies with a high level of evidence), several second-generation multigene tests have been developed. In the previous issue, Martin and colleagues report the third clinical validation study for EndoPredict, a distributed assay to assess risk of distant recurrences in estrogen receptor-positive/human epidermal growth factor receptor 2-negative women. The authors confirm the assay’s independent prognostic value in premenopausal and postmenopausal, node-positive women treated with contemporary chemotherapy. EndoPredict did not, however, predict benefit from adding paclitaxel. Predictive signatures for selecting among chemotherapy regimens remain an area needing further development.
| Assay: platform, clinical material | Training parameter | Approval or endorsement | Analytical validity: published assay validation | Clinical validity: prognosis validation | Predicting treatment benefit using randomized clinical trials | Randomized prospective trials |
|-----------------------------------|---------------------|-------------------------|-----------------------------------------------|--------------------------------------|-------------------------------------------------|-----------------------------|
| Breast Cancer Index; RT-PCR, FFPE (central) | Outcome (ER+, pN0, endocrine-treated women) MGI component – biology (tumor grade related genes) H3 component – outcome (recurrence in tamoxifen-treated women) | No | No | ATAC [13], Stockholm [17], multiple nonrandomized trial cohorts | No* | No |
| EndoPredict; RT-PCR, FFPE (distributed) | Outcome (distant recurrence in endocrine-treated ER+/HER2- pN0/pN+ women) | CE Mark | Yes [18,19] | ABCSG6 [2], ABCSG8 [2], GEICAM/9906 [1] | No | No |
| IHC4; IHC, FFPE (distributed) | Outcome (distant recurrence in ER+ endocrine-treated women) | No | No | ATAC [14], TEAM [20] | No | No |
| MammaPrint; microarray, fresh and FFPE (central) | Outcome (5-year metastasis rate in pN0 women) | FDA (fresh): risk for distant metastasis, <61 years, stage I and II, tumor ≤5 cm and node-negative | No | Multiple nonrandomized trial cohorts including RASTER | No | No |
| Mammastrat; IHC, FFPE (central) | Outcome (unselected cohort of breast cancer patients) | No | No | NSABP-B14, NSABP-B20 [15] multiple nonrandomized trial cohorts | No | No |
| Oncotype DX; RT-PCR, FFPE (central) | Outcome (recurrence in mainly tamoxifen-treated ER+, pN0 women) | NCCN, ASCO, St. Gallen (role for identifying women that may benefit from chemotherapy) | Yes [21] | NSABP-B14 [9], NSABP-B28 [22], SWOG8814 [23], multiple nonrandomized trial cohorts | No | No |
| PAM50 (research based assay); RT-PCR and microarray, FFPE and fresh (distributed) | Biology (identification of major molecular subtypes) | N/A research assay | No | NCIC-MA5 [26], NCIC-MA12 [27], GEICAM/9906 [28], multiple nonrandomized trial cohorts | NCIC-MA12 [27] (luminal subtype predicts benefit) NOAH [29] (HER2-enriched benefits the most) | No |
| | | | | | NCIC-MA5 [26] (CMF vs. CEF; epirubicin benefit in HER2-enriched subtype only) | No |

*No evidence of benefit from randomized clinical trials.
Table 1 Overview of selected multigene signatures for breast cancer (Continued)

| Prosigna; nCounter; FFPE (distributed) | Biology (subtype); outcome (ROR score) | CE Mark, Health Canada, FDA: prediction of 10-year DRFS in ER+, node 0 to 3, postmenopausal women treated with endocrine therapy | ATAC [32], ABCSG08 [33] | No | No | No | No | No | RxPONDER (one to three nodes, recruiting; embedded additional analysis) |
|----------------------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|---------------------------|----|----|----|----|----|---------------------------------------------------------------------|

CAF, cyclophosphamide, doxorubicin, fluorouracil; CEF, cyclophosphamide, epirubicin, fluorouracil; CMF, cyclophosphamide, methotrexate and fluorouracil; DRFS, distant relapse-free survival; ER, estrogen receptor; FDA, US Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; HER2, human epidermal growth factor receptor 2; H3, HOXB13/IL17BR; IHC, immunohistochemistry; MGI, molecular grade index; N/A, not applicable; pN0, pathological lymph node-negative; pN+, pathological lymph node-positive; ROR, risk of recurrence; RT-PCR, reverse transcription polymerase chain reaction. Breast Cancer Index: bioTheranostics, San Diego, CA, USA; EndoPredict: Sividon Diagnostics GmbH, Cologne, Germany; IHC4: MammaPrint: Agendia, Amsterdam, The Netherlands; Mammostrat: Clarient, Inc., Aliso Viejo, CA, USA; Oncotype: Genomic Health, Redwood City, CA, USA; PAM50: NanoString Technologies Inc., Seattle, WA, USA; Prosigna: NanoString Technologies Inc., Seattle, WA, USA. *Nested cohort study using material from NCIC CTG MA.17 – HOXB13/IL17BR predictive of benefit from extended letrozole.
ABCSG-8 trial [4]. This promising idea must be interpreted cautiously given that only 16 of the 74 EPclin low-risk patients had 10-year follow-up data. Both the aTTom and ATLAS trials have shown that survival benefits of extended hormonal therapy become more apparent after year 10 [5,6].

Several multigene prognostic assays have now been developed for use in ER+/HER2− breast cancers. First-generation assays including MammaPrint (MammaPrint: Agenda, Amsterdam, The Netherlands) and Oncotype DX (Oncotype: Genomic Health, Redwood City, CA, USA) suffered from early methodological issues, most seriously a failure to maintain rigorous separation between training and validation sets, and inclusion of nonluminal and/or HER2+ tumors in their training sets, thereby allowing these high-risk tumors to skew outcome-related gene selection away from the relevant patient group [7-9]. MammaPrint was specifically trained around early relapse (within 5 years) in node-negative women, most having received no adjuvant systemic therapy, and has not been shown to predict late recurrence outside the original training-validation cohort. Oncotype DX heavily weighed the tamoxifen-only arm of the NSABP-B20 trial in its training set, where most recurrences occurred within 5 years, and has diminished prognostic ability beyond year 5 [10].

More recently, building upon biological and technical advances and more rigorous approaches to validation, second-generation multigene tests have been developed, including the Breast Cancer Index (BCI: bioTheranostics, San Diego, CA, USA), PAM50 (PAM50: NanoString Technologies Inc., Seattle, WA, USA) and EP. The Breast Cancer Index combines a molecular grade index (quantifying tumor grade-associated genes) and a two-gene ratio, HOXB13:IL17BR, related to estrogen signaling [11]. PAM50, unlike signatures trained around outcome, was developed as a biological classifier of the major intrinsic molecular subtypes of breast cancer [12]. These three assays predict both early and late recurrences [4,10,13].

IHC4 and Mammostrat (Mammostrat: Clarient, Inc., Aliso Viejo, CA, USA) immunohistochemical panels are also prognostic in early breast cancer [14,15]. IHC4 uses standard pathology markers (ER, progesterone receptor, HER2 and Ki67) to provide prognostic information comparable with Oncotype DX [14]. Immunohistochemical staining and scoring does suffer from limited analytical reproducibility, probably contributing to Martin and colleagues’ identification of low Ki67 scores (<14%; a published cutoff point for good-prognosis luminal A tumors) in a surprisingly high fraction (almost three-quarters) of this node-positive cohort [16].

Each of these gene expression and immunohistochemical panels identifies a good prognosis group that may not need chemotherapy. Emerging evidence suggests that some panels identify women at such low risk of late recurrence that they may safely avoid extended endocrine therapy. For high-risk women, however, the question is not one of chemotherapy versus no chemotherapy, but rather a question of which chemotherapy agent(s) will be most effective for which patients – a true predictive indication. In Martin and colleagues’ report, the EP score did not predict benefit from adding weekly paclitaxel to fluorouracil–epirubicin–cyclophosphamide chemotherapy. Outcome-trained signatures from nonchemotherapy populations are unlikely to predict between chemotherapy regimens; Table 1 summarizes some relevant features of the referenced molecular signatures, including predictive studies.

What does the future hold for gene expression signatures? Cheaper and faster next-generation sequencing has been touted as the pinnacle of personalized medicine, destined to render multigene expression assays obsolete. However, the genetic complexity of tumors (copy number variations, chromosome-scale structural changes, thousands of mutations, epigenetic changes and intratumoral genetic heterogeneity) is proving even more complex than anticipated. Much as the increased detail from electron microscopy never did replace light microscopy for cancer diagnosis, the broader signatures detected by representative gene expression profile assays, reflecting clinically significant patterns common across many patients, are likely to remain relevant for important treatment decisions.

Abbreviations
EP: EndoPredict; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2.

Competing interests
TON reports a proprietary interest in the PAM50 assay, which has been licensed to Nanostring Technologies. ZK has no competing interests.

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