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**Predictive markers of endocrine response in breast cancer**

Mosly D *et al.* Predictive markers of endocrine response in breast cancer

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**Abstract**

Ongoing clinical and research efforts seek to optimise the use of endocrine therapy in the treatment of breast cancer. Accurate biomarkers are needed that predict response for individual patients. The presence of the estrogen receptor (ER) as the direct (for tamoxifen and fulvestrant) or indirect (for aromatase inhibitors) target molecule for endocrine therapy remains the foremost biomarker and determinant of response. However, ER expression only poorly predicts outcome and further indicators of response or resistance are required. The development and application of molecular signature assays such as Oncotype Dx, Prosigna, Mammaprint and Endopredict have provided valuable information on prognosis and these are being used to support clinical decision making on whether endocrine therapy alone alongside surgery is sufficient for ER-positive early stage breast cancers or whether combination of endocrine with chemotherapy are also warranted. Ki67, the proliferation marker, has been widely used in the neo-adjuvant (pre-operative) setting to help predict response and long term outcome. Gene expression studies within the same setting have allowed monitoring of changes of potential predictive markers. These have identified frequent changes in estrogen-regulated and proliferation genes. Specific molecules such as mutant ER may also prove helpful biomarkers in predicting outcome and monitoring response to treatment.

**Key words:** Predictive; Biomarker; Breast cancer; Estrogen; *IL6ST*

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**Core tip:** The expression level of estrogen receptor remains the major determinant of response for endocrine therapy in breast cancer. Molecular signatures provide increasing confidence for helping identify breast cancers for which endocrine therapy alone is likely to be sufficient. Estrogen and proliferation related genes have come to the fore in many of the molecular signatures. In neo-adjuvant studies, Ki67 expression at baseline and after 2 wk treatment can provide useful prognostic and predictive information. Neo-adjuvant studies continue to seek new markers that relate to tumor response.

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**INTRODUCTION**

Breast cancer is the second most frequently diagnosed cancer worldwide with an estimated 1676000 new cases each year[1]. Of these cancers, approximately 70%-80% will have estrogen receptor (ER) expression and be considered candidates for endocrine therapy. Tamoxifen and the aromatase inhibitors represent the major endocrine treatments in use worldwide. Tamoxifen was first approved in 1977 for treatment of breast cancer and continues to be used in many post-menopausal women, but is primarily recommended for use in pre-menopausal women[2]. The 3rd-generation aromatase inhibitors (anastrazole, letrozole and exemestane) have demonstrated superiority over tamoxifen in post-menopausal ER-positive breast cancer and have become the preferred option for this group of cancers[3]. Fulvestrant, a “pure”anti-estrogen and ER down-regulator, is an alternative after treatment failure in post-menopausal women and being considered in other settings[4]. Meta-analyses of multiple clinical trials have demonstrated that these endocrine agents can halve the risk of breast cancer relapse and reduce the risk of breast cancer death by 40%[5].

With recognition of the molecular heterogeneity present both within and between individual breast cancers, strenuous efforts have been undertaken to optimise individual patient management. This has led to the search for predictive biomarkers that might identify ER-positive breast cancers which are sensitive to endocrine therapies and those in which endocrine therapy is likely to be insufficient, hence requiring either chemotherapy or new agents. Since prognostic molecular signatures are now also helping to stratify patient groups into those for which endocrine therapy alone is likely to be sufficient, these will be mentioned briefly as well.

**ER, PROGESTERONE RECEPTOR AND HER2**

Foremost and most powerful of the biomarkers identified to predict response to endocrine therapy is the ER itself, specifically ER-alpha (ESR1)[6]. The routine classification of breast cancers into ER-positive and ER-negative categories was based on the early identification of the requirement for ER expression for response to tamoxifen with 60%-70% of ER-positive patients responding to this endocrine agent compared to only 5%-10% responding with ER-negative metastatic disease[2]. Consistent with this, the likelihood of response increased with increasing ER concentration with ER-rich tumors responding better than ER-poor cancers[7]. However, even for responders, up to 50% will eventually relapse hence predictive biomarkers are required that will identify ER-positive patients most likely to respond to therapy and those for whom endocrine therapy is likely to be insufficient[2].

Other forms of ER include ER-beta, G-protein coupled ER (GPER1) (previously GPR30) and mutated versions of ER-alpha and these have all been investigated as predictive markers of response to endocrine therapy. The role of ER-beta appears complex and dependent on whether ER-alpha is present leading to a bi-faceted role[8], however several clinical studies have suggested predictive effects for specific ER-beta isoforms[8,9]. Low expression of the membrane-bound GPER1 is associated with favourable outcome to tamoxifen[10] while high expression has been associated with tamoxifen resistance[11]. The role of ER mutants is discussed below.

The ER is one of 3 markers (ER, PR and HER2) routinely measured at diagnosis to help determine potential treatment options. Expression of the progesterone receptor (PR), an estrogen-regulated protein, is highly estrogen dependent and has therefore been regarded as an indicator of estrogen-drive and signaling. It has been associated with both disease-free as well as overall survival in tamoxifen-treated breast cancers with PR-positive breast cancers responding better than PR-negative[12] cancers, but this is not a universal finding[13]. Breast cancers that are both ER-positive and PR-positive have > 70% likelihood of response to endocrine therapy and these two receptors have become the prototypic predictive markers of endocrine response in this disease[14]. The third molecule routinely assessed at diagnosis, HER2 (assessed for amplification or overexpression), while developed as a predictor for anti-HER2 targeted therapies, *e.g*., trastuzumab or lapatinib, is generally associated with poor response to endocrine therapy[15,16]. One multi-protein assay tool using immunohistochemistry, the IHC4 score, combines information from ER, PR, HER2 and the proliferation index Ki67 into a score that helps estimates the risk of distant recurrence at 10 years in post-menopausal women with ER-positive breast cancer who have received 5 years of endocrine therapy[17]. These same 4 markers are also components of the Oncotype Dx and Prosigna assays which will be described later.

**DEVELOPMENT OF ENDOCRINE RESISTANCE**

A major limitation of endocrine therapy is the development of resistance and markers that reflect these resistance mechanisms may predict outcome[14]. Resistance may be present at the outset (*de novo*) or develop on drug treatment (acquired) and can arise in multiple ways[18]. Two well defined mechanisms of endocrine resistance are the loss of ER function and the development of estrogen-insensitivity.

ER function can be lost as a result of decreased ER expression or ER co-activator expression or function. ER expression is lost in approximately 10% or so of breast cancers on neo-adjuvant treatment[14], and these cancers have a poorer outcome than where ER expression is maintained[19]. This would be reflected in reduced down-stream signalling such as decreased PR expression or estrogen-regulated gene expression in the absence of an inhibitor and these can be indicative of a lack or loss of estrogen signaling.

The development of estrogen-independent signaling can lead to insensitivity to estrogen. This can occur via ER gain-of-function mutations[19-21] or by indirect activation of ER phosphorylation or ER-coactivator phosphorylation (hence avoiding the need for estrogen activation) via growth factor pathways including EGF receptor, HER2 and IGFIR[18]. Gain-of-function mutations in ER may bypass inhibition produced by endocrine agents. Although these ER mutations are infrequent in initially diagnosed disease, a much higher mutation rate has been observed in metastases (up to 20%) and circulating tumor DNA (up to 40%) in metastatic breast cancers[19-21]. This may be a cause of endocrine resistance to aromatase inhibitors (since production of estrogen is no longer needed to activate the receptor) and tamoxifen or fulvestrant therapy may be more effective in these cancers[19].

Increased expression of EGFR, HER2 or IGFIR have all been associated with reduced or loss of endocrine regulation and are potential indicators of endocrine resistance[18]. Moreover, the pathways they use, *i.e.*, the PI3K/AKT and Ras/Raf/MEK/ERK pathways, may have activating mutations, *e.g*., in components such as PI3K, which in turn may lead to ER activation[22]. To date, this information has been used to develop combination drug approaches that combine an endocrine agent with an inhibitor (*e.g.*, HER2, PI3K, mTOR, CDK inhibitor, *etc.*) that targets a component of the growth factor driven pathway. Although this has been valuable for the strategic development of inhibitory strategies in endocrine-resistant disease, it hasn’t yet led to the development of specific markers to predict endocrine resistance. Even for ER-positive/HER2-positive breast cancers, wherein many cancers are responsive to endocrine treatment, it remains unclear which tumors are sensitive and which are resistant indicating the need for further markers of response.

The detection of ER mutations in circulating tumor DNA is promising and supports the use of plasma sampling to help monitor the changing status of the disease in the patient. Retrospective analyses of ER mutations in baseline plasma circulating tumor DNA from completed clinical trials suggest that these mutations are prognostic and predictive of resistance to aromatase inhibitors in metastatic disease[23] however prospective studies will be needed to validate clinical utility.

**MULTIGENE SIGNATURES**

It is nearly 20 years since the first detailed molecular portrait of breast cancer was published by Perou *et al*[24] that stratified breast cancers into molecular subtypes based on gene expression data. Four groups (luminal, HER2, basal and normal breast like) were identified with the luminal group describing the ER-positive group. Further studies by the same investigators demonstrated that the ER-positive luminal group could usefully be sub-divided into luminal A and luminal B cancers[25-27]. Luminal A cancers comprise about 40%-75% (cf. large geographical variation) of breast cancers with relatively higher levels of estrogen signalling and lower proliferation. Luminal B cancers represent approximately 10%-20% of breast cancers and tend to have lower estrogen signaling and higher proliferation or HER2 over-expression. Over time further ER-negative subgroups such as claudin-low and molecular apocrine clusters have been suggested along with the so-called 4-6 Lehman TNBC subtypes[28-30], however luminal cancers remain the endocrine-sensitive group with luminal A in general being sensitive to endocrine therapy alone while luminal B cancers may require both endocrine therapy and chemotherapy. As further molecular portraits were characterised, a number of gene sets were developed as prognostic signatures and have been useful to help stratify groups of patients (Table 1). Several commercial assays have been developed that generate risk of recurrence scores that can be used to help determine the likely risk of relapse. These have been particularly valuable in clinical decision making to help identify which early stage ER-positive HER2-negative patients without lymph node spread (encompassing over half of all breast cancer patients) should receive endocrine therapy alone and which should receive chemotherapy or novel treatments as well in the adjuvant setting.

The multigene test most widely used in the clinic to date is the Oncotype Dx signature. Oncotype DX is a 21-gene recurrence score assay initially developed to predict likelihood of recurrence of tamoxifen-treated, node negative breast cancer[31]. This assay includes proliferation-related genes (*Ki67, STK15*, *Survivin, CCNB1*, *MYBL2*), estrogen-related genes (*ER, PGR, BCL2, SCUBE2*), HER2-related genes (*HER2, GRB7*), invasion-related genes (*MMP11, CTSL2*) and 3 others (*GSTM1, CD68, BAG1*) alongside 5 reference genes (*ACTB, GAPDH, RPLPO, GUS, TFRC*). Levels of expression of these genes are combined into an algorithm to generate a recurrence score between 0 and 100 which is predictive of overall survival[31]. If the score is high (> 31) then chemotherapy has been shown to be beneficial. If the score is low (< 10), then this is prognostic of a very low rate of recurrence (< 2%) and endocrine therapy alone is likely to be sufficient. Until recently, it was unclear whether endocrine therapy alone was adequate for patients with cancers with intermediate scores (10-25) since these can comprise 2/3 of patients, but the TAILORx trial has now demonstrated that endocrine therapy alone without added chemotherapy produces the same outcome suggesting that endocrine therapy alone is sufficient for this large group of patients[32]. The trial results though did not exclude a benefit of chemotherapy for patients aged < 50 years with a high-intermediate score[32].

Several other multigene signatures have been shown to produce similar prognostic data for this group of ER-positive, HER2-negative patient group. These include Prosigna (based on PAM50), Mammaprint and Endopredict.

The Prosigna classifier uses the PAM 50 (Prediction Analysis of Microarrays) set of 50 genes together with a set of 8 reference genes to identify the intrinsic gene expression subtype (*i.e*., luminal A, luminal B, HER2 or basal-like)[33]. This classifier identifies the cancer subtype based on comparison of the cancer’s gene expression profile to the characteristic subgroup profiles and generates a risk of recurrence score. Its prognostic value has been demonstrated in multiple cohorts of breast cancer patients including those with tamoxifen or anastrazole alone[34,35] and tamoxifen plus anastrazole[36]. A recently developed PAM50-based chemoendocrine score has been developed that highlights luminal to basal differences and response to treatment[37].

The Mammaprint assay is a classifier based on the 70-gene Amsterdam signature[38] developed to help identify early stage breast cancer patients most likely to develop distant metastases and therefore benefit from adjuvant chemotherapy[39]. Its value has been tested in multiple clinical trials, but the largest trial has been the 6693 patient MINDACT trial[40]. In this trial, it was demonstrated that the group of patients identified as high risk for recurrence according to clinical and pathological factors but who were classified as Low Risk by MammaPrint were unlikely to benefit from chemotherapy[40].

The Endopredict test measures 8 genes of which 3 are proliferation associated (*BIRC5, UBE2C, DHCR7*) and 5 are estrogen-related genes (*RBBP8, IL6ST, AZGP1, MGP, STC2*) by RT-PCR from fixed tissue and generates a score between 0 and 15 (< 5 is low risk; > 5 is high risk)[41]. This data is combined with nodal status and tumor size information to provide an EPclin score[41,42]. The test has been validated within a number of trials[41,42].

**DYNAMIC NEO-ADJUVANT STUDIES**

Neo-adjuvant (pre-operative) studies, wherein breast cancer patients are treated with endocrine therapy prior to surgery, have provided opportunities to study and identify predictive biomarkers of endocrine response. In these studies, tumors have commonly been serially sampled at diagnosis, after 14 d and at 3 mo of treatment and assayed for gene or protein expression levels[43]. These studies have demonstrated that several parameters may be informative including the expression level of a biomarker at diagnosis prior to treatment, the change in expression over time during treatment and the residual level compared to baseline value after a period of treatment.

The most extensively studied pharmacodynamics marker in neo-adjuvant endocrine trials is Ki67 (MKI67) which is a nuclear protein expressed only in proliferating cells[44]. The pre-treatment value of Ki67 reflects prognosis, while the change in Ki67 relates to response to treatment, hence is predictive[45]. The 14 d value then provides an indicator of residual risk[44]. This biomarker has already been incorporated into the IHC4, Oncotype Dx and Prosigna tests and is currently being studied in the POETIC phase III multicentre trial. The POETIC trial is the largest study to assess the validity of Ki67 as a marker of response and long-term outcome in a pre-surgical window-of-opportunity setting and has recruited 4500 women with early stage ER-positive breast cancer. The study is assessing whether time to recurrence and overall survival are influenced by 2 wk of aromatase inhibitor therapy prior to and after surgery to improve outcome compared to standard adjuvant therapy alone 44]. To date, the trial has provided evidence that measurement of Ki67 at baseline and at 2 wk is informative. If baseline Ki67 is low (value < 10%), prognosis is good and pre-operative treatment and a second measurement aren’t needed. However, if baseline Ki67 is high (value > 10%) and stays high at 2 wk, then prognosis is poorer and patients should be considered for further therapy (chemotherapy or new agents)[46].

Gene sets associated with both aromatase inhibitor sensitivity and resistance have been identified within neo-adjuvant studies and gene expression changes after 14 d and 3 mo of treatment linked to tumor growth response[47,48]. A common finding in many of the gene expression changes is that both estrogen-dependent genes and proliferation-associated genes can be down-regulated on treatment, however there can be discordant patterns of change as well. These changes can occur in resistant as well as sensitive treated cancers suggesting different mechanism of resistance[49]. Higher basal expression of certain immune-related genes such as SLAMF8 and TNF as well as lymphocytic infiltration have been associated with poor anti-proliferative response and resistance[50] while high expression of ribosomal proteins is associated with response to letrozole[48].

A four-gene classifier of clinical response to the aromatase inhibitor letrozole has recently been described with an accuracy of 96% based on the expression levels of two genes (*IL6ST* and *NGFRAP1*) at baseline and two proliferation associated genes (*ASPM* and *MCM4)* after 2 wk of therapy[51]. This gene set was then validated in an independent group of patients treated with anastrazole[51]. This is now being evaluated in prospective studies. It will be important to understand the roles and functions of these genes if they are to be used alongside more traditional markers such as the estrogen-regulated PR or proliferation associated Ki67. Measurement of proliferation after endocrine treatment is also a component of the Preoperative Endocrine Prognostic Index (PEPI), that was developed to identify patients at low risk of relapse after neoadjuvant endocrine therapy so that adjuvant chemotherapy can safely be avoided[52,53].

**CONCLUSION**

ER expression together with PR expression continues to be the major determinant of endocrine response in breast cancer, but further markers to more accurately guide treatment would be valuable. Markers of endocrine sensitivity are helpful to provide confidence that the use of endocrine therapy alone is sufficient treatment for a tumor and there are now multiple molecular signatures that can do this. Markers of endocrine resistance will help direct change of therapy and dependent on the marker used may provide some insight into potential inhibitory strategies that may be helpful. The use of on-treatment sampling (serial biopsy or circulating tumor cells) ideally in comparison with baseline sampling will provide the best information to aid this.

**REFERENCES**

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 **Osborne CK**. Tamoxifen in the treatment of breast cancer. *N Engl J Med* 1998; **339**: 1609-1618 [PMID: 9828250 DOI: 10.1056/NEJM199811263392207]

3 **Dowsett M**, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, Buyse M, Baum M, Buzdar A, Colleoni M, Coombes C, Snowdon C, Gnant M, Jakesz R, Kaufmann M, Boccardo F, Godwin J, Davies C, Peto R. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. *J Clin Oncol* 2010; **28**: 509-518 [PMID: 19949017 DOI: 10.1200/JCO.2009.23.1274]

4 **Nathan MR**, Schmid P. A Review of Fulvestrant in Breast Cancer. *Oncol Ther* 2017; **5**: 17-29 [PMID: 28680952 DOI: 10.1007/s40487-017-0046-2]

5 **Early Breast Cancer Trialists' Collaborative Group (EBCTCG)**. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* 2015; **386**: 1341-1352 [PMID: 26211827 DOI: 10.1016/S0140-6736(15)61074-1]

6 **Thomas C**, Gustafsson JÅ. The different roles of ER subtypes in cancer biology and therapy. *Nat Rev Cancer* 2011; **11**: 597-608 [PMID: 21779010 DOI: 10.1038/nrc3093]

7 **Allegra JC**, Lippman ME, Thompson EB, Simon R, Barlock A, Green L, Huff KK, Do HM, Aitken SC, Warren R. Estrogen receptor status: an important variable in predicting response to endocrine therapy in metastatic breast cancer. *Eur J Cancer* 1980; **16**: 323-331 [PMID: 7371687 DOI: 10.1016/0014-2964(80)90348-5]

8 **Leygue E**, Murphy LC. A bi-faceted role of estrogen receptor β in breast cancer. *Endocr Relat Cancer* 2013; **20**: R127-R139 [PMID: 23533249 DOI: 10.1530/ERC-12-0389]

9 **Speirs V**, Viale G, Mousa K, Palmieri C, Reed SN, Nicholas H, Cheang M, Jassem J, Lønning PE, Kalaitzaki E, van de Velde CJ, Rasmussen BB, Verhoeven DM, Shaaban AM, Bartlett JM, Bliss JM, Coombes RC; PathIES Sub-Committee. Prognostic and predictive value of ERβ1 and ERβ2 in the Intergroup Exemestane Study (IES)-first results from PathIES†. *Ann Oncol* 2015; **26**: 1890-1897 [PMID: 26002610 DOI: 10.1093/annonc/mdv242]

10 **Sjöström M**, Hartman L, Grabau D, Fornander T, Malmström P, Nordenskjöld B, Sgroi DC, Skoog L, Stål O, Leeb-Lundberg LM, Fernö M. Lack of G protein-coupled estrogen receptor (GPER) in the plasma membrane is associated with excellent long-term prognosis in breast cancer. *Breast Cancer Res Treat* 2014; **145**: 61-71 [PMID: 24715381 DOI: 10.1007/s10549-014-2936-4]

11 **Ignatov A**, Ignatov T, Roessner A, Costa SD, Kalinski T. Role of GPR30 in the mechanisms of tamoxifen resistance in breast cancer MCF-7 cells. *Breast Cancer Res Treat* 2010; **123**: 87-96 [PMID: 19911269 DOI: 10.1007/s10549-009-0624-6]

12 **Bardou VJ**, Arpino G, Elledge RM, Osborne CK, Clark GM. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol* 2003; **21**: 1973-1979 [PMID: 12743151 DOI: 10.1200/JCO.2003.09.099]

13 **Early Breast Cancer Trialists' Collaborative Group (EBCTCG)**, Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C, Wang YC, Dowsett M, Ingle J, Peto R. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* 2011; **378**: 771-784 [PMID: 21802721 DOI: 10.1016/S0140-6736(11)60993-8]

14 **Clarke R**, Tyson JJ, Dixon JM. Endocrine resistance in breast cancer--An overview and update. *Mol Cell Endocrinol* 2015; **418** Pt 3: 220-234 [PMID: 26455641 DOI: 10.1016/j.mce.2015.09.035]

15 **Borg A**, Baldetorp B, Fernö M, Killander D, Olsson H, Rydén S, Sigurdsson H. ERBB2 amplification is associated with tamoxifen resistance in steroid-receptor positive breast cancer. *Cancer Lett* 1994; **81**: 137-144 [PMID: 7912163]

16 **Carlomagno C**, Perrone F, Gallo C, De Laurentiis M, Lauria R, Morabito A, Pettinato G, Panico L, D'Antonio A, Bianco AR, De Placido S. c-erb B2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph node metastases. *J Clin Oncol* 1996; **14**: 2702-2708 [PMID: 8874330 DOI: 10.1200/JCO.1996.14.10.2702]

17 **Cuzick J**, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, Zabaglo L, Mallon E, Green AR, Ellis IO, Howell A, Buzdar AU, Forbes JF. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. *J Clin Oncol* 2011; **29**: 4273-4278 [PMID: 21990413 DOI: 10.1200/JCO.2010.31.2835]

18 **Musgrove EA**, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. *Nat Rev Cancer* 2009; **9**: 631-643 [PMID: 19701242 DOI: 10.1038/nrc2713]

19 **Ellis MJ**, Tao Y, Luo J, A'Hern R, Evans DB, Bhatnagar AS, Chaudri Ross HA, von Kameke A, Miller WR, Smith I, Eiermann W, Dowsett M. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst* 2008; **100**: 1380-1388 [PMID: 18812550 DOI: 10.1093/jnci/djn309]

20 **Jeselsohn R**, Buchwalter G, De Angelis C, Brown M, Schiff R. ESR1 mutations—a mechanism for acquired endocrine resistance in breast cancer. *Nat Rev Clin Oncol* 2015; **12**: 573-583 [PMID: 26122181 DOI: 10.1038/nrclinonc.2015.117]

21 **Angus L**, Beije N, Jager A, Martens JW, Sleijfer S. ESR1 mutations: Moving towards guiding treatment decision-making in metastatic breast cancer patients. *Cancer Treat Rev* 2017; **52**: 33-40 [PMID: 27886589 DOI: 10.1016/j.ctrv.2016.11.001]

22 **Araki K**, Miyoshi Y. Mechanism of resistance to endocrine therapy in breast cancer: the important role of PI3K/Akt/mTOR in estrogen receptor-positive, HER2-negative breast cancer. *Breast Cancer* 2018; **25**: 392-401 [PMID: 29086897 DOI: 10.1007/s12282-017-0812-x]

23 **Jeselsohn R**, De Angelis C, Brown M, Schiff R. The Evolving Role of the Estrogen Receptor Mutations in Endocrine Therapy-Resistant Breast Cancer. *Curr Oncol Rep* 2017; **19**: 35 [PMID: 28374222 DOI: 10.1007/s11912-017-0591-8]

24 **Perou CM**, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. *Nature* 2000; **406**: 747-752 [PMID: 10963602 DOI: 10.1038/35021093]

25 **Sørlie T**, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001; **98**: 10869-10874 [PMID: 11553815 DOI: 10.1073/pnas.191367098]

26 **Sorlie T**, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003; **100**: 8418-8423 [PMID: 12829800 DOI: 10.1073/pnas.0932692100]

27 **Hu Z**, Fan C, Oh DS, Marron JS, He X, Qaqish BF, Livasy C, Carey LA, Reynolds E, Dressler L, Nobel A, Parker J, Ewend MG, Sawyer LR, Wu J, Liu Y, Nanda R, Tretiakova M, Ruiz Orrico A, Dreher D, Palazzo JP, Perreard L, Nelson E, Mone M, Hansen H, Mullins M, Quackenbush JF, Ellis MJ, Olopade OI, Bernard PS, Perou CM. The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics* 2006; **7**: 96 [PMID: 16643655 DOI: 10.1186/1471-2164-7-96]

28 **Lehmann BD**, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 2011; **121**: 2750-2767 [PMID: 21633166 DOI: 10.1172/JCI45014]

29 **Lehmann BD**, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, Moses HL, Sanders ME, Pietenpol JA. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS One* 2016; **11**: e0157368 [PMID: 27310713 DOI: 10.1371/journal.pone.0157368]

30 **Pareja F**, Reis-Filho JS. Triple-negative breast cancers - a panoply of cancer types. *Nat Rev Clin Oncol* 2018; **15**: 347-348 [PMID: 29555966 DOI: 10.1038/s41571-018-0001-7]

31 **Paik S**, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; **351**: 2817-2826 [PMID: 15591335 DOI: 10.1056/NEJMoa041588]

32 **Sparano JA**, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, Geyer CE Jr, Dees EC, Goetz MP, Olson JA Jr, Lively T, Badve SS, Saphner TJ, Wagner LI, Whelan TJ, Ellis MJ, Paik S, Wood WC, Ravdin PM, Keane MM, Gomez Moreno HL, Reddy PS, Goggins TF, Mayer IA, Brufsky AM, Toppmeyer DL, Kaklamani VG, Berenberg JL, Abrams J, Sledge GW Jr. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med* 2018; **379**: 111-121 [PMID: 29860917 DOI: 10.1056/NEJMoa1804710]

33 **Parker JS**, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009; **27**: 1160-1167 [PMID: 19204204 DOI: 10.1200/JCO.2008.18.1370]

34 **Chia SK**, Bramwell VH, Tu D, Shepherd LE, Jiang S, Vickery T, Mardis E, Leung S, Ung K, Pritchard KI, Parker JS, Bernard PS, Perou CM, Ellis MJ, Nielsen TO. A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. *Clin Cancer Res* 2012; **18**: 4465-4472 [PMID: 22711706 DOI: 10.1158/1078-0432.CCR-12-0286]

35 **Dowsett M**, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, Ferree S, Storhoff J, Schaper C, Cuzick J. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol* 2013; **31**: 2783-2790 [PMID: 23816962 DOI: 10.1200/JCO.2012.46.1558]

36 **Gnant M**, Filipits M, Greil R, Stoeger H, Rudas M, Bago-Horvath Z, Mlineritsch B, Kwasny W, Knauer M, Singer C, Jakesz R, Dubsky P, Fitzal F, Bartsch R, Steger G, Balic M, Ressler S, Cowens JW, Storhoff J, Ferree S, Schaper C, Liu S, Fesl C, Nielsen TO; Austrian Breast and Colorectal Cancer Study Group. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol* 2014; **25**: 339-345 [PMID: 24347518 DOI: 10.1093/annonc/mdt494]

37 **Prat A**, Lluch A, Turnbull AK, Dunbier AK, Calvo L, Albanell J, de la Haba-Rodríguez J, Arcusa A, Chacón JI, Sánchez-Rovira P, Plazaola A, Muñoz M, Paré L, Parker JS, Ribelles N, Jimenez B, Bin Aiderus AA, Caballero R, Adamo B, Dowsett M, Carrasco E, Martín M, Dixon JM, Perou CM, Alba E. A PAM50-Based Chemoendocrine Score for Hormone Receptor-Positive Breast Cancer with an Intermediate Risk of Relapse. *Clin Cancer Res* 2017; **23**: 3035-3044 [PMID: 27903675 DOI: 10.1158/1078-0432.CCR-16-2092]

38 **van 't Veer LJ**, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; **415**: 530-536 [PMID: 11823860 DOI: 10.1038/415530a]

39 **Mook S**, Van't Veer LJ, Rutgers EJ, Piccart-Gebhart MJ, Cardoso F. Individualization of therapy using Mammaprint: from development to the MINDACT Trial. *Cancer Genomics Proteomics* 2007; **4**: 147-155 [PMID: 17878518]

40 **Cardoso F**, van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, Pierga JY, Brain E, Causeret S, DeLorenzi M, Glas AM, Golfinopoulos V, Goulioti T, Knox S, Matos E, Meulemans B, Neijenhuis PA, Nitz U, Passalacqua R, Ravdin P, Rubio IT, Saghatchian M, Smilde TJ, Sotiriou C, Stork L, Straehle C, Thomas G, Thompson AM, van der Hoeven JM, Vuylsteke P, Bernards R, Tryfonidis K, Rutgers E, Piccart M; MINDACT Investigators. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med* 2016; **375**: 717-729 [PMID: 27557300 DOI: 10.1056/NEJMoa1602253]

41 **Filipits M**, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CF, Dietze O, Greil R, Jelen A, Sevelda P, Freibauer C, Müller V, Jänicke F, Schmidt M, Kölbl H, Rody A, Kaufmann M, Schroth W, Brauch H, Schwab M, Fritz P, Weber KE, Feder IS, Hennig G, Kronenwett R, Gehrmann M, Gnant M; EP Investigators. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res* 2011; **17**: 6012-6020 [PMID: 21807638 DOI: 10.1158/1078-0432.CCR-11-0926]

42 **Buus R**, Sestak I, Kronenwett R, Denkert C, Dubsky P, Krappmann K, Scheer M, Petry C, Cuzick J, Dowsett M. Comparison of EndoPredict and EPclin With Oncotype DX Recurrence Score for Prediction of Risk of Distant Recurrence After Endocrine Therapy. *J Natl Cancer Inst* 2016; **108** [PMID: 27400969 DOI: 10.1093/jnci/djw149]

43 **Miller WR**, Larionov AA. Bridging the gap between translational research and clinical application. *J Natl Cancer Inst Monogr* 2011; **2011**: 134-137 [PMID: 22043060 DOI: 10.1093/jncimonographs/lgr020]

44 **Klintman M**, Dowsett M. Early Surrogate Markers of Treatment Activity: Where Are We Now? *J Natl Cancer Inst Monogr* 2015; **2015**: 24-28 [PMID: 26063881 DOI: 10.1093/jncimonographs/lgv002]

45 **Dowsett M**, Smith IE, Ebbs SR, Dixon JM, Skene A, Griffith C, Boeddinghaus I, Salter J, Detre S, Hills M, Ashley S, Francis S, Walsh G; IMPACT Trialists. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. *Clin Cancer Res* 2005; **11**: 951s-958s [PMID: 15701892]

46 **Robertson JFR**, Dowsett M, Bliss JM, Morden JP, Wilcox M, Evans A, Holcombe C, Horgan K, Kirwan C, Mallon E, Sibbering M, Skene A, Vidya R, Cheang M, Banerji J, Kilburn L, Dodson A, Smith I. Peri-operative aromatase inhibitor treatment in determining or predicting long-term outcome in early breast cancer - The POETIC Trial (CRUK/07/015). Abstracts: 2017 San Antonio Breast Cancer Symposium; December 5-9, 2017; San Antonio [DOI: 10.1158/1538-7445.SABCS17-GS1-03]

47 **Miller WR**, Larionov A, Anderson TJ, Evans DB, Dixon JM. Sequential changes in gene expression profiles in breast cancers during treatment with the aromatase inhibitor, letrozole. *Pharmacogenomics J* 2012; **12**: 10-21 [PMID: 20697427 DOI: 10.1038/tpj.2010.67]

48 **Miller WR**, Larionov A, Renshaw L, Anderson TJ, Walker JR, Krause A, Sing T, Evans DB, Dixon JM. Gene expression profiles differentiating between breast cancers clinically responsive or resistant to letrozole. *J Clin Oncol* 2009; **27**: 1382-1387 [PMID: 19224856 DOI: 10.1200/JCO.2008.16.8849]

49 **Miller WR**, Larionov A. Changes in expression of oestrogen regulated and proliferation genes with neoadjuvant treatment highlight heterogeneity of clinical resistance to the aromatase inhibitor, letrozole. *Breast Cancer Res* 2010; **12**: R52 [PMID: 20646288 DOI: 10.1186/bcr2611]

50 **Dunbier AK**, Ghazoui Z, Anderson H, Salter J, Nerurkar A, Osin P, A'hern R, Miller WR, Smith IE, Dowsett M. Molecular profiling of aromatase inhibitor-treated postmenopausal breast tumors identifies immune-related correlates of resistance. *Clin Cancer Res* 2013; **19**: 2775-2786 [PMID: 23493347 DOI: 10.1158/1078-0432.CCR-12-1000]

51 **Turnbull AK**, Arthur LM, Renshaw L, Larionov AA, Kay C, Dunbier AK, Thomas JS, Dowsett M, Sims AH, Dixon JM. Accurate Prediction and Validation of Response to Endocrine Therapy in Breast Cancer. *J Clin Oncol* 2015; **33**: 2270-2278 [PMID: 26033813 DOI: 10.1200/JCO.2014.57.8963]

52 **Ellis MJ**. Lessons in precision oncology from neoadjuvant endocrine therapy trials in ER+ breast cancer. *Breast* 2017; **34** Suppl 1: S104-S107 [PMID: 28669712 DOI: 10.1016/j.breast.2017.06.039]

53 **Ellis MJ**, Suman VJ, Hoog J, Goncalves R, Sanati S, Creighton CJ, DeSchryver K, Crouch E, Brink A, Watson M, Luo J, Tao Y, Barnes M, Dowsett M, Budd GT, Winer E, Silverman P, Esserman L, Carey L, Ma CX, Unzeitig G, Pluard T, Whitworth P, Babiera G, Guenther JM, Dayao Z, Ota D, Leitch M, Olson JA Jr, Allred DC, Hunt K. Ki67 Proliferation Index as a Tool for Chemotherapy Decisions During and After Neoadjuvant Aromatase Inhibitor Treatment of Breast Cancer: Results From the American College of Surgeons Oncology Group Z1031 Trial (Alliance). *J Clin Oncol* 2017; **35**: 1061-1069 [PMID: 28045625 DOI: 10.1200/JCO.2016.69.4406]

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**Table 1 Summary of the multigene tests**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Test name | Samples | Key references | Method | Genes No. | Genes |
| Oncotype DX | FFPE tumor tissue | [31,32] | QRT-PCR | 16 + 5 | *MKI67, AURKA, BIRC5, CCNB1, MYBL2, ERBB2, GRB7, ESR1, PGR, BCL2, SCUBE2, MMP11, CTSL2, GSTM1, CD68, BAG1* (+ ref genes *ACTB, GAPDH, RPLPO, GUS, TFRC*) |
| MammaPrint | Fresh or freshly frozen breast cancer tissue or FFPE tissue | [38-40] | DNA microarray | 70 | *AA555029\_RC, ALDH4A1, AP2B1, AYTL2, BBC3, C16orf61, C20orf46, C9orf30, CCNE2, CDC42BPA, CDCA7, CENPA, COL4A2, DCK, DIAPH3, DTL, EBF4, ECT2, EGLN1, ESM1, EXT1, FGF18, FLT1, GMPS, GNAZ, GPR126, GPR180, GSTM3, HRASLS, IGFBP5, JHDM1D, KNTC2, LGP2, LIN9, LOC100131053,LOC100288906, LOC730018, MCM6, MELK, MMP9, MS4 A7, MTDH, NMU, NUSAP1, ORC6L, OXCT1, PALM2, PECI, PITRM1, PRC1, QSCN6L1, RAB6B, RASSF7, RECQL5, RFC4, RTN4RL1, RUNDC1, SCUBE2, SERF1A, SLC2A3, STK32B, TGFB3,TSPYL5, UCHL5, WISP1, ZNF533* |
| Endopredict | FFPE tumor tissue | [41,42] | QRT-PCR | 8 + 4 | *BIRC5, UBE2C, DHCR7, RBBP8, IL6ST, AZGP1, MGP, STC2* (+ ref genes *CALM1, OAZ1, RPL37A, HBB*) |
| Prosigna (based on PAM50) | FFPE tumor tissue | [33-37] | Nanostring | 50 + 8 | *MIA, SFRP1, KRT14, KRT17, KRT5, FGFR4, GRB7, ERBB2, BAG1, MDM2, ACTR3B, BLVRA, CXXC5, TMEM45B, MMP11, FOXC1, EGFR, CDH3, PHGDH, MYC, CCNE1, CDCA1, CDC20, KIF2C, TYMS, KNTC2, UBE2T, MELK,PTTG1, CCNB1, CDC6, MYBL2, BIRC5, CENPF, EXO1, ORC6L, ANLN, UBE2C, RRM2, MKI67, CEP55, PGR, NAT1, SLC39A6, BCL2, ESR1, MAPT, GPR160, MLPH, FOXA1* (+ 8 reference genes) |

FFPE: Formalin-fixed paraffin-embedded; qRT-PCR: Quantitative reverse transcriptase-PCR.