

Predictive markers of endocrine response in breast cancer

Duniya Mosly, Arran Turnbull, Andrew Sims, Carol Ward, Simon Langdon

Duniya Mosly, Arran Turnbull, Andrew Sims, Applied Bioinformatics of Cancer, University of Edinburgh Cancer Research Centre, MRC Institute of Genetics and Molecular Medicine, Edinburgh EH4 2XR, United Kingdom

Duniya Mosly, Arran Turnbull, Carol Ward, Simon Langdon, Cancer Research UK Edinburgh Centre and Division of Pathology Laboratory, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, United Kingdom

Carol Ward, the Royal (Dick) School of Veterinary Studies and Roslin Institute, University of Edinburgh, Easter Bush, Roslin, Midlothian, Edinburgh EH25 9RG, United Kingdom

ORCID number: Duniya Mosly (0000-0001-8477-5871); Arran Turnbull (0000-0002-2287-0889); Andrew Sims (0000-0001-9082-3665); Carol Ward (0000-0002-9969-959X); Simon Langdon (0000-0002-7200-9237).

Author contributions: All authors contributed their views in this editorial; all authors read and agreed to the final manuscript.

Conflict-of-interest statement: None.

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Manuscript source: Invited manuscript

Correspondence to: Simon Langdon, PhD, Reader (Associate Professor), Cancer Research UK Edinburgh Centre and Division of Pathology Laboratory, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh EH4 2XR, United Kingdom. simon.langdon@ed.ac.uk
Telephone: +44-131-5371763
Fax: +44-131-5373159

Received: June 29, 2018

Peer-review started: June 30, 2018

First decision: July 17, 2018

Revised: July 26, 2018

Accepted: August 4, 2018

Article in press: August 4, 2018

Published online: August 30, 2018

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, Mammprint 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: Estrogen; *IL6ST*; Biomarker; Breast cancer; Predictive

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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.

Mosly D, Turnbull A, Sims A, Ward C, Langdon S. Predictive markers of endocrine response in breast cancer. *World J Exp Med* 2018; 8(1): 1-7 Available from: URL: <http://www.wjgnet.com/2220-315X/full/v8/i1/1.htm> DOI: <http://dx.doi.org/10.5493/wjem.v8.i1.1>

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 (anastrozole, 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^[9,9]. 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 year 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 downstream 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 α -positive group. Further studies by the same investigators demonstrated that the ER α -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 α -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

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	<i>MKI67, AURKA, BIRC5, CCNB1, MYBL2, ERBB2, GRB7, ESR1, PGR, BCL2, SCUBE2, MMP11, CTSL2, GSTM1, CD68, BAG1</i> (+ ref genes <i>ACTB, GAPDH, RPLPO, GUS, TFRC</i>)
MammaPrint	Fresh or freshly frozen breast cancer tissue or FFPE tissue	[38-40]	DNA microarray	70	<i>AA555029_RC, ALDH4A1, AP2B1, AYTL2, BBC3, C16orf61, C20orf46, C9orf30, CCNE2, CDC42BPA, CDCA7, CENPA, COL4A2, DCK, DIAPH3, DTL, EBF4, ECT2, EGLN1, ESM1, EXT1, FGF18, FLT1, GMP5, 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, RUNC1, SCUBE2, SERF1A, SLC2A3, STK32B, TGFB3, TSPYL5, UCHL5, WISP1, ZNF533</i>
Endopredict	FFPE tumor tissue	[41,42]	QRT-PCR	8 + 4	<i>BIRC5, UBE2C, DHCR7, RBBP8, IL6ST, AZGP1, MGP, STC2</i> (+ ref genes <i>CALM1, OAZ1, RPL37A, HBB</i>)
Prosigna (based on PAM50)	FFPE tumor tissue	[33-37]	Nanostring	50 + 8	<i>MIA, SFRP1, KRT14, KRT17, KRT5, FGF4, 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</i> (+ 8 ref genes)

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

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 treated with tamoxifen or anastrozole alone^[34,35] and tamoxifen plus anastrozole^[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 anastrozole^[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.

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