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Gene expression signatures for tailoring adjuvant chemotherapy of luminal breast cancer: the pathologists' perspective

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Gene expression signatures for tailoring adjuvant chemotherapy of luminal breast cancer: the pathologists' perspective.

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LoE: Level of Evidence, according to Oxford Centre for Evidence-based Medicine.

GR: Grades of Recommendations.

Introduction

Recommendations regarding the administration of adjuvant systemic treatment to patients with invasive breast carcinoma (BC) rely on the morphological assessment of key histopathological indices including tumour subtype, size, histological grade, lymphovascular invasion and lymph node status combined with ER, PR and HER2 receptor biomarker profile determined using immunohistochemistry and ISH. The presence of tumour infiltrating lymphocytes (TILs) is becoming increasingly important in triple negative BC (TNBC). In recent years, gene expression signatures or profile (GEP) assays have been developed to help to predict the risk of recurrence and to estimate the potential benefit from adjuvant systemic chemotherapy (CT) in patients with luminal BC.

Three prospective, randomized, clinical trials have demonstrated the reliability of MammaPrint® (MINDACT) and Oncotype DX Breast Recurrence Score® (TAILORx and RxPONDER) in evaluating the benefit of adjuvant CT based on the risk of recurrence in luminal BC. The MINDACT trial reported the value of MammaPrint® in the “high clinical risk” and “low genomic risk” population, while RxPONDER and TAILORx demonstrated clinical utility irrespective of formal clinical risk estimation [1-5]. The results of these assays may assist the clinician and the patient in a mutual shared decision-making model on the added value of systemic CT [6].

Does daily practice reflect the results of phase 3 clinical GEP trials?

The use of GEP assays in the routine assessment of luminal BC is clearly not uniform across the globe. Due to the high cost per test of these assays and the current centralized system of testing, many countries are simply unable to resource this form

of testing for patients with BC. In many wealthier countries these tests are reimbursed by the state or by health insurers. However, uptake by clinicians is not uniform. Some question the definition of 'high clinical risk', others argue that similar information can be obtained from in-house assessment of the Ki67 index or from other alternatives like MAGEE-equations [7]. How individual GEP assays have been constructed is not generally known by many physicians and the perception that GEP assays are interchangeable is widespread, a perception that is not substantiated by evidence when stratifying patients into risk categories.[8].

Assay selection varies according to local practice, knowledge and the personal experience of clinicians and pathologists with the different assays, and this differs from country to country. In general, there are no uniform criteria agreed upon nationally, e.g. in Spain or internationally regarding assay selection or patient eligibility for testing. This impedes an informative Health Technology Assessment (HTA) of the individual assays by national governments, which is a common practice in European countries before an assay is considered for reimbursement [9], in contrast to the US where HTA assessments are not usually performed, except by private payers. In countries where private insurance companies drive the reimbursement of GEP assays, the criteria for testing and assay selection may differ from those specified by the government. These differences do not always appear to have a scientific basis.

The current situation illustrates that a positive phase 3 trial using a GEP assay, with excellent scientific merit, does not necessarily translate into implementation of that assay in daily practice, and this applies to all types of assays used in phase 3 clinical

trials. This disparity between science and practice highlights the need for socio-economic considerations to be factored into clinical trials from the outset.

This divergence between the scientific benefit demonstrated by a clinical trial and the realities of daily practice settings is further highlighted by the following two points. First, there is no uniform definition among oncologists across or within countries regarding what constitutes a “clinically high risk patient”. Secondly, “clinically high risk”, as defined in the MINDACT trial, does not include assessment of Ki67 index. In fact, none of the large phase 3 trials have used Ki67 for patient selection. In some countries the MINDACT criteria are not used to select patients; rather, selection is based on intermediate risk as per TAILORx, and this is recommended by some HTA bodies as a criterion for reimbursement. Despite concerns regarding reproducibility and appropriate ‘cut-off’ levels, Ki67 is used in many countries to add to the tumour biological profile and, in some institutions, to classify luminal BC as Type A or B. However, the management implications of these subtypes is unclear, as well as what actually defines each subtype, with ever-changing definitions. Furthermore, the Ki67 cut-off level required to make this distinction is ever-changing and the potential therapeutic significance of such classification is not clearly defined. The International Ki67 Breast Cancer Working Group (www.ki67inbreastcancerwg.org) has recently completed a multi-year Ki67 project and concluded that Ki67 can be a reliable marker if 1) as for ER and HER2 testing, pre-analytical handling considerations are considered as critical; 2) a standardized visual scoring method, developed by the Working Group is used; 3) participation in and evaluation of quality assurance and quality control programs is ensured to maintain analytical validity. The Working Group recognized that Ki67 IHC as a prognostic marker in BC has clinical validity but concluded that clinical

utility is evident only for prognosis estimation in patients with anatomically favourable ER positive and HER2 negative BC, to identify those less likely to benefit from adjuvant CT. In these T1-2, N0-1 patient groups, the consensus is that Ki67 $\leq 5\%$ or $\geq 30\%$ may be used to estimate prognosis. Although this suggests that Ki67 may be useful in this setting, the St Gallen consensus conferences from 2017 through 2021 failed to agree on any specific Ki67 cut-off in luminal early BC, pointing to the (dis)illusion on ever finding the ideal Ki67 cut-off. Nevertheless, in resource poor countries where there are a limited number of assays reimbursable, or for clinicians who still rely on Ki67, it may be useful in restricting the GEP assays to those patients whose tumours fall in the 5%-30% Ki67-category. It is noteworthy that in Italy and Germany, a 15%-30% category is used in this regard, illustrating the difficulties in harmonizing clinical practice

The definition of a “clinically low risk” BC patient might achieve International consensus more easily than for the “high risk”-category. For example, a pT1, lymph node negative, ER and PR positive (Allred score > 4), HER2 negative, histological grade 1, tumour without lymphovascular invasion and a Ki67 index $<10\text{-}15\%$ is likely to be considered low risk in most centers.

The Belgian Health Care Knowledge Centre (www.kce.fgov.be), in partnership with oncologists and pathologists and the Belgian Cancer Registry, initially developed several reports on how GEP assays could be implemented in Belgium. Following this preparatory work and the publication of the results of the MINDACT and TAILORx trials, the Belgian Government established a National pilot study on the use of GEP assays specific for the Belgian context ([Remboursement des tests de profilage d'expression génétique \(GEP\) en cas de cancer du sein au stade précoce - INAMI \(fgov.be\)](#)). One of the key objectives is to assess how GEP testing is used in routine

settings and to what extent it influences the decision, by the physician and patient to initiate adjuvant CT or not.

This study has specified a number of assays, restricted to those with level IA evidence, to be reimbursed by the Belgian Government on a yearly basis, for a limited period of 3 years. It is acknowledged that assays without level I evidence to date, including Prosigna®, Breast Cancer Index® (BCI®) and Endopredict®, may provide useful prognostic information, but also acknowledges that in a societal context where there are budgetary constraints, not all assays can be reimbursed. Strict criteria on how to select patients have been defined. A GEP assay will only be reimbursed by the Belgian government when the decision to request the test on an individual patient's tumour is discussed and registered at a multidisciplinary meeting, attended by oncologists and pathologists and only in officially recognized BC centers by the Belgian government. All relevant information in this pilot study is collected by the Belgian Cancer Registry and will be used to inform the Belgian Government on the use and legislative reimbursement of GEP assays on an on-going basis. A similar system of patient selection for Oncotype DX® BC tumour testing operates in Ireland (National Cancer Control Programme. Breast Chemotherapy Regimens - HSE.ie).

In general, it is still unclear that the introduction of GEP assays in daily practice will lead to a decrease in the prescription of chemotherapy, as some patients who may not have received CT due to being low clinical risk, may be recommended to receive treatment due to a "high-genomic risk" on GEP, and this effect may differ according to which assay is used. While a reduction in the administration of adjuvant CT may offset the high costs of these assays, possibly with a net financial gain, as the tendency is

indeed that net CT use is less with GEP, the primary motivation is to assist the development of a personalized treatment plan for patients with luminal BC.

The role of the pathologist?

There are several GEP assays available, developed using different methodologies and validated in different trials. In practice, medical oncologists may prefer one assay based on the results of the clinical trials and the specific clinical need but also due to personal preference, familiarity with a particular assay and negative experience with one assay over another. Different Guideline groups have issues varying recommendations regarding the use of GEP assays e.g., the NCCN recommend the Oncotype Dx® as the preferred assay based on its predictive value for CT benefit in the US and the European Commission Initiative on Breast Cancer Guideline Development Group endorse both Oncotype Dx® and MammaPrint® based on availability of level I evidence [10]. Different recommendations by guideline groups do not help to achieve uniformity of testing across regions, countries, or continents.

All available assays, including Prosigna®, Oncotype Dx®, BCI® and EndoPredict®, are prognostic in postmenopausal luminal N0-disease setting, albeit with different hazard ratios [11]. It is important to note that tumour size and nodal status are required by Prosigna® and EndoPredict®, which may influence a physician's choice of assay. Two GEP assays have level I evidence with a considerable duration of follow-up: TAILORx (9 years) and RxPONDER (5 years) for Oncotype DX®, and MINDACT (8 years) for the MammaPrint® assay. The MINDACT study, with an 8-year median follow up, demonstrated that MammaPrint® could help to identify N0 and N1 postmenopausal patients with high-clinical risk who had good outcomes without CT. The TAILORx

study, with a 9-year median follow-up demonstrated that Oncotype DX® can identify N0 pre- and postmenopausal patients who can avoid CT irrespective of baseline clinical risk. The RxPONDER study reported initial results with a 5-year median follow up suggesting that the Oncotype DX® identifies postmenopausal N1 patients who can be spared CT independent of baseline clinical risk. Nevertheless, MammaPrint® and Oncotype DX® provide nearly similar estimates of CT benefit, both in pre- and postmenopausal women. Consequently, in the realities of daily practice, these assays are used interchangeably.

However, depending on the information required, some assays may be more informative than others and provide different risk scores if tested in parallel on the same tumor sample [8]. For example, the BCI® (Breast Cancer Index®) is a gene-expression based signature comprised of 2 functional biomarker panels, HOXB13 and IL17BR (H/I), which is a 2-gene ratio measuring ER signaling, combined with the Molecular Grade Index (MGI), which is a 5-gene measurement of cancer cell proliferation [8, 12]. In the Trans-aTTOM study, BCI® was able to predict benefit from extended tamoxifen use, suggesting that the prognostic component is able to define the risk of late (5-10 year) and overall (1-10 year) distant recurrence [12]. This information is not provided by other signatures, suggesting that the information provided by BCI® may be complementary to other GEP assays, depending on the clinical need. A thorough understanding of the information provided by each assay is important as, in some instances, it is the pathologist who may decide which assay to use, having received a request by the oncologist for “a GEP test”. Furthermore, while risk information provided by multiple assays like Prosigna®, IHC4 NexCourse Breast®, MammaPrint® and Oncotype DX® is broadly similar for luminal BC, for the individual patient different

assays may provide different risk categorization, complicating matters even more for the individual oncologist and pathologist [13]. All the above suggests that specific clinical indications may guide which assay is best to use, conceptually urging for uniform and internationally agreed indication-specific guidelines. Table 1 is a detailed methodologically and clinical summary of the assays discussed, including levels of evidence.

Some clinicians select an assay, expecting that it will be used precisely according to trial protocol. In the MINDACT trial, the assay was based on micro-arrays and used on frozen tissue which differs from routine practice in most histopathology laboratories. The pathologist is responsible for implementing the assay locally or sending the tumour material for testing in a centralized laboratory. Currently, both assays with level I-evidence, MammaPrint® and Oncotype DX®, both can be performed centrally [8, 14, 15] but to date only Mammapiint® can be performed in the local setting. This presumes the implicit knowledge of the pathologist on how different GEP assays were constructed, what trials were applied to validate their prognostic and predictive power and how to select the optimal assay for an individual patient. Due to General Data Protection Regulatory (GDPR) and preferences of the local regulatory or reimbursement agencies in several countries local testing is now preferred, limiting the range of assays that are used.

The pathologist is also responsible for careful selection of the tumour material to be tested. Mitotically active, cellular tumour stroma and/or highly inflammatory stroma with a high Ki67 index may contribute to a high-risk score in low grade invasive BC [2]. Inclusion of biopsy cavities in breast cancer specimens is associated with significant

changes in the expression of individual (proliferative) genes driving the risk score of GEP [16]. Multigene signature results may be different in core needle biopsy vs resection specimens when patients are receiving hormone replacement therapy [17]. The DCIS component should not exceed 30% of the tumour sample as the molecular profile of DCIS may differ from that of the invasive component (e.g., HER2 status). Accurate histological typing is also important in view of the inherent good outcome associated with some breast cancer subtypes, e.g., cribriform, tubular, and mucinous cancer, may preclude the use of GEP assays in some luminal BC [18]. Finally, intratumor heterogeneity (ITH) based on Ki67 and ER may affect the GEP result, resulting in an under- or overestimated prognostic risk, emphasizing the importance of the selection of FFPE-block by the pathologist [19].

The different levels of evidence required by the local regulatory authorities and reimbursement agencies, the number of tests available, the lack of uniformity within the clinical community on what constitutes a patient who would benefit from testing, potential subjectivity in selection of GEP assay and inconsistencies regarding the use of proliferation markers such as Ki67 in addition to economic considerations are examples of real-world facts that contribute to lack of a standardized approach to the use of GEP assays.

It is the role of the pathologist, together with clinicians and health care authorities, to develop the most appropriate framework for use of GEP assays in clinical practice. Taking all real-world realities into consideration, the oncologist, pathologists as well as industry have a moral obligation to urge that a GEP assay can be provided for all patients who would benefit from testing, pre- or post-menopausal, a GEP assay is

requested, irrespective of the constraints of daily clinical practice. In Table 2 a risk-management perspective is proposed that may inform pathologists as well as oncologists, government, patients, and industry to develop the most appropriate and realistic framework possible [20].

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Table 1 Summary of the gene expression assays discussed, including methodological, clinical and evidence levels (adapted and updated from references n°8, n°14, n°15).

Multigene Test	Mammaprint	Oncotype Dx	Breast Cancer Index	Prosigna	Endopredict
Evidence Levels :					
NCCN	1	1 (pN0 (all ages) 1 (pN1, postmenopausal), 2A (pN1, premenopausal)	2A	2A	2A
LoE	1B	1B	2B	2B	2B
GR	A	A	B	B	B
Provider	Agendia	Exact Sciences (former Genomic health)	Biotheranostics	NanoString	Sividon (Myriads)
Year validated	2002	2004	2008	2009	2011
Validation set	n= 97 cases (LN -, < 5cm, <55 years)	N=668 cases (LN -, ER +, adjuvant ET)	n=410 and n=323 (ER +, HER2 -, adjuvant ET)	N=189 cases (LN +/-, all subtypes) Postmenopausal status	N=964 cases (LN +/-, >55 years, adjuvant ET, HER2 -) Postmenopausal status
Nr. of genes	70 genes	21 genes (16+5)	5 + 2 genes	58 genes (50+8)	11 (8+3) +1 genes
Tissue type used	frozen tumor tissue	FFPE tissue	FFPE tissue	FFPE tissue	FFPE tissue
Genes tested (# of genes)	12 groups Apoptosis (2) Antigrowth signalling (1) Growth factors (6)	6 groups ER-related (4) Proliferation (5) HER2-related (2) Invasion (2) Others (3) Reference genes (5)	3 groups H:I index (2 genes) Molecular grade (5) Reference genes (4)	13 groups Proliferation (18) Cytoskeletal function (4) Aptosis (2) RTK- and proliferation- signalling (3)	3 groups Proliferation (3) Hormone-receptor related (5) Reference genes (4)

	Proliferation and oncogenic transformation (12) Cell cycle (15) Adhesion and remodeling (5) Motility (3) Altered metabolism (7) Angiogenesis (6) Unknown function (9) Miscellaneous (7)* *3 genes were assigned to more than one group			ECM & adhesion (5) HER2 signaling (2) Cell division (3) Hormone signaling (3) WNT-pathway (2) Metabolism (4) Ubiquitin ligase (1) Miscellaneous (3) Reference genes (5)	
Methodology	DNA microarrays	qRT-PCR	qRT-PCR	Nanostring nCounter	qRT-PCR
Central lab	yes	yes	yes	no	no
Genomic scores	Genomic risk score	Recurrence Score (RS 0-100)	Risk score (0-10)	ROR Score (0-100) Intrinsic subtype	EP Score (0-15) EPclin Score (1 - 6,9)
Risk category	Low High	Low Intermediate High	Low High	Low Intermediate High	Low High
Clinical parameters	No clinical parameters taken into account of risk scores	Interpretation of RS using pN +/- RSPC= RS + age+ pT + Grade (NO)	No clinical parameters taken into account in risk scores	ROR = PAM50 + pT (+/- 2cm) + pN (-/+)	EPclin = EP score + pT + pN

Indication for testing	Invasive breast cancer pT1-2 pN0/pN1	Invasive breast cancer pT1-2 ER+ / HER2- pN0/pN1	Invasive breast cancer pT1-2-3 ER+ / HER2- pN0 adjuvant ET	Invasive breast cancer pT1-2 / pN0 or pT2 / pN1 ER+ adjuvant ET postmenopausal status	Invasive breast cancer pT1-2 ER+ / HER2- pN0/pN1 postmenopausal status
Clinical validation	Yes	Yes	Yes	Yes	Yes
Validated for prognosis (after 5 years resp late recurrences)	Not separately shown	Yes	Yes	Yes	Yes
Validated for prediction	Yes	Yes	No	No	No
Prospective evidence	Mindact	TailorX PlanB RxPonder ADAPT	No	No	No
Prospective-retrospective evidence	Multicenter validation	NSABP-B-14 NSABP-B-20 ECOG 9127 SWOG 8814 ATAC	TransATTOM	MA.12 MA.5 ABCSG 8 ATAC	ABCSG 6 ABCSG 8 GEICAM-9906 ATAC

Table 2 Risk-management framework for implementation of Gene-expression profile assays in daily practices

Type of Risk	Risk	Possible consequences	Solution
Risk to patients with luminal breast cancer	Not receiving a GEP-analysis	Patients can be over- or undertreated with systemic chemotherapy, with a high burden of inappropriate use of chemotherapy for patients and society.	Raise awareness at government level and patient organizations or discuss with private insurance companies of the societal and ethical importance to get these assays reimbursed.
	The price of the assay is too high	No GEP will be reimbursed, and consequently no patient will be tested.	Raise awareness at government level of the positive budget-impact of reimbursing the assays based on potential net reduction in chemotherapy use. Raise awareness at government level and patient organizations or discuss with private insurance companies of the societal and ethical importance to get these assays reimbursed.
		Inconsistent use of GEP-assay uses and health care inequalities with a GEP performed only for those patients that can afford paying for the assay.	Raise awareness with industry that the price may be unaffordable in certain health care settings, ensuring that there is an equitable access to these assays across regions or countries. Find a societal framework between governments, oncologists, patients, and pathologists that avoids over- and underuse of GEP in daily practice, and that is independent of the wealth of the patients.
	Overreliance on Ki67	Suboptimal selection of patients to be tested with GEP and/or receive chemotherapy, leading to over- or undertreatment	Education of patients, oncologists, government bodies and industry on the need to define a pragmatic approach for GEP-assay reimbursement, that considers and accepts the different realities in daily practices. National and International oncology and pathology societies need to come up with enforceable guidelines that take into consideration the realities of daily practice,

			considering that an abolishment of Ki67 will probably never happen, neither will a complete implementation of Ki67 in all daily practices.
		Divergence in KI67-assessment between centers, hence less uniform approach of patients	Educate clinicians to define clearly how to use Ki67 in their setting; use reference materials as well as monitor yearly Ki67 index of the given pathology institution as mean and median Ki67 levels differ from one pathology center to another, but that have proven to remain stable within the same pathology center [21]
	Suboptimal knowledge of the clinician and pathologist on the different GEP-assays	Subjective reliance on one assay over the other	Development of educational evidence-based materials for pathologists and oncologists. Further research to understand the specific biology of luminal tumors that have an indolent clinical course. Investigate further the biology of GEPs.
	Different Guidelines with different recommendations on how to use GEP	No uniformity in use of GEP across regions, countries and even continents.	Within a region, country or continent define which guideline is used. Urge professional societies like NCCN, ESMO, ASCO to aim for uniformity, and use scientific and unbiased evidence for the recommendations, including uniform and internationally agreed indication-specific guidelines.
Risk to assay integration in society	Insufficient knowledge on the importance of GEP-assays at government-level	Inconsistent use of GEP in daily practices, with well-informed patients requesting it, while less-informed patients may be denied an assay.	Educate patients, government, pathologists, and clinicians.

	Insufficient knowledge on the underlying biology of the different GEP-trials	Not asking the appropriate assay for the appropriate clinical question.	
Risk to society	Inequalities in cancer care	Matheus-effect in health care settings	Develop a framework with National Cancer Registries and Health Technology Assessments bodies (HTA's) to calculate the benefits for society in an unbiased manner.
	Different health care settings worldwide	Disparate use of GEP-assays in daily practices within and between continents.	Propose to industry and the collaborative trial-groups to share and pool the trial-data of the different trials that led to level I-evidence, find commonalities of use between the assays, including specific indications, and enforce the use of GEP-assays in daily practice, whereafter the market- and societal driven pressures will determine which assay will eventually be used.