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

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

R. Salgado[1], V. Peg[2], J. Rüsschoff[3], A Vincent- Salomon[4], I. Castellano[5], S. Perner[6], K. Van De Vijver[7], CM. Quinn[8], Z. Varga[9].

Word count: 2078

References: 15

Table: 1

Introduction

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

Three key prospective …. of which the latter has been presented at…

Two prospective, randomized, clinical trials, ie. MINDACT, TAILORx, and a third one, RxPONDER, presented at the last 2020 SABCS but not yet published, have demonstrated the reliability of GEP assays in predicting the risk of recurrence in luminal breast cancer, mostly in the "high clinical risk" and "low genomic risk" population (1). 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 chemotherapy (2).

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

The use of GEP assays in the routine assessment of luminal breast cancer 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 breast cancer. 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 (15). Knowledge on how individual GEP assays have been constructed is not generally known by many physicians and the belief that GEP assays are interchangeable is widespread but this belief is not substantiated by evidence when stratifying patients into risk-categories is concerned (11).

Assay selection varies according to local practice, knowledge and the personal experience of clinicians and pathologists with the different assays, and this is different from country to country. In general, there are mostly no uniform nationally,

e.g. Spain or even internationally agreed criteria regarding assay selection or patient eligibility for testing. The Spanish experience for example is reflective of the practice in other countries, and impedes an informative Health Technology Assessment (HTA) of the assays by national governments, which is a common practice across the EU before an assay is considered for reimbursement (3), while in the US HTA-assessments are not usually performed. 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 to implementation of that assay in daily practice, and this observation accounts for all types of assays used in phase 3 clinical trials. This disparity between science and practice highlights the need for integration of socio-economic considerations to be factored into clinical trials from the outset.

The definition of a low risk breast cáncer patient can reach a larger consensus tan high and intermediate risk. In practice pT1, node negative, ER and PR positive (with an Allred score > 4), HER2 negative, histological grade according to Elston and Ellis I, without any vascular invasión and KI67 < 15% breast cancers are considered at low risk.

This divergence between the scientific benefit demonstrated by of a clinical trial and the realities of daily practice settings is further exemplified by two examples. 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. Despite concerns of 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 breast cancer as Type A or B. The International Ki67 Breast Cancer Working Group -www.ki67inbreastcancerwg.org- has recently concluded a multi-year KI67 project and concluded that Ki67 can be a reliable marker if 1) as for estrogen receptor 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 accepted that Ki67 IHC as a prognostic marker in breast cancer has clinical validity but concluded that clinical utility is evident only for prognosis estimation in anatomically favorable ER-positive and HER2-negative patients, to identify those who will not benefit from adjuvant chemotherapy. In these T1-2, N0-1 patient groups, the consensus is that Ki67 ≤ 5% or ≥ 30% may be used to estimate prognosis. This suggests that Ki67 may be useful in this setting, however the St Gallen consensus conferences from 2017 through 2021 failed to agree on any specific Ki67 cut-off in luminal early breast cancer, pointing to the (des)illusion on ever finding the ideal Ki67 cut-off. Nevertheless, in resource-poor regions or countries where there are no unlimited number of assays reimbursable, or even for clinicians that still rely on Ki67, Ki67 may be useful to restrict the GEP-assay only for those patients that fall between the 5%-30% Ki67-category. (This is what we follow in our Institution: Ki67 between 15% and 30% get GEP)

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 clinical trials, the Belgian Government established a National pilot-study on use of GEP 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)-. 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 IA evidence to date, including Prosigna, Breast Cancer Index and Endopredict, may provide useful prognostic information, 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, and a GEP assay will only be reimbursed when this is discussed and registered at multidisciplinary meetings, attended by oncologists and pathologists in officially recognized breast cancer centers. 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.

In general, it is however 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 chemotherapy due low clinical risk, may be recommended to receive this treatment due to a "high-genomic risk" on GEP. While a reduction in the administration of adjuvant chemotherapy may offset the high costs of these assays, possibly with a net financial gain, the primary motivation is to assist the development of a personalized treatment plan for patients with luminal breast cancer.

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 guidelines produce different recommendations regarding the use of GEP assays e.g. the NCCN in the US and the European Commission Initiative on Breast Cancer (4) which does not help in achieving uniformity of testing across regions, countries or continents.

All of the available assays, including ROR, Breast Cancer Index (BCI) and EpClin, are prognostic in the luminal N0-disease setting, albeit with different hazard ratios (5). This nearly similar prognostic information is corroborated by the level IA evidence of the assays used in the TAILORx, MINDACT and RxPONDER, while not all of them have the same duration of follow-up, with for example, no 10-year follow-up for RxPONDER. Mammaprint and Oncotype-DX provide similar estimates of chemotherapy benefit, both in pre- and post-menopausal women, suggesting that based on this criterium of benefit of chemotherapy, these assays may be interchangeable. However, depending on the information required, some assays may be more informative than others and provide different risk score if tested parallel on the same tumor sample (11). For example, the BCI is a gene-expression based

signature comprised of 2 functional biomarker-panels, HOXB13 and IL17BR (H/I), which is a 2-gene ratio measuring estrogen signaling, combined with the Molecular Grade Index (MGI), which is a 5-gene measurement of cancer cell proliferation (6). 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 (6). This information is not provided by other signatures, suggesting that the information provided by different assays can be complementary, 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 for "a GEP-test". (so far most pathologists & clinicians follow the rule: don´t change the system. It would be very helpful setting up criteria for decision making about how to use different GEP assays – indication specific.)

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 carries the responsibility to implement the assay and decentralize locally or to send the tumour material for testing in a centralized laboratory. Currently, of the assays with level IA-evidence, Mammaprint and Oncotype DXx, both can be performed centrally, but so far only Mammaprint can be performed in the local setting. This decision presumes the implicit knowledge of the pathologists on how different GEP were constructed and what trials were applied to validate their prognostic and predictive power and also the ultimate choice for a given GEP. However, due to GPDR-regulations and preferences of the local regulatory or reimbursement agencies in several countries local testing is preferred, limiting the range of assays, and excluding some GEP than can be used.

The pathologist is also responsible for careful selection of the tumour material to be tested. Mitotically active, cellular tumor stroma and/or highly inflammatory stroma with high Ki67 index may contribute to a high-risk score in low-grade invasive breast carcinomas (7). 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 (8). Multigene signature results may be different in core needle biopsy vs resection specimen when patients are under hormone replacement therapy (9). The DCIS component should not exceed 30% of the tumour sample as the molecular profile of DCIS may differ from invasive component (e.g. HER2 status). Furthermore, the inherent good outcome in specific histological subtypes of breast cancer, like cribriform, tubular or mucinous breast cancer, may preclude the systemic GEP-use in all luminal breast cancer cases (13). Finally, intratumor heterogeneity based on Ki67 and ER for example may affect the GEP-result, resulting in an under- or overestimated prognostic risk, suggesting the assessment of multiple samples (both representative and atypical cores) to fully account for the ITH-driven variation in risk prediction, which underlies again the importance of the choice of the FFPE-block by the pathologist (14).

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 "clinically high-risk" patient, 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 the 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 for the “clinically intermediate risk”-patients, pre- or post-menopausal, a GEP-assay is requested, whatever this assay may be and how different the realities of daily clinical practice are. In Table 1, 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 as possible (10).

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Corresponding author

Roberto Salgado

Department of Pathology, GZA-ZNA Hospitals, Antwerp, Belgium/ Division of Research, Peter Mac Callum cancer Centre, Melbourne, Australia.

E-mail: roberto@salgado.be

Affiliations

[1]Department of Pathology, GZA-ZNA Hospitals, Antwerp, Belgium/ Division of Research, Peter Mac Callum Cancer Centre, Melbourne, Australia.

[2]Universidad Autónoma de Barcelona, Barcelona, Spain; Department of Pathology, Vall D'Hebron University Hospital, Barcelona, Spain; Spanish Biomedical Research Network Centre in Oncology (CIBERONC), Madrid, Spain.

[3]Institute of Pathology Nordhessen and Targos Molecular Pathology GmbH, Kassel, Germany.

[4] Department of Pathology and Department of Diagnostic and Theranostic Medicine, , Institut Curie, PSL Research University, Paris, France.

[5]Department of Medical Sciences, University of Turin, Turin, Italy.

[6]Institute of Pathology, University Hospital of Schleswig-Holstein, Campus Lübeck, Lübeck; Pathology, Research Center Borstel, Leibniz Lung Center, Borstel, Germany.

[7]Department of Pathology, Ghent University Hospital, Ghent, Belgium.

[8] Department of Pathology, St. Vincent's University Hospital, Dublin, Ireland.

[9]Department of Pathology and Molecular Pathology, University and University Hospital Zurich, Zurich, Switzerland.

Table 1 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	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 expensive	No GEP will be reimbursed, and consequently no patients' tumor will be tested.	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. 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 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.
		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.	
	Overreliance on Ki67	Suboptimal selection of patients, with 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. Find a societal framework between governments, oncologists, patients, and pathologists that avoids over- and underuse of GEP in daily practice, independent of the wealth of the patients. 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 remain stable within the same pathology center (12)**
	Suboptimal knowledge by the clinician and pathologist on the different GEP-assays	**Subjective reliance on one assay over the other**	**Development of educational materials for pathologists and oncologists.**
	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.**
Risk to assay integration in society	**Insufficient knowledge on the importance of GEP-assays at government-level**	**Chaotic and 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.**	**Further research to understand the specific biology of these luminal, LN+ tumours that have an indolent clinical course. What is the role of the proliferation- and oestrogen pathways in the different assays, the role of stroma, for example tumor infiltrating lymphocytes and cancer-associated fibroblasts, menopausal status and hormonal balance in these patients' luminal cancer?**
Risk to	**Inequalities in**	**Matheus-effect in health**	

society	**cancer care**	**care settings**	**Develop a framework with Cancer Registries to calculate the benefits for society.**
	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 IA-and IB-evidence and draw conclusions that are irrespective of the assay used, but that enforce the use of GEP-assays in daily practice, whereafter the market- and societal driven pressures will determine which assay will eventually be used.**