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REVIEW ARTICLE

Clinical Utility of Multigene Assays for Guiding Treatment Decisions in Early Breast Cancer Patients

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ABSTRACT

The clinical management of invasive breast cancer has changed during the last decade with the use of molecular-based multigene assays (MGAs).They are increasingly used to gain additional prognostic and predictive information and guide adjuvant treatment decisions. Since 2004, several MGAs have become available but, four of them are the most widely used in clinical practice: the OncotypeDX® Breast Recurrence Score, the 70-gene signature MammaPrint®, the Prosigna® (PAM50) and the EndoPredict® (EP/EPclin Scores) assay. However, MGAs are not all the same and they do not provide interchangeable information. They differ in terms of the technological platform used for their development, the number and specific genes assessed, and the patient populations in which they were validated. Furthermore, although they are all validated for providing prognostic information, not all of them are supported with data from prospective randomised trials confirming the clinical value of their use in chemotherapy treatment decisions in certain groups of breast cancer patients; in this regard, so far there are published data only for OncotypeDX and MammaPrint, whilst PAM-50 (Prosigna) and EndoPredict assays are currently not supported by entirely prospective randomized trials evaluating their predictive value of chemotherapy benefit. As such, inclusion of these MGAs in major international treatment guidelines differs in indications for their use in clinical practice as prognosticators only or as predictors of chemotherapy benefit as well. Use of MGAs in clinical decision making can lead to de-escalation of chemotherapy recommendations and thus save a large number of patients from unnecessary side effects and decrease the cost of breast cancer treatment to National Health systems. This review provides an overview of the four most widely used MGAs in clinical practice, including basic information on their development and validation, as well as recent data on the information they can provide.

Keywords: Breast cancer, Multigene assays, OncotypeDX, Mammaprint, Prosigna, Endopredict

Introduction

The realisation that Breast Cancer (BC) is not a single entity but a heterogeneous group of diseases characterised by distinct molecular profiles[1] and that multi-gene assays (MGAs) can provide insights regarding tumour biology, transformed treatment decision-making in early-stage BC. In the past, clinicians based their treatment recommendations on traditional clinical-pathological prognostic factors such as age, lymph node status, tumour size and grade, oestrogen receptor (ER), Ki67 and human epidermal growth factor receptor 2 (HER2). However, MGAs can provide additional prognostic and predictive information in order to guide treatment decisions and offer chemotherapy only to patients who are likely to benefit from it, while sparing other patients unnecessary treatment. Since 2004, several MGAs have become available but, four of them are the most widely used in clinical practice: Oncotype DX® Breast Recurrence Score Assay (21-gene assay), MammaPrint® (70-gene panel, "Amsterdam Signature"), Prosigna® (PAM50) and EndoPredict® (EP/EPclin Scores); basic information on their development and validation, as well as recent data on the information they can provide is included in this review (Table 1).

Table 1. Most widely used Multi-gene Assays

Assay	Technological platform	Number of Genes involved	Indication (patient/tumour characteristics)	Prognosis	Prediction of adjuvant chemotherapy benefit
OncotypeDX Breast Recurrence Score	qRT-PCR	21 (16 cancer-related and 5 reference genes)	• All ages • N0 and N1(1-3) • HR+ • HER2 negative	√	√
MammaPrint	Mircroarray	70	• Tumour size ≤5 cm • N0 and N1(1-3) • HR+ or HR- • HER2 negative or positive	√	√[a]
Prosigna (PAM50)	Hybridization-based (for the Prosigna Assay)	58 (50 classifier and 8 reference genes)	• N0 • HR+ • Postmenopausal	√	-
EndoPredict	qRT-PCR	11 (8 cancer-related and 3 reference genes)	• N0 and N1 • HR+ • HER2-negative • Postmenopausal	√	-

[a] see restrictions in Table 4

HR, hormone receptor; HER2, human epidermal growth factor receptor 2; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction.

OncotypeDX Breast Recurrence Score Assay

The Recurrence Score assay is a quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR)-based assay performed on RNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue samples that were developed using 3 cohorts of patients with early BC and long-term follow up[2]. The assay, which is performed in a central laboratory, provides a Recurrence Score result (range: 0-100), based on expression of 21 genes (16 cancer-related, 5 reference genes), which represents a point estimate of the 10-year risk of distant recurrence, classifying patients into low, intermediate and high risk group (Table 2).

Table 2. Patient Classification of Multi-gene Assays

Assay			
OncotypeDX *(Recurrence Score)*	**MammaPrint**	**Prosigna (PAM50)** *(Risk of Recurrence [ROR], Prosigna Score)*	**EndoPredict** *(EP and EPclin Scores)*
Initial classification: – Low-risk (score <18) – Intermediate-risk (score: 18-30) – High-risk (score ≥31) *In regards to Prediction of Chemotherapy benefit (TAILORx and RxPONDER):* – Low-risk (score <11) – Intermediate-risk (score: 11-25) – High-risk (score ≥26)	– Low-risk – High-risk	• Node-negative: – Low-risk (score: 0-40) – Intermediate-risk (score: 41-60) – High-risk (score: 61-100) • 1-3 positive nodes: – Low-risk (score: 0-40) – High-risk (score: 41-100) Also provides intrinsic tumour-subtype classification	• EP: – Low-risk (score <5) – High-risk (score ≥5) – • EPclin: – Low-risk (score <3.3) High-risk (score ≥3.3)

- **Prognosis**

The prognostic utility of the assay in ER+ node-negative early BC patients treated with endocrine therapy alone was retrospectively validated in samples from 4 prospective studies, all of which used archival FFPE tissue samples: analysis of patients receiving 5 year of tamoxifen in the National Surgical Adjuvant Breast and Bowel trial (NSABP) B-14 study; analysis of node-negative patients in transATAC (the translational arm of the ATAC trial); the Kaiser Permanente case-control study; and the Japan BC Research Group cohort study[3-6]. The assay was also validated in node-positive patients (in node-positive patients in transATAC and the tamoxifen-treated arm in Southwest Oncology Group [SWOG] 8814)[4,7]. Notably, the Recurrence Score result also predicts late recurrence (beyond 5 years), which has become clinically relevant with the recent evidence for the benefit of 10 years of tamoxifen treatment in ER+ patients[8].

Several recent outcome studies with consistent results have further validated the Recurrence Score assay. The Trial Assigning Individualized Options for Treatment (TAILORx) phase III study[9] examined the non-inferiority of endocrine treatment alone vs endocrine therapy plus chemotherapy in node-negative hormone receptor (HR) positive patients with Recurrence Score results of 11-25. All patients with scores >25 received endocrine therapy plus chemotherapy and those with scores <11 received only endocrine therapy. Findings from the nonrandomised arm with patients with results <11 showed that these patients have excellent clinical outcomes (rate of freedom from distant recurrence at 5 years: 99.3%; overall survival at 5 years, 98.0%).

The TAILORx findings are consistent with results from the endocrine therapy-only treated patients (node-positive/high-risk node-negative) with Recurrence Score results ≤11 in the West German Study Group (WSG) PlanB study[10]. In addition, 2 large cohort studies (the Clalit study and the Surveillance, Epidemiology, and End Results [SEER]-based analysis) confirmed and extended the results of TAILORx and WSG PlanB by demonstrating excellent clinical outcomes in node-negative low (<18) Recurrence Score patients[11,12].

- **Prediction of Adjuvant Chemotherapy benefit**

The predictive ability of Oncotype DX in ER+ early BC patients was retrospectively tested using archival samples from the prospective, randomised NSABP B-20 (node-negative patients) and SWOG 8814 (node-positive patients) studies[7,13]. In both studies, high Recurrence Score patients derived a large benefit from chemotherapy (NSABP B20: 10-year distant recurrence-free [DRF] rates of 88% vs 61%; SWOG 8814: 10-year disease-free survival [DFS] rates of 55% vs 43%); low Recurrence Score patients derived minimal, if any, benefit (NSABP B20: 10-year DRF rates of 96% vs 97%; SWOG 8814: 10-year DFS rates of 64% vs 60%); and the statistical test for interaction between the Recurrence Score result and chemotherapy treatment was significant. Also, in both studies, intermediate Recurrence Score patients did not derive a significant benefit from chemotherapy; although, a clinically relevant effect could not be ruled out.

The question of whether Node-negative, early BC patients with an intermediate Recurrence Score (RS: 11-25) benefit from adjuvant chemotherapy has being addressed in the prospective, randomised TAILORx trial[9]. The results of this study established the non-inferiority of withholding CT from adjuvant treatment in the study population of HR+, Node-negative, HER2-negative patients with RS 11-25. However, there was a statistically significant interaction observed between age and CT benefit assessed by invasive disease free survival - IDFS (*p* 0.003) and recurrence free interval - RFI (*p* 0.02). An exploratory analysis of TAILORx patients ≤50 years of age, which also incorporated clinical risk stratification (based on tumour size and histologic grade), showed a potential benefit, particularly for patients with RS 16-20 and high clinical risk as well as for patients with RS 21-25 irrespective of clinical risk. Patients ≤50 years of age with RS ≤15 as well as patients >50 years with RS ≤25 derived no benefit from the addition of chemotherapy to endocrine treatment.

The prospective, randomised RxPONDER trial addressed the question of whether N1 (1-3 positive nodes), early BC patients with a Recurrence Score ≤25 benefit from adjuvant chemotherapy[14]. The results from the RxPONDER study showed that the 21-gene RS 0-25 was prognostic but did not show a treatment interaction with chemotherapy; the relative benefit of chemotherapy was similar across RS 0-25. However, analysis according to menopausal status of the patients showed that postmenopausal women with RS 0-25 did not benefit from adjuvant chemotherapy in any subgroup, whilst premenopausal women with RS 0-25 had benefit from the addition of chemotherapy to endocrine therapy. Specifically, a benefit of 46% decrease in IDFS events was observed across premenopausal subgroups and a 53% decrease in deaths, leading to a 5-year OS absolute improvement of 1.3%. The findings led to the conclusion that postmenopausal patients with 1-3 positive nodes and RS 0-25 can safely forego adjuvant chemotherapy without compromising IDFS but, premenopausal patients with positive nodes and RS 0-25 likely benefit significantly from chemotherapy, although it is unclear if the benefit is due to the ovarian suppressor effects promoted by chemotherapy.

Taking together the results of these two large prospective, randomised clinical trials - TAILORx and RxPONDER – can lead to a more precise individualisation of treatment and a significant de-escalation of chemotherapy use for the majority of patients with early breast cancer (Table 3).

Table 3. Prediction of Chemotherapy benefit by OncotypeDX Recurrence Score based on TAILORx and RxPONDER trial results

N0	**RS[1] >50 years old**	**RS ≤50 years old**
	0-25 No CT[2] benefit 26-100 Increasing CT benefit[3]	0-15 No CT benefit 16-25 Increasing CT benefit (1.6% – 6.5%) 26-100 Increasing CT benefit
N1+	**Post-menopausal**	**Pre-menopausal**
	0-25 No CT benefit 26-100 Increasing CT benefit	0-25 2.4% CT benefit[4] 26-100 Increasing CT benefit

[1] Recurrence Score; [2] CT, Chemotherapy; [3] based on SWOG 8814 study (Albain *et al*, Lancet Oncol 2010); [4] on Distance Recurrence Free Interval – DRFI (Kalinsky SABCS 2021)
[+] N1, 1-3 positive lymph nodes

MammaPrint

MammaPrint is a 70-gene expression profile signature developed using a cohort of 78 patients >55 years with ER+, HER2-negative or positive, as well as triple negative early BC who had surgery, no systemic therapy, and long-term follow up[15]. MammaPrint, which is performed by a central laboratory (in the Netherlands and US), is microarray-based and assesses the expression of genes that regulate cell cycle, invasion, metastasis and angiogenesis to classify patients as having either good or poor prognosis (Table 1 and 2).

- **Prognosis**

MammaPrint was first validated with fresh frozen tissue samples from the tumour bank of the Netherlands Cancer Institute[16]. Several additional validation studies (using various cohorts) followed (i.e., TRANSBIG Consortium, which was an independent validation study, the MicroarRAy PrognoSTics in Breast CancER study [RASTER], and hospital tissue banks including the Massachusetts General Hospital)[17-19]. Subsequently, the assay has also been optimized and analytically validated for

FFPE, and equivalence to the assay on fresh frozen tissue was demonstrated[20].

MINDACT (the Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy), a large prospective, randomized, phase III clinical trial, included 6,693 patients from 2007 to 2011 in Europe with ER+/ER-, HER2+/HER2- or triple negative tumours and investigated whether patients classified as low-risk by MammaPrint can be spared chemotherapy[21]. Only those whose MammaPrint risk assessment was discordant with their Adjuvant!Online (a tool that assesses risk based on clinical factors)-based risk assessment were randomised to receive or not chemotherapy.

The first analysis of MINDACT results showed that the trial met its primary endpoint, showing that the 644 clinical-high/genomic-low patients who received hormonal therapy alone had a 5-year survival rate without metastases of >92% (94.7%, CI 92.5-96.2), confirming the utility of the genomic assay to stratify risk. Patients with high risk according to Adjuvant!Online and low risk according to MammaPrint have excellent clinical outcomes without adjuvant chemotherapy, thus further validating (level 1A evidence) MammaPrint as a prognosticator.

MammaPrint was also evaluated in samples from a pooled study series (not a randomised trial) where tissue samples were not re-analysed (RASTER study[19]). The study showed that in the MammaPrint high-risk group, breast cancer-specific survival and distant disease-free survival were significantly longer for patients treated with endocrine therapy plus chemotherapy vs those treated with endocrine therapy alone, whereas for the low-risk group, this difference was not statistically significant. Nonetheless, the interaction test for survival was non-significant, suggesting that the assay is not predictive.

- **Prediction of Adjuvant Chemotherapy benefit**

The primary objective of MINDACT trial[21] was to assess the prognostic utility of the 70-gene signature (MammaPrint) and validate prospectively MammaPrint as a prognosticator. MINDACT also evaluated N0 and N1 (1-3 positive LNs) patients with discordant clinical risk and genomic risk with a randomization to CT versus no CT (intention-to-treat population); however, this endpoint was secondary, and the trial was not specifically sized to ensure adequate statistical power for conclusive analysis. In a recent exploratory analysis by age of the MINDACT results on 8-year distant metastasis-free survival, clinical-high risk patients ≤50 years of age with genomic-low risk had a clear chemotherapy benefit of an absolute difference of 5% (±2.8) whilst clinical-high/genomic-low patients >50 years of age had a difference of only 0.2% (±2.1)[22]. However, comparisons between Chemotherapy and no-Chemotherapy groups are again low powered and therefore, any treatment selection must be based solely on the prognostic value of the assay.

Prosigna (PAM50) and EndoPredict

The Prosigna assay, performed on RNA extracted from FFPE tissue, is based on PAM50 (a 50-gene set originally developed to classify intrinsic BC subtypes[1]), and uses a hybridization technique to assess 58 genes (50 cancer-related, 8 reference genes). The assay can be performed locally (using the Nanostring nCounter DX Analysis System) and provides a BC intrinsic subtype based on the similarity of gene expression to prototypical expression, and risk of recurrence (ROR) score (Prosigna score; range: 0-100), which is calculated based on a subset of 54 genes (46 cancer-related, 8 reference genes), a proliferation score and tumour size[23]. The ROR score is used to classify node-negative patients into low-, intermediate- and high-risk groups, and node-positive (1-3 positive nodes) patients into low- and high-risk groups (Table 1 and 2).

EndoPredict is an 11-gene (8 cancer-related, 3 reference genes) qRT-PCR-based assay performed on RNA extracted from FFPE tissue[24]. The assay, which can be performed by local laboratories, calculates a continuous risk score, EP (range: 0-15). When combined with nodal status and tumour size, an EP clinical score (EPclin) is calculated. The EP and EPclin scores are used to classify postmenopausal ER+ HER2-negative early BC patients who are treated with endocrine therapy into low- and high-risk groups (Table 1 and 2).

- **Prognosis**

The Prosigna assay was first validated as a prognosticator using samples from ER+ endocrine therapy-treated postmenopausal patients with node-negative/node-positive early BC in the Austrian Breast and Colorectal Cancer Study Group (ABCSG)-8 trial[25]. Several additional validation studies included samples from transATAC, transATAC plus ABCSG-8, and the NCIC CTG MA2.1 trial[26,27]. The ROR score was shown to predict early (years 0-5) and late (years 5-10) recurrence in a dataset from the transATAC trial

(including node-negative and node-positive patients)[28].

EndoPredict was validated independently using archived samples from 2 randomised trials (ABCSG-6 and -8)[29]. Additional analyses on a cohort including patients from these 2 randomised trials who were treated with endocrine therapy for only 5 years demonstrated that EP and EPclin were predictive of both early (first 5 years) and late (years 5 to 10) risk of recurrence.

- **Prediction of Adjuvant Chemotherapy benefit**

PAM-50 (Prosigna) and EndoPredict assays are currently not supported by entirely prospective randomized trials evaluating their predictive value of chemotherapy benefit (Table 1).

Discussion

MGAs development and their application in breast cancer have transformed the landscape in BC treatment by suggesting that the clinical risk determined by traditional clinico-pathological parameters may be inadequate and by providing independent information that reflects the tumour's underlying biology. In this respect, they can provide additional prognostic and predictive information in order to guide treatment decisions, minimize over- and under-treatment and offer chemotherapy only to patients who are likely to benefit from it, while sparing other patients unnecessary treatment. Reducing overtreatment in particular in node-negative, HR-positive, HER2-negative BC patients is of great importance, as this population is generally of favourable prognosis and constitutes nearly half of all newly diagnosed BC patients.

However, MGAs are not all the same; they differ in the technology used for their development, the number and specific genes assessed, as well as the patient populations in which they were validated (Table 1). OncotypeDX was validated in HR+ HER2-negative patients of all ages, MammaPrint in HR+/- and HER2-positive or negative patients, whilst PAM50 and EndoPredict in HR+ HER2-negative post-menopausal only patients.

This led to an interest in comparing risk classifications on the same tumour samples using different MGAs. Interestingly, the assays utilized in these studies often produce discordant results in the same tumour specimen, as each measures different components of tumour biology. Several such studies have been published, comparing Oncotype DX classification to those by MammaPrint, Prosigna and Endopredict[30-33] and they were all consistent in showing differences in risk classification between the assays. The review by Varga et al.[34] examined studies in which tumours were classified by OncotypeDX and other MGAs, including MammaPrint and in the 4 studies where the 21-gene assay and 70-gene signature were directly compared, the overall discordance in risk classification was 44-58%. Furthermore, it was demonstrated that the 21-gene assay classifies only 12% of tumours as high risk (RS 31-100) vs 46% by the 70-gene signature. Overall, Oncotype DX classified less patients as high risk compared to the other evaluated assays. It is also noteworthy that, a wide range of Recurrence Score results was observed in each of the risk classifications, as determined by the comparator MGA. Thus, these direct comparisons demonstrate that MGAs do not provide interchangeable information.

Most importantly, not all MGAs provide data from prospective, randomised trials confirming the clinical value of their use in predicting chemotherapy benefit in certain groups of breast cancer patients. Notably, the ability of an assay to predict adjuvant chemotherapy benefit should only be determined preferably in appropriately designed prospective trials or in archived samples from clinically relevant prospective randomised trials by using a statistical test for the interaction between chemotherapy treatment and risk group classification. At present, only for the OncotypeDX Recurrence Score assay there is a strong body of evidence from two large, prospective randomised trials (TAILORx for N0 patients and RxPONDER for N1 patients) supporting its ability to predict adjuvant chemotherapy and for MammaPrint (MINDACT with N0 and N1 patients) to a lesser extent[35]. In the absence of such data, any treatment selection based solely on the prognostic value of an assay leaves uncertainty about adjuvant Chemotherapy use in the management of an individual patient with early BC and mixed – low, Intermediate and high – clinicopathologic parameters.

The above has led to the inclusion of MGAs in international BC treatment guidelines with different indications in regards to their clinical application in treatment decisions. At present, only Oncotype DX and MammaPrint are included in all major international guidelines, whereas the other MGAs are included in some of them. ESMO-2019 guidelines for Breast Cancer Management[36] endorsed that both, Oncotype DX and MammaPrint assays may be used to gain additional prognostic and/or predictive information with level 1A

evidence to complement pathology assessment and to predict the benefit of adjuvant chemotherapy, whilst Prosigna and Endopredict were endorsed as assays that may be used only to gain additional prognostic information with 1B evidence to complement pathology assessment. The St. Gallen 2021 consensus referred only to the prospective randomized studies (MINDACT, TAILORx, and RxPONDER) and to MammaPrint and the new Oncotype DX cut-offs and there was a majority endorsement for N0 and N1, HR+, HER2- early stage breast cancer patients, irrespective of grade and menopausal status[37]. The results of MINTACT, TAILORx and RxPONDER trials were included in the last 2022-update of ASCO[38] and NCCN Breast Cancer treatment guidelines[39] (Table 4).

Table 4. Inclusion of MGAs in major International Guidelines for Breast Cancer Treatment

Assay	ASCO (April 2022)	NCCN (version 4.2022)	ESMO-2019	St. Gallen 2021
OncotypeDX	Sufficient evidence to support clinical utility in all patients groups but premenopausal N1 patients[a]. - *Evidence quality*: high - *Recommendation*: strong	Included as the best-validated / preferred prognostic and predictive assay.	May be used: • to gain additional prognostic and/or predictive information with level 1A evidence *and* • to predict the benefit of adjuvant chemotherapy	• Referred only to the prospective randomized studies (MINDACT, TAILORx, RxPONDER) and to MammaPrint and the new Oncotype DX cut-offs • A majority endorsement for N0 and N1, HR+, HER2- early-stage breast cancer patients, irrespective of grade and menopausal status
MammaPrint[b]	Should only be used in >50 years, N0-N1[a] and high clinical risk *patients*. - *Evidence quality*: intermediate - *Recommendation*: strong	Acknowledged as a clinically-validated prognosticator, *but* the ability to predict chemotherapy benefit is unknown.		
Prosigna	Should only be used in postmenopausal, N0 patients. - *Evidence quality*: intermediate - Recommendation: moderate	Acknowledged as a clinically-validated prognosticator, *but* the ability to predict chemotherapy benefit is unknown.	May be used only to gain additional prognostic information with level 1B evidence	
EndoPredict	Should only be used in postmenopausal, N0- N1 patients. - *Evidence quality*: intermediate - *Recommendation*: moderate			

[a] N1, 1-3 positive lymph nodes; the assay is prognostic and may be used for shared patient-physician treatment decision making.

[b] Received US FDA clearance. Note that MammaPrint received FDA clearance for the assay using fresh frozen samples and more recently on the assay that uses FFPE sample.

Abbreviations: ASCO, American Society of Clinical Oncology; FDA, Food and Drug Administration; FFPE, formalin-fixed, paraffin-embedded; NCCN, National Comprehensive Cancer Network.

Finally, MGAs not only present an opportunity to offer chemotherapy only to those BC patients who are likely to benefit from it but, by sparing chemotherapy and its associated costs - both direct and indirect: cost of treatment itself and cost of managing treatment-related toxicity - from all other patients, they have the potential for cost effectiveness/cost saving. Health economics (HE) studies were conducted for some of the available MGAs, particularly for those that have been commercially available for the longest period (Oncotype DX and MammaPrint). For Oncotype DX, multiple studies demonstrated cost effectiveness/cost saving and for MammaPrint, such studies suggested cost effectiveness in the evaluated countries[40-43]. A HE study showed cost saving from the perspective of the German healthcare system for EndoPredict test[44] as well. In a recently published model-based cost-effectiveness study based on the results of the RxPONDER trial, it was shown that OncotypeDX test is highly likely to be cost-effective in node-positive early breast cancer as well[45].

Conclusion

MGAs developments and their application in BC have transformed the landscape in BC treatment. Next to clinical burden and tumour biology (ER and

PR status, HER2 expression, Ki67, and tumour Grade), MGAs may help in decision making and significantly de-escalate chemotherapy recommendations minimizing over- and under-treatment. Omitting chemotherapy and avoiding over-treatment can save a large number of patients from unnecessary side effects and decrease the cost of breast cancer treatment to National Health systems. However, MGAs are not all the same, they do not give interchangeable information and most importantly, they do not provide the same amount of data supporting their clinical value of their use in guiding treatment decisions. Clinicians should be aware of these significant differences and use the right MGA for each patient / case scenario. Undoubtedly, the development of complex molecular tools will continue in the upcoming years, eventually enabling high-level individualised treatment for all BC patients.

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