Introducing new molecular technologies into routine clinical cancer care: is there an impact on the treatment of breast cancer?

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Introduction

In the past decade, high-throughput technologies (HTTs) were refined to achieve unprecedented levels of dependability, speed and number of assays, and they are changing the process of scientific discovery greatly. The resulting 'omics' sciences are affecting the field of oncology well beyond the simple quantitative aspect and are contributing to the effective development of new drugs and useful biomarkers. Indeed the application of HTTs offers the opportunity for extensively improving current knowledge of the molecular basis of clinical and biological heterogeneity, thus leading to the possibility of tuning the definition of prognosis and prescription to the characteristics of individual patients. It is reasonably expected that a combination of several molecular biomarkers selected from thousands of candidate biomarkers could describe and predict significant clinical behaviours and phenotypes better than a single or few markers. The impact of these new tools on daily clinical practice in breast cancer treatment is becoming relevant. The scope of the present work is to describe results and challenges of the current wave of 'omics' applications.

'Omics' sciences and their tools

Genomics studies the complete collection of the genes that the genome contains. It is widely accepted that human cancer is a genetic disease, caused by sequential accumulation of mutations, deletions and amplifications either of oncogenes or of tumour suppressor genes. Several technologies with partially overlapping aims have been used in genomics. Methods for high-throughput identification of tumour-specific (somatic) mutations in breast cancers (mutational analyses) identify candidate genes in a rapid and scalable fashion [1]. Careful functional studies of mutated genes are required for ultimate proof of cancer gene status and translation into practical useful information. Microarray-based comparative genomic hybridization (CGH) is a molecular–cytogenetic method capable of detecting loss, gain and amplification of the gene copy number. The method was used, for example, to study genomic changes after neo-adjuvant chemotherapy [2]. Single nucleotide polymorphism (SNP) array is a type of DNA microarray used to detect variations at a single site in DNA with a frequency of >1% within a population. These polymorphisms significantly affect drug toxicity and efficacy (pharmacogenetics) in a population [3,4]. Hybridization to SNP arrays is an efficient method to detect genome-wide cancer loss of heterozygosity (LOH), the form of allelic imbalance that can result from the complete loss of an allele or from an increase in copy number of one allele relative to the other. While CGH arrays can detect only genomic gains or deletions, SNP array has the additional advantage of also detecting copy number. In addition, high-density SNP array (to 10 000 SNPs) permits detection of smaller regions of LOH [5].

Epigenomics is the study of heritable changes other than those in the DNA sequence and encompasses two major modifications of DNA or chromatin: DNA methylation and post-translational modification of histones. These 'epigenetic changes' induce patterns of altered gene expression. A unique DNA methylation 'fingerprint' has been described in various normal biological processes and diseases, in particular cancer [6]. The emergence of highly reproducible quantitative high-throughput microarray technology is permitting epigenomics research to be read on microarray platforms [7].

Transcriptomics attempt to analyse whole gene expression profiling. There is a wide range of available techniques. DNA microarray platforms differ in terms of material used (short oligonucleotides, long oligonucleotides or cDNA) and number of samples per array (single-channel or two-channel). Multiplexed quantitative real-time PCR (qRT–PCR) is based on the quantification of a fluorescent reporter molecule generated using PCR, which reflects the abundance of the mRNA target. The dynamic range of real-time PCR to measure gene expression is broader than the dynamic range of DNA arrays. On the other hand, current qRT–PCR systems can measure up to a few hundred genes simultaneously, which is substantially less than the comprehensive profiling that DNA microarrays can provide.

MicroRNAs (miRNAs) are an abundant class of small non-protein-coding RNAs that function as negative gene regulators. Their alterations are involved in the initiation and progression of human cancer [8]. miRNA expression profiling of human tumours either by oligonucleotide miRNA microarray or by RT–PCR analysis has identified signatures
associated with staging, prognosis and response to treatment [9,10].

Proteomics examines how, when and where proteins are expressed; it includes two-dimensional gel electrophoresis, mass spectrometry and protein microarrays. Mass spectrometry is typically used for comprehensive proteomic surveys [11]. At present the approach may lead to artefactual results, but some proof-of-principle studies have also been performed in breast cancer [12].

The brief list of HTT methods that is far from complete illustrates the range of new possibilities for profiling the biology of cancer in individual cases. Indeed the new technologies have widened the boundaries of molecular biomarker discovery and represent a new opportunity with great potential use in the clinic. However, the number of markers that have emerged as clinically useful is so far small. The reasons for this low yield of clinically successful development have been widely discussed for single molecular markers, but the same reasoning also fully applies in the case of new technologies [13]. Poor study design, inadequate sample size, lack of standardization of the assays and inappropriate or misleading statistical analyses are commonplace [14]. Reproducibility and wide applicability are additional problems that limit the success of predictive or prognostic new markers in the era of ‘omics’ science. However, some findings from transcriptomic research are ready for widespread application, and they will be discussed in more detail after introducing the concept of clinical utility.

clinical utility of biomarkers

A biomarker should assist a specific and relevant therapeutic decision. To develop a biomarker it is therefore necessary to define which clinical phenotypes are associated with a useful prediction. One important aspect is the appropriate tag of different classifiers as prognostic or predictive. Prognostic classifiers aim at the identification of patients who were cured by local treatment and sometimes they refer to a subset of patients selected on the basis of clinical presentation (e.g. node negative) [15] or specific biological subtype [e.g. estrogen receptor (ER) status of the tumour] [16]. Predictive classifiers aim to identify patients who do or do not benefit from a specific treatment [17–19]. Finally, context-specific predictors aim at predicting outcome in a well-defined set of patients who receive a well-defined therapy (‘context’) without taking into account the distinction between prognostic and predictive contribution [20].

All the above classifiers must be therapeutically relevant. The relevance for therapeutic decisions and associated clinical utility must be formally proved by demonstrating that a patient’s benefit improved as a result of using the classifier. Examples of clinical utility and patient’s benefit vary from better tumour control, improved therapeutic ratio because fewer adverse events, or even avoidance of ineffective drugs. An independent clinical validation of the utility of new classifiers in a defined clinical context is a requirement before implementing the new classifier for therapeutic decision [21]. In addition to clinical benefit, a new classifier should also provide novel information that is independent of that already available from established classifiers. The clinical utility will depend greatly on the ability of the new classifier to predict more accurately than other prognostic and predictive factors or to add predictive accuracy to that provided by standard prognostic factors [22].

Finally, the new biomarker should be easily accessible for the broadest clinical application. The source of material for performing the assay of a new biomarker has a number of implications for its development. A biomarker that can be studied only in fresh-frozen tissue suffers the limitation of the logistic procedures for its collection and analysis. In the case of classifiers obtained from formalin-fixed and paraffin-embedded (FFPE) material the advantage is 2-fold: the initial development of the FFPE-derived classifier is facilitated by the availability of archival material associated with a clinical database and a clinical experiment that is already completed at the time of analysis. The second advantage is the immediate and wide applicability of the marker. Indeed, formalin fixation and paraffin embedding are standard procedures in any pathology laboratory, so that procurement of source material for the classifier is formidably facilitated. A notable example of the advantage of working on FFPE material is the rapid development of the recurrence score (RS) assay (Oncotype DX™) [20].

Finally, the clinical utility of a classifier is closely linked to the ease of reproducing it with standardized accuracy. This potential pitfall is commonly addressed by conducting quality controls and appropriate tests of inter- and intra-laboratory reproducibility [22]. More recently the approach for complex and proprietary assays such as the Oncotype DX™ or MammaPrint was that of having the assay performed in a central commercial laboratory. This approach provides improved quality and reproducibility, but it also results in increased costs.

gene expression profiling and breast cancer

potential impact on treatment decision making in breast cancer

The decision to prescribe adjuvant systemic therapy to patients with early breast cancer is based on the estimate of the risks of relapse and death and on the expected benefit from treatment. NCI consensus recommendations [23] and NCCN practice guidelines (at least before the last version V1.2008) called for considering adjuvant chemotherapy for most node-negative breast cancers >1 cm regardless of ER status. These guidelines consider prognosis per se as the most relevant factor for chemotherapy administration. In light of the continuum nature of the risk of relapse, an informatic tool (AdjuvantOnline, http://www.adjuvantonline.com/) based on standard prognostic factors was developed [24]. In a completely different approach the St Gallen Expert Consensus recommendation [25] suggested that the clinically relevant aspect is the risk, as well as the degree, of endocrine responsiveness that according to the experts is inversely related to the likelihood of benefit from chemotherapy.

Both approaches are useful tools, but the first approach is limited by the assumption that the relative benefit from chemotherapy is the same for all patients who receive the same
treatment, ignoring the fact that chemosensitivity may be different for breast cancer subtypes. The second approach overcomes this limit, but the definition of endocrine responsiveness is not robust enough, reliable and reproducible. In addition, both approaches imply the overtreatment of a high number of patients. Dependable predictive biomarkers for individual decision making are warranted in clinical practice. Some of the most successful or relevant examples of gene expression profiling for clinical application are described in detail in Table 1.

70-gene signature (Amsterdam signature, MammaPrint®)

Using an oligonucleotide-based Agilent platform in a series of 78 frozen tumours belonging to untreated node-negative breast cancer patients younger than 55 years, The Netherlands Cancer Institute in Amsterdam and Rosetta Inc. identified a 70-gene signature to predict the 5-years distant metastasis-free survival [26]. The clinical purpose was to identify a low-risk group of patients otherwise candidates to receive chemotherapy who could be safely spared administration of cytotoxics. The signature was initially validated on a larger data set of 295 patients that included patients with node-negative and -positive disease and patients younger than 53 years undergoing or not undergoing systemic therapy. Moreover, 61 patients used as the training set were included in that validation set [15]. These limitations prompted a collaborative validation study conducted by the TRANSBIG consortium in 302 untreated women, younger than 61 years, with node-negative disease [27]. The estimated 10-year survival for the good profile group supported a higher discrimination of prognosis of the gene signature over that of Adjuvant!Online [28]. However, the hazard ratios in the first validation study [15] were much higher than those in this validation series [27]. Furthermore, the signature did not seem informative in ER-negative tumors. In light of the partial discordance in the prognostic strength between the first and the second validation study and the retrospective nature of the investigations, the clinical utility of this signature above and beyond the use of standard clinico-pathological prognostic variables is still uncertain, as also concluded by recent ASCO guidelines [29]. Another potential limitation of this signature in clinical practice is the requirement for snap-freezing the samples in liquid nitrogen within 1 h of surgery, and it is unclear whether the method is applicable to diagnostic core biopsies. A large prospective randomized clinical trial (Microarray In Node-negative Disease may Avoid Chemotherapy Trial) is currently ongoing to evaluate the benefit-to-risk ratio of chemotherapy when the risk assessment based on clinicopathological factors (AdjuvantOnline) differs from that provided by the gene signature (MINDACT-EORTC Trial 10041 (BIG 3-04) http://www.eortc.be/services/units/mindact/).

76-gene signature (Rotterdam signature)

A 76-gene signature to predict distant metastases at 5 years irrespective of age and tumour size in patients with node-negative and previously untreated breast cancer was developed by the Erasmus Medical Center (Rotterdam) and Veridex [16] using an oligonucleotide-based Affymetrix chip and frozen specimens. A notable feature of the experimental design was that the training set to develop the signature considered separately ER-positive and ER-negative tumours. In so doing, the Rotterdam investigators defined a 60-gene prognostic signature for ER-positive tumours and a 16-gene prognostic signature for ER-negative tumours. The two signatures had no common genes. The rationale for this innovative approach was that mechanisms of disease progression could be different for the two subgroups of breast cancer that have well known different clinical course and different patterns of gene expression [30].

A multicentre retrospective validation study was performed in 180 cases of node-negative untreated breast cancer [31]. The 10-year distant metastasis-free survival was 94% for the good profile group. Unfortunately, the analysis according to ER status could not be performed because the ER-negative group was too small. The TRANSBIG consortium decided to perform an independent validation of the signature in the same patients in whom they had validated the 70-gene signature [32]. The 10-year times to distant metastasis were 94% and 73% for the good and poor risk group, respectively. The signature’s performance was a better predictor of earlier than later relapse. However, the adjusted hazard ratios of 13.58 at 5 years and 5.11 at 10 years for time to distant metastasis confirmed this signature as a strong and robust prognostic factor. Of note and very importantly, 25% of ER-negative tumours were classified for the good profile group. Unfortunately, the analysis according to ER status could not be performed because the ER-negative group was too small. The TRANSBIG consortium decided to perform an independent validation of the signature in the same patients in whom they had validated the 70-gene signature [32]. The 10-year times to distant metastasis were 94% and 73% for the good and poor risk group, respectively. The signature’s performance was a better predictor of earlier than later relapse.

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Table 1. Gene expression signatures related to prognostication

<table>
<thead>
<tr>
<th>Gene expression signatures</th>
<th>Microarray platform</th>
<th>Tumour specimens</th>
<th>Number of genes in the signature</th>
<th>Independent validation</th>
<th>Guidelines recommendation</th>
<th>Prospective clinical validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MammaPrint</td>
<td>Agilent (oligonucleotides)</td>
<td>Frozen tissue (surgery)</td>
<td>70-gene</td>
<td>Yes</td>
<td>No</td>
<td>Ongoing (MINDACT)</td>
</tr>
<tr>
<td>Rotterdam signature</td>
<td>Affymetrix (oligonucleotides)</td>
<td>Frozen tissue (surgery)</td>
<td>76-gene [60-gene (ER pos)+16-gene (ER neg)]</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Oncotype DX</td>
<td>qRT–PCR</td>
<td>FFPE (surgery and core biopsy)</td>
<td>21-gene (16 cancer-related; 5 reference)</td>
<td>Yes</td>
<td>Yes (ASCO, NCCN)</td>
<td>Ongoing (TAILORx)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; FFPE, formalin-fixed paraffin-embedded; qRT–PCR, quantitative reverse transcriptase-polymerase chain reaction.
**recurrence score (Oncotype DX™)**

Paraffin-embedded tissue (PETT) from women enrolled in three different studies was the source material for conducting an RT–PCR gene expression analysis and establishing a 21-gene assay (16 cancer-related, five reference genes) and a score algorithm called the RS (Oncotype DX™; Genomic Health Inc.) [20]. The RS was validated in 668 patients with ER-positive and node-negative breast cancer who were treated with tamoxifen in the National Surgical Adjuvant Breast and Bowel (NSABP) trial B-14 [20]. The validation is nominally retrospective but makes use of a prospectively collected clinical database from a controlled trial that ASCO guidelines have considered level of evidence I [29]. In the collected clinical database from a controlled trial that ASCO Breast and Bowel (NSABP) trial B-14 [20]. The validation is significantly retrospective, but makes use of a prospectively collected clinical database from a controlled trial that ASCO guidelines have considered level of evidence I [29]. In the low-risk group with RS <18, the rate of distant recurrence and death at 10 years was 6.8% and 3.1%, respectively. In a multivariate Cox model, the RS was a classifier independent of age and tumour size, and more accurate than Adjuvant!Online. Similar results were described in a large population-based external validation study [33]. Remarkably, a prognostic predictor for ER-positive and tamoxifen-treated patients can be more useful than a prognostic predictor in ER-positive untreated patients given the undisputed utility and widespread use of tamoxifen [34]. On clinical grounds it is more useful to identify those patients who are likely to be cured by tamoxifen monotherapy to spare them the additional useless toxicity of chemotherapy.

The ASCO guidelines for the use of tumour markers in breast cancer [29] and the last version of the NCCN guidelines (2008) have considered the above evidence solid enough to suggest that Oncotype DX™ (Genomic Health Inc.) can be used to predict the risk of recurrence in patients treated with tamoxifen and identify those women who may not require adjuvant chemotherapy in newly diagnosed patients with node-negative and ER-positive breast cancer. The NCCN indication only applies to HER2-negative cases.

The performance of the RS was also tested in the placebo arm of the randomized trial B-14 used for the validation [35]. The RS was also prognostic in untreated patients, and the comparison with the treated arm of the study revealed that cases with low and intermediate RS (RS <31) but not those with RS ≥31 derived substantial benefit from tamoxifen. Prediction of prognosis in untreated patients was confirmed in another study on ER-positive, node-negative tumours [33] but not in a study where both ER-positive and ER-negative tumours were included [36]. More recently, evidence indicated that RS predicts long-term outcome in women with ER-positive and node-negative breast cancer who received tamoxifen [37]. The RS added prognostic information over that provided by classical variables and Adjuvant!Online. Finally, the RS it also capable of predicting survival after first metastatic relapse [38].

In a very relevant series of studies, the RS was also associated with benefit from chemotherapy. It predicted the likelihood of pathological complete response [17] and clinical complete response [39] after neo-adjuvant chemotherapy. It also predicted benefit from adjuvant treatment in ER-positive, tamoxifen-treated, node-negative [40] and node-negative disease [37,41]. The predictive value in spite of the heterogeneity of the chemotherapy regimens [docetaxel, paclitaxel-docetaxel, CAF and (C)MF] suggests that low RS tumours show a common pattern of drug insensitivity to several cytotoxic agents and should not be treated with such drugs.

A large prospective trial (Trial Assigning Individualized Options for Treatment, TAILORx; http://www.cancer.gov/clinicaltrials/digestpage/TAILORx) as part of the US National Cancer Institute (NCI) Program for the Assessment of Clinical Cancer Tests (PACCT) is underway to confirm the clinical utility of Oncotype DX™.

**‘bottom-up approach’: selecting genes according to a specific biological hypothesis**

Several researchers have developed prognostic and predictive signatures based on mechanistic and biological hypotheses instead of selecting genes directly through their association with outcome. This in part was also the case for the development of the RS.

The Stanford group hypothesized that features of the molecular programme of normal wound healing might also be associated with cancer progression. They identified a ‘wound-response signature’ from the transcriptional response of normal fibroblasts to serum [42] and tested the signature in 295 women with early breast cancer. Those patients whose tumours expressed the wound response signature had a markedly worse clinical outcome [43]. The wound signature also improved the risk stratification over that of other prognostic signatures (‘molecular subtypes’ and ‘70-gene signature’).

A different hypothesis-driven approach focused on the identification of gene expression patterns associated with histological grade. Although high- and low-grade tumours have a clearly different clinical course, the majority of tumours are categorized as intermediate grade. A gene expression grade index (GGI) based on 97 unique genes mostly related to proliferation was developed, and its application revealed that intermediate grade tumours have expression patterns and clinical outcome that closely match those of low- or high-grade cases [44]. In 650 untreated or tamoxifen-only-treated ER-positive breast cancer patients, GGI appeared to be the strongest predictor of clinical outcome, highlighting the prognostic role of proliferation genes in ER-positive tumours [45].

Another very interesting approach was based on the concept that tumour progression and metastasis depend on cancer stem cells. By comparing the gene expression profiles of normal mammary tissue and a highly tumorigenic breast cancer subpopulation (CD44+/CD24–) from six patients, a 186-gene invasiveness gene signature (IGS) was developed [46]. This IGS was significantly associated with overall and metastasis-free survival in breast cancer and other tumour types. IGS was not dependable for stratifying tumours with poor or good differentiation and ER-negative status. Moreover, the 10-year metastasis-free survival of patients with a good IGS was 81%, a value too low to be useful for clinical application. However, the IGS was more strongly associated with outcomes when combined with the wound response
signature. The latter observation confirmed that different signatures could be combined to provide a more robust and accurate classification for use in clinical practice [47]. Despite these attractive results, some statistical weaknesses of this approach suggest the need for additional validation [48].

gene expression profiling to predict response to neo-adjuvant chemotherapy

The response of the primary tumour to neo-adjuvant therapy is a surrogate marker of chemosensitivity in occult distant metastatic sites [49]. This view is supported by the favourable long-term outcome of patients who achieve an eradication of invasive breast cancer (pathological complete response, pCR) [50,51].

Several studies addressed the ability of gene expression profiles to predict response to primary chemotherapy reliably (Table 2). None of the reported gene signatures is robust enough to be clinically useful on its own. Furthermore, many of these signatures lack appropriate validation [22]. Other common limitations are the small number of patients included in the studies, the discordant and questionable clinical phenotype to be predicted and significant statistical faults [14]. However, these studies show that gene expression profiles may predict response to chemotherapy.

Another possibility proposed by the M. D. Anderson Group is that of using different predictors of pCR after different types of treatment and apply them so as to offer the highest likelihood of tumour eradication to the largest number of cases.

Table 2. Gene expression profiling for predicting response to neoadjuvant chemotherapy

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients$^1$</th>
<th>Stage of disease</th>
<th>Drugs</th>
<th>Material</th>
<th>Arrays platform</th>
<th>Purpose of Prediction</th>
<th>Signature (finding)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang JC (2003) [52]</td>
<td>24</td>
<td>LABC</td>
<td>Docetaxel × 4</td>
<td>Frozen</td>
<td>cDNA</td>
<td>Responsive Tumour (25% or less residual disease)</td>
<td>92-gene signature</td>
</tr>
<tr>
<td>Ayers M (2004) [54]</td>
<td>42</td>
<td>I – III</td>
<td>wT x12 → FAC x4 AC x 4</td>
<td>Frozen</td>
<td>cDNA</td>
<td>pCR (breast)</td>
<td>74-gene model</td>
</tr>
<tr>
<td>Folgueira MA (2005) [55]</td>
<td>51</td>
<td>II or III</td>
<td>(or to PD for LABC)</td>
<td>Frozen</td>
<td>c-DNA (2 platform)</td>
<td>Responsive Tumour (cCR + PR according to RECIST)</td>
<td>3-gene signature</td>
</tr>
<tr>
<td>Iwao-Koizumi K (2005) [56]</td>
<td>70</td>
<td>II or III</td>
<td>Docetaxel × 4</td>
<td>Frozen</td>
<td>c-DNA</td>
<td>Responsive Tumour (cCR + PR according to WHO)</td>
<td>85-gene signature</td>
</tr>
<tr>
<td>Hannemann J (2005) [57]</td>
<td>48</td>
<td>LABC</td>
<td>AC × 6 AD × 6</td>
<td>Frozen</td>
<td>cDNA</td>
<td>npCR (pCR breast and residual scattered tumour cells)</td>
<td>No</td>
</tr>
<tr>
<td>Gianni L (2005) [17]</td>
<td>89</td>
<td>LABC</td>
<td>AT × 3 → wT × 12</td>
<td>FFPE</td>
<td>384 genes qRT–PCR</td>
<td>pCR (breast)</td>
<td>Genes list</td>
</tr>
<tr>
<td>Cleator S (2006) [58]</td>
<td>18</td>
<td>T(2–10cm)</td>
<td>AC × 6</td>
<td>Frozen</td>
<td>Affy U133A</td>
<td>cCR Progressive Disease vs PR</td>
<td>No</td>
</tr>
<tr>
<td>Sorlie T (2006) [59]</td>
<td>81</td>
<td>T(2–10cm)</td>
<td>Doxorubicin (weekly) × 16; 5-FU + mitomycin</td>
<td>Frozen</td>
<td>cDNA</td>
<td>pCR (breast)</td>
<td>No</td>
</tr>
<tr>
<td>Harris LN (2007) [61]</td>
<td>22</td>
<td>II–III</td>
<td>wVH x12</td>
<td>Frozen</td>
<td>Affy U133 Plus 2.0</td>
<td>pCR (breast and nodes): Resistant Tumours</td>
<td>No</td>
</tr>
</tbody>
</table>

$^1$Referring to the number of patients available for the analysis.

T, paclitaxel; wT, weekly paclitaxel; FAC, 5-fluorouracil+doxorubicin+cyclophosphamide; AC, doxorubicin+cyclophosphamide; AD, doxorubicin+docetaxel, AT, doxorubicin+paclitaxel; 5-FU, 5-fluorouracil; GED, gemcitabine+epirubicine+docetaxel; wVH, weekly vinorelbine+trastuzumab; FFPE, formalin-fixed paraffin-embedded; pCR, pathological complete response; PR, partial response, cCR, clinical complete response.

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After molecular profiling with an Affymetrix gene chip on fine needle aspirations, the individual sensitivity to HER2-targeted treatment by ERBB2 gene expression measurement [62], sensitivity to neo-adjuvant T/FAC chemotherapy by the DLD-30 probes signature [19] and sensitivity to endocrine therapy by the SET index [63] will be defined and used to decide treatment. This strategy is currently pursued in a trial that represents a distinct departure from the common approach of ‘one size fits all’ ([http://ctep.cancer.gov/bcmeeting/pusztai.pdf](http://ctep.cancer.gov/bcmeeting/pusztai.pdf)).

**Conclusion**

Tailored therapy based on individual risk assessment and prediction of sensitivity to pharmacological interventions is one of the most formidable goals of modern oncology. Several HTTs offer a new and unique opportunity to seek and eventually reach the goal of tailoring treatments. In this rapidly evolving field, technologies for gene expression profiling have provided the first and soundest approaches. New multigene biomarkers are quickly translating from proof-of-principle to successful clinical tools. Testimony of the success of such approaches is the inclusion of a commercially available gene signature, Oncotype DX™, in the latest versions of the ASCO and NCCN guidelines for deciding on adjuvant treatment of early breast cancer. This probably represents the tip of the iceberg of a number of applications. The risk is the generation of a plethora of signatures because of the ever growing accessibility of the techniques without a discriminating analysis of the true benefit associated with the markers. One of the challenges of future and ongoing studies is therefore the application of a successful roadmap to discover clinically useful biomarkers that complement or substantially improve the performance of the first generation of signatures [22]. Another formidable task is the identification of biomarkers capable of predicting response or resistance to individual cytotoxic drug or regimens, a quest that so far has been elusive but would represent a major achievement of immediate clinical utility. No matter how many challenges the future will propose, new molecular technologies are already affecting routine clinical care, and their role is destined to grow.

**Disclosures**

L.G. has a relationship as advisor with Roche, Novartis, BMS, Sanofi and Genentech.

**References**


