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Review

High-throughput techniques in breast cancer: A clinical perspective

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ARTICLE INFO

Article history:
Received 10 June 2005
Accepted 22 November 2005
Available online 23 January 2006

Keywords:
Breast cancer
Clinical trials
DNA microarrays
PCR
Treatment outcome
Prognosis
Proteomics
Review

ABSTRACT

High-throughput technologies such as DNA-microarrays, RT-PCR and proteomics can improve the prognostic and predictive information acquired from classical parameters. Unlike information gathered by classical methods, high-throughput technologies can accurately inform clinicians on patient response to adjuvant therapy or those who will resist the effect of that therapy. Studies performed in breast cancer with high-throughput techniques have focused on tumour biology, prognosis, prediction of response to a few agents and, more recently, early diagnosis. However, further refinement is needed before these techniques become part of clinical routine. In the meantime, they will be used in clinical investigation, particularly in the areas of hormonal therapy and adjuvant chemotherapy, where modest improvements in the capacity of prediction can benefit many women. Close cooperation among clinicians, pathologists and basic investigators is essential to take high-throughput techniques to daily practice. New diagnostic tools will be complex but they will provide valuable patient information.

1. Introduction

The outcome of patients with breast cancer has improved in the last 20 years due to early diagnosis and the widespread use of adjuvant therapies. This outcome can be predicted with the help of clinical and pathological parameters, the so-called prognostic factors. However, currently available prognostic factors are far from accurate and they must be improved. New high-throughput technologies are now being used in an attempt to enhance our prognostic and predictive capacities, which could help in determining the best adjuvant treatment for every patient.

In this review, we shall deal with the limitations of classical prognostic factors, the main features of high-throughput techniques and what they should offer before being incorporated to daily clinical practice. Our objective is to offer a global perspective that is useful for the physician and basic investigator.

2. Prognostic factors and adjuvant treatment of breast cancer

2.1. *Early-stage disease*

Women with localized tumours usually undergo a conservative surgical procedure, followed by radiotherapy and adjuvant systemic treatment. The latter consists of chemotherapy, hormonal therapy or both, and is given with the

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2005.11.021

aim of decreasing the chance of relapse and death. The likelihood that a patient will benefit from adjuvant therapy depends on several classical prognostic factors: number of affected lymph nodes, size of the tumour, grade of differentiation and expression of hormonal receptors and ERBB2.[1–6] Table 1 shows disease-free survival values according to the lymph node status and the size of the tumour, the two main prognostic factors.

Nowadays, most patients receive some kind of adjuvant chemotherapy. Although chemotherapy improves the long-term outcome of women with breast cancer, it induces alopecia, nausea, vomiting and fatigue, among other side-effects. On the other hand, patients whose tumours express hormonal receptors are treated with hormones for at least five years. Hormones may produce hot flushes, loss of libido, vaginal discharge, muscular or joint pain and accelerated loss of bone density. Noteworthy, toxicity derived from chemotherapy or hormonal therapy appears in all women, not only in those obtaining a benefit.

The main limitation of both chemotherapy and hormonal therapy is that the absolute gain in survival is small in many instances, even with very active schemes[7] (Fig. 1). This means that most patients receiving systemic therapy will not benefit

Table 1 – Disease-free survival for breast cancer depending on the size of the tumour and the lymph-node status[1–5]

Size (cm)	Disease-free survival (%)
Negative lymph nodes	
Up to 2	80–90
2–5	70
Over 5	60
Positive lymph nodes	
Up to 2	63–50
2–5	50–35
Over 5	35–21
No. of positive lymph nodes	**Disease-free survival (%)**
1–3	55
4–9	35
10 and over	15

For any given size of the primary tumour, the presence of axillary lymph nodes decreases the likelihood of disease-free survival. Lymph node status is the most powerful clinical prognostic factor. Regardless of the size of the tumour, the number of affected lymph nodes is directly related to the outcome. Patients with four or more lymph nodes are considered to have locally-advanced disease.

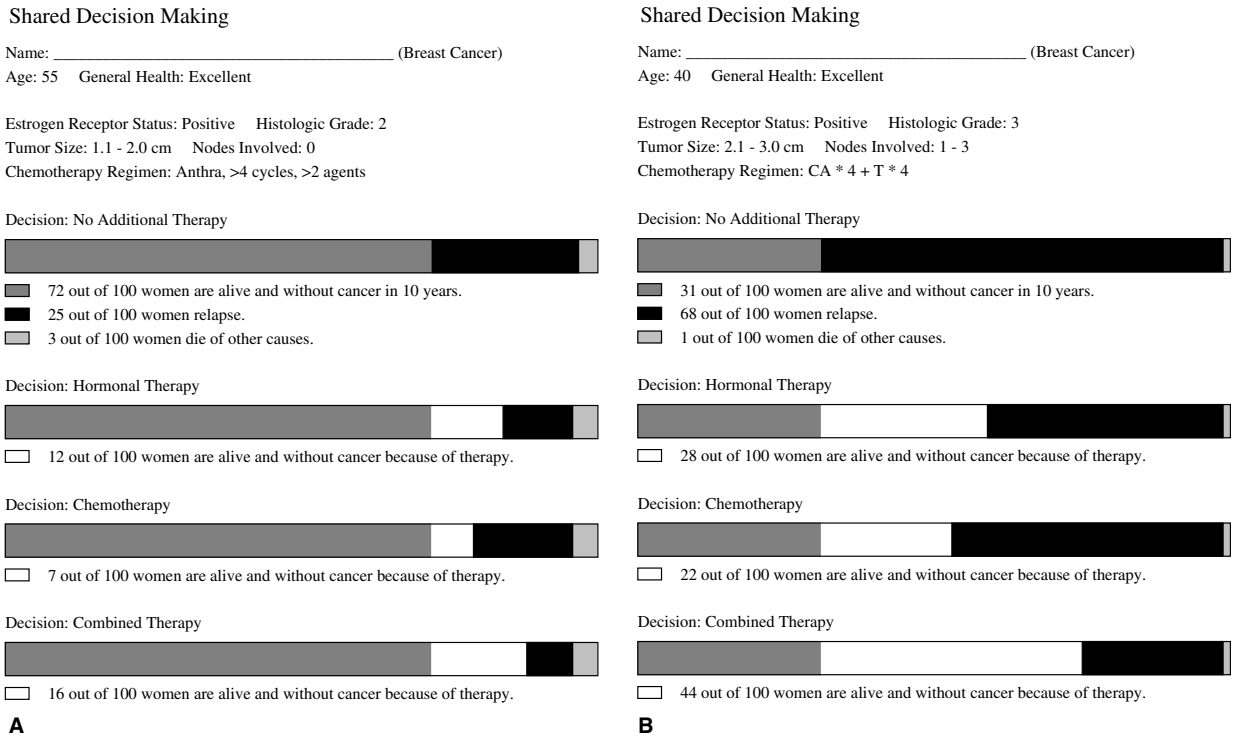

Fig. 1 – Estimation of disease-free survival and gain with adjuvant therapy in two patients by using Adjuvant™ Software[7] (with permission). (A) A 55-year-old patient with a good prognosis. Adjuvant therapy consists of fluorouracil–doxorubicin–cyclophosphamide followed by anastrozole. Anastrozole alone provides a 12% reduction in the rate of relapse, whereas chemotherapy alone contributes with 7%. If both therapies are combined, the relapse rate decreases by 16%: this is less than the sum of those separately (the effect is not additive), indicating that, if the patient is going to receive hormones – benefit 12% –, chemotherapy will add only an absolute benefit of 4%. (B) A 40-year-old woman with a poor prognosis tumour. Adjuvant therapy consists of doxorubicin–cyclophosphamide + paclitaxel followed by anastrozole. The absolute gain (white area) is greater for the second patient whatever the combination of adjuvant therapy, but even so only one out of six patients will benefit from chemotherapy if they are also treated with hormones. The first situation is far more common than the second, so the absolute gain with adjuvant therapy is moderate for the average patient.

from it, either because they will be cured just with local treatments or because they will relapse anyway. Currently available prognostic factors are not good enough to predict outcome accurately, hence the difficulty in selecting patients who will benefit most from adjuvant systemic therapy.

2.2. *Locally-advanced disease*

Women with tumours greater than 5 cm or with $\geqslant$4 or palpable axillary lymph nodes have locally-advanced disease. They may undergo either a mastectomy or preoperative chemotherapy followed, if the response is good, by conservative surgery and radiotherapy. Preoperative (neoadjuvant) chemotherapy has two main objectives: to allow a conservative surgical procedure and to improve survival. Of note, survival improvement is greater in patients achieving a pathological complete response, approximately 10–20% of those with locally-advanced disease.[8–10]

Obviously, tumours with primary resistance to chemotherapy will not shrink with neoadjuvant therapy. Drug resistance may depend on many factors, but we have limited information in this regard.[11] So far, there is no way to identify who will have a complete response, nor is it possible to predict lack of response to chemotherapy, a situation where delaying surgical therapy might be deleterious. It follows that, in locally-advanced disease, prognostic factors, i.e., those related to survival, are less important than predictive factors, i.e., those indicating the likelihood to respond to a given therapy.

2.3. *Disseminated disease*

Metastatic breast cancer is an incurable disease. Although few women survive for five years or longer, most of them have a life expectancy of two to three years from the diagnosis of the metastasis.[12,13] Many patients experience some kind of benefit from first or second line therapies (either hormones or chemotherapy), but the chances of responding decrease thereafter with every new line of treatment. As in the cases of early-stage and locally-advanced disease, however, we cannot identify who will respond to a particular treatment (with the obvious exception of lack of response to hormones in cases with negative hormonal receptors). Something similar applies to trastuzumab, a monoclonal antibody directed against ERBB2: positive FISH or immunohistochemistry staining is required to begin therapy with the antibody, but responses appear only in one third of these selected patients.[14,15]

As a summary, clinical and pathological factors do not accurately indicate which patients will require adjuvant therapy or which tumours will be resistant to anticancer drugs. New factors are obviously needed to optimize cancer therapy and high-throughput techniques could be of help in this regard.

3. A brief explanation of high-throughput techniques

3.1. *Genomics*

DNA microarrays and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) have been used to perform gene profiling in breast cancer. Profiles from different samples can be compared to each other: for instance, normal versus tumoural tissues; or samples coming from patients with poor versus good outcome; or those who responded versus those who did not respond to a given therapy. A microarray is a solid support containing thousands of different gene fragments called probes. Probes consist of complementary DNA or oligonucleotides. The device is put in contact with a mixture of complementary RNA or complementary DNA derived from the study sample. If the sample contains a gene that is present in the microarray, hybridization will take place. The signal present in each probe is automatically detected, quantified and integrated with specialized hardware and software, which yields the gene expression profile for that sample. Data can be used to improve either our understanding of oncogenesis (discovering key genes or characterization of pathways) or the management of patients, as we shall discuss later. Arrays for gene expression profiling can now accommodate the whole genome.[16–18]

On the other hand, qRT-PCR capitalizes on the fact that there is a quantitative relationship between the amount of starting target sample and the amount of PCR product at any given PCR cycle number. The concept of 'real-time' PCR consists of the detection of PCR products as they accumulate.[19,20] Current qRT-PCR systems are based on a set of primers and fluorogenic probes, which accounts for the high specificity of the technique. The amount of fluorescence produced from these fluorogenic probes is measured at each amplification cycle, providing a look at the "real-time" changes in the amplification product as the PCR process develops. Identification of the PCR cycle when the exponential growth phase is first detectable provides extremely accurate quantitation of gene expression in the starting samples. This cycle number is called the threshold cycle and is inversely proportional to the starting amount of target genetic material.

From a clinical point of view, qRT-PCR has some advantages over DNA-microarrays: it requires smaller quantities of valuable tumour tissue and provides accurate, easily reproducible and quantitative results with less manipulation of the sample.[21,22] Unlike microarrays, many pathologists are familiar with this technique. Moreover, recent reports suggest the possibility of using paraffin-embedded tumour tissue with qRT-PCR.[23,24] Quantitative-PCR devices analyse a limited number of genes simultaneously, so that they are not ideal for a first approach, but once screening has been performed with microarrays, they have the advantage of being easier to manufacture and standardize. For these reasons, if a profile containing a limited number of genes would be accepted for use in the clinic, qRT-PCR or a similar technique would be a practical choice.[22]

Other techniques have also been used to study breast tumours. Comparative genomic hybridization (CGH) is a method to detect chromosomal copy number by comparing hybridization intensity of a tumour and a normal control DNA sample.[25] Array-based CGH makes it possible to scan the whole genome for copy number alterations with high resolution by hybridizing genomic representative DNA to arrayed oligonucleotides, BAC (bacterial artificial chromosome) or

cDNA clones that correspond to multiple regions of the genome.[26–28]

Finally, single nucleotide polymorphism (SNP) arrays have also been developed.[29] SNPs are single-letter variations in DNA base sequence and represent the most common source of genetic variation in the human genome (about one in 300 bases). SNP analysis is useful for a variety of applications, such as copy number[30] or linkage analysis to identify disease-related genes.[31,32]

3.2. *Proteomics*

Protein-microarrays rely on the same principle as their DNA counterparts, thus allowing the simultaneous assessment of hundreds to thousands of proteins. The results can offer information about the functional state of the encoded proteins, as well as information about protein to protein interactions.[33] For this reason, protein-microarrays are becoming more relevant in this field. A new type of protein array, reverse-phase protein array, has been successfully used with small amounts of tissues.[34]

Tissue microarrays are means of combining tens to hundreds of specimens from paraffin-embedded tissue and, less commonly, from frozen tissue. A tissue microarray slide can be processed like an ordinary tissue section, and used for histochemical, immunohistochemical staining or in situ hybridization.[35] Whereas gene arrays can examine thousands of genes per sample, tissue microarrays generally study only a single gene product in tens to hundreds of samples. Thus, the technologies are in some respects opposed, although highly complementary. The system may allow the fast validation of multiple biomarkers, a process that would take weeks if performed by classical immunohistochemistry.[36] A common criticism is that assessment of an entire tissue section is more accurate than a small spot of tissue on an array, but some groups have shown excellent concordance between tissue microarray spots and whole sections in immunohistochemistry.[37,38]

The detection of the whole set of proteins can be accomplished with mass spectrometry.[39] A mass spectrometer separates proteins (and other analytes) according to their mass-to-charge (m/z) ratio. Mass spectrometers generally couple three devices: an ionization device, a mass analyzer, and a detector. The molecule is ionized by one of several techniques, and the ion is propelled into a mass analyzer by an electric field that resolves each ion according to its m/z ratio. The detector passes the information to the computer for analysis. The most common ionization techniques used in biology are matrix-assisted laser desorption ionization (MALDI), its derivative surface enhanced laser desorption/ionization (SELDI) and electrospray ionization (ESI). Mass spectrometry technology is fast and requires small amounts of the protein sample. Samples can consist of any fresh or frozen tissue samples, including blood or tumour specimens. Promising evaluations of diagnostic proteomic profiles have been reported from serum samples in patients with tumours of the ovary and prostate.[40,41] SELDI-TOF MS ProteinChip technology has recently been used for the diagnosis of breast carcinoma.[42] In spite of that, experts agree that mass spectrometry needs further validation before it is adopted in clinical practice.[34]

3.3. *Limitations*

The requirement of frozen samples to process the genetic material with high-throughput techniques limits their widespread use. This outlines the importance of tumour banks, although some investigators have successfully used paraffin-embedded tumour tissue to circumvent the problem.[43] Another important limitation is the overwhelming volume of data generated in these studies. Specialized software has been developed, but there is still much room for improvement and an experienced statistician must analyse the data. Finally, high-throughput techniques are very expensive compared to those traditionally used for the pathological work-up. These difficulties explain why new technologies will not gain access to daily clinical practice until they prove their superiority with regard to classical factors. This will require close cooperation among clinicians, basic investigators and statisticians.

4. Studies in breast cancer

High-throughput techniques have been used in normal breast tissue and tumours with different clinical or pathological features. Studies most interesting for clinicians relate to prognosis and response prediction. The main results with clinical implications can be summarized as follows.

4.1. *Breast cancer biology*

1. The expression profiles of distinct pathological stages of breast cancer, i.e., early versus advanced disease have remarkable similarities.[44–46] In contrast, benign tissue shows quite a different expression of genes. This suggests that the capacity to metastasize appears soon in the natural history of breast cancer, so that the gene expression profile of early-stage disease reflects the metastatic potential of the lesion. The hypothesis has been confirmed in further studies that demonstrate a correlation between the profile of the primary tumour and the prognosis.[46,47] From a clinical point of view, it is well known that even small primaries may produce metastases.
2. Some sets of genes correlate with the presence of oestrogen receptors.[48,49] This includes not only genes related to the hormonal pathway, but also genes encoding proteins that synergise with oestrogens. It follows that the hormonal status does not only define the adjuvant treatment, but also divides tumours into genetically different categories. It has long been recognized that tumours with expression of hormonal receptors produce less relapses and have a more indolent course.
3. Microarrays can identify at least five types of tumour subclasses in ductal carcinomas: normal breast-like, basal-like, ERBB2 and luminal types A and B.[50] The basal-like and ERBB2 subclasses are associated with shortest survival times, as opposite to the luminal A type. Tumours in carriers of BRCA-1 mutations usually correspond to the basal-like subclass.[51] This is one of the first proposals for a genetic classification of breast cancer. Our current classification relies on pathological description, where most tumours are ductal, followed by the lobular type, but this

division does not provide much information about the clinical outcome. A genetic taxonomy supports the observation that the clinical evolution of breast cancer is extremely heterogeneous. So far, clinicians can only speculate that there are different diseases with a similar pathological appearance. A new classification also opens the possibility to explore the pathogenesis of the disease and to develop targeted therapies in the future. Specific profiling of *BRCA* tumours has also been performed,[52,53] confirming that *BRCA* tumours are genetically different from other varieties of breast cancer.

4.2. *Prognosis and adjuvant treatment*

A 70-gene expression profile predicts outcome in premenopausal women with early-stage breast cancer even better than the lymph node status.[47] We have reproduced these results with qRT-PCR.[54] Besides, a 21-gene profile predicts the likelihood of distant recurrence in tamoxifen-treated patients with node-negative breast cancer.[24] This profile identifies up to 50% of patients whose prognosis with hormonal therapy is so good that chemotherapy could be avoided.[24,55] Finally, a 76-gene signature does so in women with node-negative disease and no adjuvant therapy.[56] For the first time, physicians may use genetic factors that rival node status in the capacity to predict outcome, but unlike the situation of the lymph nodes, genetic tests may also provide information to customise adjuvant treatment, as we shall discuss later.

Some protein profiling studies have also been performed with tissue microarrays. A French group identified a set of 21 proteins which correlated with metastasis-free survival.[57] A British group used a large panel of markers to delineate five groups with distinct patterns of expression.[58] In both cases, correlation was found with the five subclasses previously determined by DNA-microarray technology.

4.3. *Response prediction and advanced disease*

Some studies have found gene expression profiles predicting response to taxanes[59–61] or tamoxifen.[23,62,63] A study of cDNA microarrays in poor-prognosis tumours treated with anthracycline-based adjuvant chemotherapy found a 23-gene set that was associated with different survival.[64] On the other hand, single-nucleotide polymorphisms can contribute to individual drug response.[65,66] This is an area of paramount importance. Current therapeutic strategies are planned with no information about the susceptibility of the tumour to anticancer drugs in a given patient, so that this patient has to face side-effects with no guarantee of success. Particularly in advanced disease, time is everything and if one line of treatment fails, the prognosis worsens dramatically. We must also consider the huge economic cost of ineffective drugs. Unfortunately, results in this area of investigation are still far from the clinic and a lot more studies will be required in the near future.

4.4. *Early diagnosis*

Proteomic techniques have been used more recently to focus on the analysis of blood samples to develop a rapid diagnostic test,[67,68] early detection of relapse in nipple aspiration,[69,70] the characterization of ductal carcinoma in situ[71] and the response to neoadjuvant chemotherapy.[72] Data from these studies are however, too preliminary.

5. Criteria to be met by molecular profiling technologies before introduction to the clinic

The aforementioned results could lead the reader to think that high-throughput techniques can already become part of routine pathological workup, particularly in early-stage disease, but some requirements still need to be fulfilled. Firstly, most centres do not have these techniques available and their staff lacks appropriate training to use them. Only when this is accomplished will results become available fast enough for clinicians and their patients. Most oncologist agree that effective adjuvant chemotherapy should begin within one month after surgery, a concept based on some classical studies.[73,74] Also, neoadjuvant chemotherapy is usually begun a few days after the initial biopsy. Even in the absence of definitive data about the ideal timing of chemotherapy, women prefer to know as soon as possible whether they will need it, as well as what kind of chemotherapy. If a high-throughput technique was to be used in the clinic, qRT-PCR would have advantages over microarrays in terms of simplicity and reproducibility, although one can imagine a future ready-to-use DNA chip that is also simple. As we said before, procedures should be refined so that paraffin-embedded or even cytology material could be used instead of frozen samples. The need for reproducible and easily available methods has been previously emphasized.[75]

Secondly, different platforms and profiles have been validated. To date, we have no data to favour one over the other. As none of them has 100% accuracy, they will need further refinement and, after that, universal profiles should be agreed. Ideally, these new markers should not only inform about prognosis but also about the probability of response to different therapies (predictive value). In the same way that pathologists routinely report on size, lymph nodes, grade of differentiation, hormonal status and ERBB2 expression, future platforms and profiles should provide standard information, so that results are comparable among studies and institutions.

Finally, methodological problems may create pitfalls in the interpretation of results. Previous studies included limited numbers of patients, which is not enough to change clinical standards based on solid trials that have recruited thousands of patients in the past. A recent analysis demonstrated that molecular signatures strongly depend on the selection of patients in the training sets, so that validation studies should be performed with all candidate profiles.[76] Besides cohort selection and validation of results, other methodological issues that should be carefully addressed are the statistical analysis and the reporting of raw data.[77]

In spite of their associated problems, these new technologies are here to stay and will certainly be used to improve our knowledge of prognosis. An open question is what kind of studies should be performed to reliably validate new molecular markers that complement classical prognostic and predictive markers.

6. Design of clinical trials using gene profiles

Molecular markers hold great promise for refining our ability to establish early diagnosis and prognosis, and to predict response to therapy. The fact that techniques and profiles should be improved does not mean that we cannot begin using them in clinical trials. High-throughput techniques will have to undergo a thorough process of validation in the same way as any other new diagnostic or therapeutic tool. So, what kind of gene profile trials could have the greatest clinical impact?

6.1. *Early diagnosis and follow-up*

Proteomics has shown promising results in the early detection of disease, but this is the least developed area. Women at high risk of having breast cancer could be screened by radiological methods as well as proteomic technologies from blood samples to assess the predictive power of the new technique. Patient sample profiling could even help in selecting patients for chemoprevention trials. In the short or medium term, similar trials could also be performed in women with aggressive tumours, to detect early relapses. However, important concerns have been raised about the validity of serum proteomic pattern analysis by mass spectrometry for early cancer diagnosis.[78]

6.2. *Trials with hormonal therapy*

Approximately 60% of patients with early-stage disease have tumours with positive hormonal receptors and they all receive hormones, even when only a fraction will benefit (Fig. 1). In the case of advanced disease, first-line hormonal therapy obtains an objective response or stabilization in approximately 60% of the patients. Although less serious than those of chemotherapy, these drugs also produce side-effects. Economical savings could be another reason to encourage studies in this regard. As a consequence, the identification of patients who do not benefit from hormones should become a priority in the next decade.

Recent reports have identified gene profiles that can predict either response to tamoxifen in metastatic breast cancer[62] or resistance in the adjuvant setting.[24] Similar studies should be performed with aromatase inhibitors. These trials will take time, particularly in the adjuvant setting, as patients treated with hormones usually have low-risk disease, which means low risk of relapse and death.

6.3. *Trials with chemotherapy in node-negative disease*

The EORTC (European Organisation for the Research and Treatment of Cancer) will shortly begin a trial to validate a 70-gene profile in women with node-negative breast cancer. Patients' risk will be defined with either the St. Gallen prognostic index (based on classical factors) or the gene profile: women having a low risk of relapse as defined with either method will not receive adjuvant chemotherapy, whereas the remaining patients will receive chemotherapy (±hormones). The purpose of the trial is to compare the outcome of patients in both low-risk groups. Twenty percent of women with negative nodes have low risk of relapse according to St. Gallen's index, as compared to 40% with the gene profile. If disease-free survival is comparable, 20% of women could be spared adjuvant chemotherapy in the future by using the molecular method. A 20% looks modest considering the sophistication of the information provided by high-throughput techniques, but it could include thousands of women across Europe and the United States every year. To demonstrate the hypothesis, the trial will recruit 5000 patients[79].

On the other hand, in the United States, the Intergroup will initiate another clinical trial using the 21-gene profile, as part of the Program for Assessment of Clinical Cancer Tests (PACCT). Women with intermediate-risk recurrence score will be randomized to receive adjuvant hormonal therapy with or without chemotherapy. This study will also include about 5000 patients. It follows that for the moment, trials using gene profiles will not include fewer patients than similar trials validating classical prognostic factors.

Even if these gene profiles are validated in the aforementioned trials, further improvements will be needed. In the EORTC trial for instance, 60% of women with an unfavourable gene signature would receive adjuvant chemotherapy based on this profile, whereas the proportion with greatest benefit is lower in node-negative disease (Fig. 1a). On the other hand, there are clinical differences in the behaviour of tumours depending on the hormonal status and histology, but we do not yet understand the genetic background that explains such differences. For instance, negative-receptor tumours usually behave more aggressively than positive tumours, and lobular carcinomas have a particular pattern of relapse. When new and supposedly better profiles are discovered in the future, they should also undergo clinical evaluation under similar conditions. Data bases and samples from big multi-institutional trials could be used to validate these improved profiles.

6.4. *Trials with chemotherapy in high-risk disease*

The next question could be if we can spare useless chemotherapy in patients defined as having high risk of relapse according to classical prognostic factors. This is more difficult. Fig. 1 shows that the risk of relapse and the benefit obtained with adjuvant chemotherapy grow in parallel, so a trial with the EORTC design for low-risk women would be unacceptable in the high-risk situation. For high-risk patients, checking chemo-resistance would be of more interest. Women with tumours that are very unlikely to respond to standard chemotherapy could be offered entering clinical trials with new agents or even no chemotherapy at all. However, this kind of trial is now difficult to implement for two main reasons: first, we have few data regarding gene profiles that predict resistance to chemotherapy; and second, trials of adjuvant therapy require many patients and take a long time (it is not possible to get conclusions until a significant proportion of the patients have relapsed).

For this purpose, the neoadjuvant setting, therapy before surgery, offers the possibility to assess the clinical response in a more simple way. Biopsies can be obtained at diagnosis for the molecular study, whereas the final response to chemotherapy is known after surgery. For example, the SPORE trial will try to identify genes that exhibit differences between

responding and non-responding tumours before treatment with neoadjuvant paclitaxel (www.cancer.gov).

However, target response groups are slightly different in neoadjuvant and adjuvant settings. In the first case, it is important to identify patients who will have a pathological response, because they experience a survival advantage. In the second case, identifying chemo-resistance would be more appropriate, and this could include a different set of genes. A profile predicting lack of response to neoadjuvant chemotherapy should then be checked in the adjuvant setting. Fig. 2 illustrates this issue.

6.5. *Trials in metastatic disease*

Many genetic factors are involved in response to anti-tumour drugs.[11] Physicians treating patients with advanced breast cancer do not know in advance whether a given tumour will respond to chemotherapy or hormones. Unlike the neoadjuvant and adjuvant settings, single agent therapy plays an important role in advanced disease, so that trials could assess resistance to individual drugs rather than to combination regimens. In first or second lines, knowing about drug sensitivity would allow to select the most active options for a particular patient. In third or subsequent lines, where the chances of response are usually low, this information could be used to give advice and share therapeutic decisions with the patient.

6.6. *Other issues*

New investigations are also considering host factors. The genetic background on which the tumour grows influences its ability to metastasize. For instance, differences of the target stroma to support angiogenic conversion in response to tumour-secreted growth factors may influence in this regard. Whenever laser microdissection is not used, samples contain cells from both the normal stroma and the tumour, so that high-throughput techniques are evaluating also host factors. In the same way, subtle variations in the ability to mount an immune response could partially explain the patient's outcome.[80]

Drug metabolism, hence drug activity or toxicity, depends on drug-metabolizing enzymes, which could be assessed either by RT-PCR or microarrays.[81] The activity of some of these enzymes is modulated by polymorphisms present in some individuals,[66,82] so that routine detection of some polymorphisms can be of interest.

Finally, epigenetic changes affecting gene expression can be related to prognosis, as demonstrated by recent investigations.[83,84] This issue could also be considered in the future to assess prognosis.

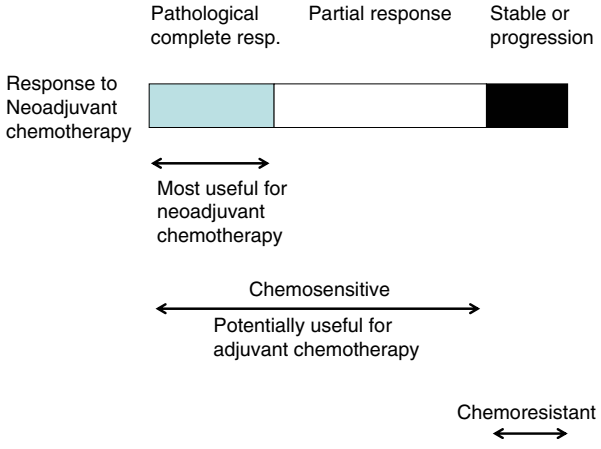

Fig. 2 – Areas of interest for neoadjuvant and adjuvant chemotherapy. In the case of neoadjuvant chemotherapy, the ideal gene profile should identify patients who obtain a pathological complete response (grey area), because these women achieve longer survival. This profile cannot be used in the adjuvant setting, as it would exclude too many people: a useful adjuvant profile should identify only patients with resistant disease (black area) i.e., women who are very unlikely to benefit from adjuvant chemotherapy. The difference in concept must be considered when designing clinical trials.

7. Future directions

High-throughput techniques are not likely to replace current pathological workup, but will rather be complementary. These new techniques should be used by pathologists, so they will have to incorporate them into their training and practice. But the most successful and efficient research about clinically useful molecular markers will require effective interdisciplinary communication and collaboration integrating the knowledge of clinicians, biochemists, and statisticians.

Consensus on platforms and profiles will be certainly needed in the future, so that we can use this information in the same way we work with standard pathological reports. As this technology is evolving very fast at the moment, it will take time to reach such a consensus. In every profile described so far, a lot of genes being apparently independent might be in an overlapping network. Before an ideal profile is accepted, functional pathways must be fully characterized. Making raw data available on the internet may help other scientists in validating profiles, so that profile accuracy is improved as data on more and more patients are incorporated. Obviously, only profiles supported by good clinical trials will have a chance of being widely accepted. Fig. 3 shows a representation of what could be an "ideal" profile for the future. Such a profile could be tailored according to the centre protocols or the clinical setting (neoadjuvant, adjuvant, advanced disease).

Gene profiling will not only be used to get prognostic and predictive data, but also to identify potential therapeutic targets. This step is required to synthesize new drugs that broaden our armamentarium against breast cancer and other tumours. It follows that a number of different profiles will be used in the future depending on the objectives pursued, either clinical or investigational, and obtained with DNA-microarrays, RT-PCR or tissue-microarrays.

Gene expression and genotyping do not provide all the information about tumoural biology: protein detection could also play a role. Standard operating procedures must be established for sample handling and processing before proteomics is broadly adopted. At this moment, it is not possible

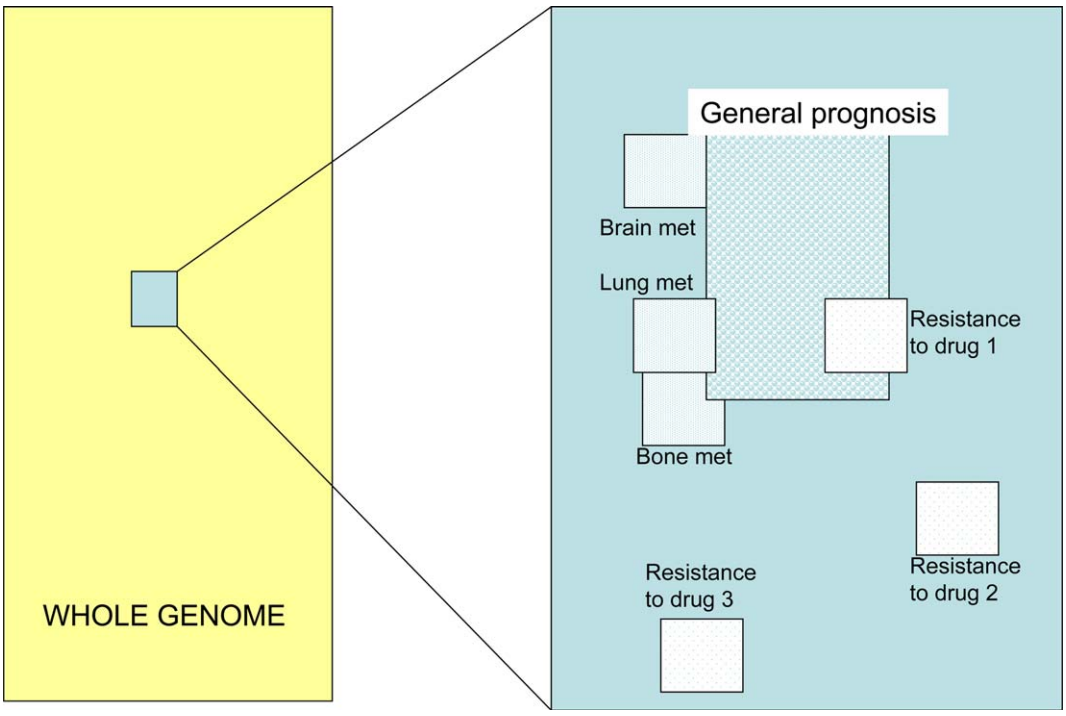

Fig. 3 – Representation of an "ideal" gene-profile providing both prognostic and predictive information. Clinical data from new trials can be incorporated into a computer-based model to improve its accuracy, adding or deleting genes from the original profile. The clinician could ask the model for specific information. The profile could be tailored to provide specific predictive information for the therapeutic protocols in a given centre.

to know whether this technique will substitute for gene profiling or if they will be complementary.

With all this in mind, we can predict that genetic tools in the future will include a mixture of tumour and host features. Considering the heterogeneity of breast cancer and the number of factors to be included, general prognosis, resistance to different drugs, these tools will certainly be complex, but they will provide valuable information to improve the outcome of our patients.

Conflict of interest statement

The authors declare that they do not have any conflict of interest.

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