Introduction

Following the seminal introduction of CMF (cyclophosphamide, methotrexate, and fluorouracil) for adjuvant breast cancer treatment more than three decades ago [1], the efficacy of adjuvant chemotherapy has gradually improved by the introduction of the anthracyclines [2] and, more recently, the taxanes [3]. However, while anthracycline-containing regimens have become standard treatment for oestrogen receptor (ER)-positive as well as ER-negative tumours, conflicting evidence has linked the use of adjuvant taxanes to improved outcome in patients with ER-positive tumours. This uncertainty has now, to a large extent, been resolved with the results from a recent study [4] revealing a benefit for ER-positive tumours with a high growth rate as evaluated by Ki67 status, but no benefit for ER-positive tumours with a low Ki67 score. These results indicate a way forward; while studies three decades ago centred on identifying prognostic factors in breast cancer, we are now starting to learn predictive factors identifying which tumours may achieve optimal benefit from defined therapeutic regimens [5]. This relates to established drug regimens and also to new experimental therapies in particular. While this is conventional wisdom with respect to targeted therapies, such as endocrine agents and anti-human epidermal growth factor receptor 2 (anti-HER-2) strategies, current data illustrate the need for predictive factors to enable optimal use of chemotherapy as well.

Realizing the need to identify predictive factors and, ideally, to understand the mechanisms causing drug resistance [6], translational research aiming at identifying such biological parameters should be part of most phase I to III trials [7]. Before discussing future perspectives and the implementation of novel drugs, a brief summary of the state of the art for predictive factors in breast cancer therapy is provided.

Predictive factors in breast cancer treatment

The topic of predictive factors in breast cancer treatment has recently been reviewed in detail by Lønning [8]; thus, a brief summary will be provided here.

While a slow tumour growth rate, as determined by Ki67 expression, has been related to a good prognosis in patients on endocrine therapy [9], high Ki67 expression, as well as high histological grade, has been related to improved efficacy of chemotherapy, in particular a better chance of having a pathological complete response to primary systemic treatment [10-13]. With regards to other single parameters, TP53 mutations have been associated with poor response to anthracycline therapy [14,15], but the sensitivity and specificity of these do not allow their implementation in routine clinical use. HER-2 amplification not only predicts efficacy of anti-HER-2 therapies but is associated with enhanced efficacy of anthracyclines at high doses [16,17], probably due to co-amplification of topoisomerase II, an anthracycline target, in a subset of tumours [18,19]. The genes for both HER-2 and topoisomerase II are located in close proximity on chromosome 17. Interestingly, recent data have suggested centromere amplification on this chromosome to be a better predictor of anthracycline sensitivity compared to amplification of either HER-2 or topoisomerase II [20].

Considering gene expression profiles, the OncotypeDX signature, initially developed as a prognostic signature in patients receiving adjuvant tamoxifen treatment [21], has been evaluated as a predictive factor with respect to chemotherapy efficacy. Notably, while a low score by this index revealed good prognosis among patients treated with tamoxifen but not chemotherapy, a high score signalled chemotherapy benefits with CMF [22] as well as anthracycline-containing treatment [23]. So far, the predictive value of this signature has been evaluated in patients harbouring ER-positive tumours exposed to tamoxifen; the potential predictive power of the signature with respect to the efficacy of CMF treatment in ER-negative tumours has not been addressed. Several of the genes included in the signature predict cellular proliferation rate; thus, it should be evaluated whether the information provided could be reflected to the same
extent by simple cell cycle parameters like Ki67. As for other supervised signatures, while some have been associated with chemotherapy response, they lack the sensitivity and specificity required for clinical implementation (see references in [5]). Moreover, the specificity of supervised gene expression signatures with respect to which genes are included has been challenged [24].

As for the hierarchical gene expression signature developed by Perou and colleagues [25,26], tumour subclases are not fully predictive of chemotherapy response in either the primary (neoadjuvant) or the adjuvant setting [27-29]. While TP53 mutations have a tendency to be present in tumours of the HER-2, luminal B and basal cell-like classes, they may also be detected among tumours belonging to the luminal A class, albeit at low incidence [26], underlining the correlation between different parameters but also tumour heterogeneity, probably the main reason why we have been unsuccessful in identifying accurate predictive factors.

**Do scientific aims need to be redefined?**

The findings summarized above reveal an emerging understanding of the mechanisms controlling tumour response to therapy. On the other hand, while some of these parameters are used clinically (such as HER-2 amplifications for anthracycline dose selection), we are still far from the goal of ‘individualized medicine’ - the selection of optimal therapy at an individual level based on predictive factors. To fully achieve such a goal will most probably require extension of our ambitions beyond identification of correlative predictive factors toward identification of the mechanisms causing drug resistance.

There may be several different entrances into this field. With regard to novel drugs in clinical trials, two important examples illustrate unexpected clinical observations that may lead translational research into a new area, and how new drugs may be designed based on molecular translational research identifying molecular defects in tumour subgroups.

The first example relates to novel drugs implemented for anti-HER-2 therapy. The tyrosine kinase inhibitor lapatinib inhibits HER-1 as well as HER-2 activity, and has been shown to have anti-tumour activity in tumours resistant to trastuzumab [30]. Surprisingly, among patients developing resistance to trastuzumab, lapatinib and trastuzumab given together improved progression-free survival compared to lapatinib monotherapy [31]. Such observations define an ideal clinical setting from which tumour samples should be systematically collected and analyzed with the aim of identifying the molecular mechanisms dictating resistance versus sensitivity to these individual compounds.

The second approach - the design of therapeutic strategies from knowledge about particular gene defects in individual tumours - is illustrated by the development of poly ADP ribose polymerase (PARP) inhibitors for breast and ovarian cancers in patients harbouring BRCA1 or BRCA2 mutations. BRCA1 and BRCA2 mutated tumours both have a defect in homologous DNA repair [32]. Thus, phase II studies have shown PARP inhibitors to have specific anti-tumour effects in breast as well as ovarian carcinomas with BRCA1 and BRCA2 mutations [33]. Interestingly, PARP inhibitors were also found to enhance efficacy of chemotherapy in triple negative breast cancers not tested for BRCA1 or BRCA2 mutations [35]. Sixty to eighty percent of all triple negative breast cancers belong to the so-called basal cell-like class [36,37], and about 80% of all breast cancers arising in BRCA1 mutation carriers reveal a gene expression profile resembling basal cell-like tumours [26,38,39]. While only 10% of all basal-like breast cancers arise in BRCA1 mutation carriers [40], the similarity with respect to gene expression profile between spontaneous basal cell-like and BRCA1 mutated tumours has raised the question whether basal cell-like tumours may harbour other defects in the ‘BRCA1’ pathway [41]. However, the role of PARP inhibitors in basal cell-like tumours harbouring wild type BRCA1 and BRCA2 remains to be determined; in a recent study [42] none of 15 patients with advanced triple negative breast cancers responded to a PARP inhibitor administered as monotherapy. Currently, we lack an explanation for the seeming disparity between the studies by Gelmon and colleagues [42] and O’Shaughnessy and colleagues [35] with respect to the efficacy of PARP inhibition in triple negative breast cancer in general. There are several dissimilarities between the studies, one evaluating PARP inhibition as monotherapy, the other addressing the effect of adding a PARP inhibitor to a defined chemotherapy regimen. Finally, the studies used different PARP inhibitors, raising the issue of whether these compounds may express additional biological effects. Clearly, more data are needed to address this topic.

**The way forward**

So far, the development of individual predictive factors, as well as multigene-expression arrays, has had limited impact on therapy. While our aim should be to identify the functional mechanisms controlling drug resistance, the complexity of the issue should not be underestimated. The finding that nonsense CHEK2 mutations may substitute for TP53 mutations as a cause of anthracycline resistance [15] is consistent with Chk2 activating p53 in response to DNA damage, and suggests a pivotal role of this mechanism in effecting anthracycline-induced cell death. On the other hand, the p53 protein is subject to multiple modifications, including ubiquitinations, deacetylations and phosphorylations at multiple sites executed by different enzymes, and may directly interact...
with proteins like MDM2 and MDMX [43]. Thus, activation of different cellular processes is most likely due to multiple factors acting in concert and modulating the effects of each other. While substantial evidence suggests mutations affecting a limited number of genes may act as ‘drivers’ of tumour progression [44,45], we do not currently know whether similar phenomena relate to either primary or acquired drug resistance in vivo. Rapidly emerging technologies may allow such analysis to be applied to large sets of tumours at reasonable costs in the not too distant future [46].

Development of novel techniques for high-throughput sequencing opens new possibilities for exploring these issues. Thus, technologies are available for screening the whole genome for point mutations as well as indels or larger deletions/duplications. Therefore, the first reported whole genome for point mutations as well as indels or sequencing opens new possibilities for exploring these effects of each other. While substantial evidence suggests mutations affecting a limited number of genes may act as ‘drivers’ of tumour progression [44,45], we do not currently know whether similar phenomena relate to either primary or acquired drug resistance in vivo. Rapidly emerging technologies may allow such analysis to be applied to large sets of tumours at reasonable costs in the not too distant future [46].

In conclusion, new knowledge and new technologies now suggest we should conduct clinical trials differently to what we have done over the past decades. The phase I to III trial ‘ladder’ remains; however, instead of confirming marginal benefits in repeated large phase III studies, we should concentrate on how we may use these new compounds optimally by exploring drug resistance. To do so, we not only need tumour tissue bank collections, but perhaps more importantly we need to design our clinical studies, whenever ethically justifiable, in a way that aims at exploring drug responsiveness in a manner that allows systematic comparisons between clinical response and biological parameters [48]. The examples provided by novel anti-HER-2 strategies as well as PARP inhibitors both illustrate ways forward.

Abbreviations
ER, oestrogen receptor; HER-2, human epidermal growth factor receptor 2; PARP, poly ADP ribose polymerase.

Competing interests
The author declares that they have no competing interests.

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