Predictive biomarkers: a paradigm shift towards personalized cancer medicine

Nicholas B. La Thangue and David J. Kerr

Abstract | Advances in our understanding of the intricate molecular mechanisms for transformation of a normal cell to a cancer cell, and the aberrant control of complementary pathways, have presented a much more complex set of challenges for the diagnostic and therapeutic disciplines than originally appreciated. The oncology field has entered an era of personalized medicine where treatment selection for each cancer patient is becoming individualized or customized. This advance reflects the molecular and genetic composition of the tumors and progress in biomarker technology, which allow us to align the most appropriate treatment according to the patient's disease. There is a worldwide acceptance that advances in our ability to identify predictive biomarkers and provide them as companion diagnostics for stratifying and subgrouping patients represents the next leap forward in improving the quality of clinical care in oncology. As such, we are progressing from a population-based empirical 'one drug fits all' treatment model, to a focused personalized approach where rational companion diagnostic tests support the drug's clinical utility by identifying the most responsive patient subgroup.

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Introduction

There is little doubt that cancer is one of the most important healthcare issues facing our society. Cancer is the second most common cause of death in the Western World, where the lifetime risk of developing cancer is approximately 40%.[1] The reasons for this high lifetime risk are multifactorial and include environmental and lifestyle changes. The average lifespan of Western populations is increasing and, therefore, so is the risk of developing cancer. The overall annual costs of cancer measured in pure economic terms, namely direct medical expenses and lost productivity, is increasing at an exponential rate, and in 2008 was estimated to be $228 billion in the USA alone, and £18.3 billion in the UK.[2,3]

The term 'cancer' defines over one hundred different diseases that can arise from virtually any tissue or organ in the body and, while sharing common properties of local invasion and distant spread, may have different causative factors, molecular composition, natural history of disease, methods for diagnosis and methods by which they are treated. Consequently, modern developmental therapeutics requires that a new treatment must not only address a disease defined by the histology and anatomical site from which it arose but also the specific molecular, genetic or immunologic subtype. Importantly, the advances in biomarker technologies and how best to deploy them in the clinical setting means that treating cancer has progressed from a 'one drug fits all' approach to a more 'personalized' strategy where treatment regimens are driven by biomarker expression profiles.[4–6]

There are different types of cancer biomarkers; prognostic, pharmacodynamic and predictive.[7] A prognostic biomarker anticipates the likely outcome of the illness and may, if appropriate, dictate whether further therapy is required. For example, the benefits of adjuvant chemotherapy for stage II colon cancer have been long debated, and various histopathological factors such as T stage, vascular invasion and tumor grade have been used to describe a subgroup considered to be of higher than average risk of recurrence and, therefore, used to select patients who might benefit from an improved absolute survival gain from adjuvant chemotherapy.[8,9] More recent versions of prognostic biomarkers include the Onco*type* DX® test (Genomic Health, Redwood City, CA), which is a transcript-based assay that forecasts the probability of breast cancer recurring after surgical intervention.[8] Pharmacodynamic biomarkers measure the effect of a drug on the disease;[10] for example, the level of proliferation and apoptosis in the tumor upon delivery of a drug, or the degree of change on a substrate regulated by an enzymatic drug target (such as phosphorylation after inhibition of a protein kinase).[11] By contrast, predictive biomarkers assess the likelihood that the tumor will respond to the drug, and thereby allow a level of personalization to be introduced into the treatment regimen. There are a small number of predictive biomarkers that have found clinical utility,[12] and others are gaining clinical acceptance as objective measurements that inform on the clinical response to the drug (Table 1).

Competing interests
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Key points

- Cancer is a diverse collection of diseases that have different causative factors, molecular composition, and natural histories
- Many recently developed cancer drugs target discrete molecular aberrations or pathways in tumor cells and consequently are active on a subset of the patient population
- Companion diagnostics that measure biomarkers that allow responsive patients to be identified and subgrouped are being increasingly integrated with the drug-development process and clinical trials
- Most response-specific biomarkers that have reached clinical validation were identified through retrospective analysis of clinical data
- Molecular techniques are available that allow biomarkers to be identified in a systematic prospectively driven fashion
- The long sought after goal where therapeutic choice is guided by an informative biomarker 'code' is now upon us

The need for predictive biomarkers

Support for the development of predictive biomarkers for drug response is wide and varied, and encompasses regulatory, commercial and clinical standpoints. These considerations are relevant to many recently developed cancer drugs that target discrete molecular aberrations, which are usually effective on only a subset of the patient population, typically in the region of 10–20%.[13] This means that for the annual spend on cancer drugs (estimated to be $60 billion in 2010), without patient selection about $45 billion will be spent on medicine that provides limited benefit. As such, there is a clear and compelling argument to develop companion diagnostics that measure biomarkers which, in turn, identify the responsive subpopulation of patients. This will improve the cost effectiveness of the therapy, and go hand-in-hand with improved clinical benefit and safer drugs. Furthermore, certain chemotherapy regimens result in death rates in the range of 0.5–2.0%, and 30–40% of patients experience grade 3 or 4 toxic effects,[14] representing a large burden of morbidity, especially if a significant fraction of this population do not benefit from treatment. Targeted therapies can cause similar levels of toxicity. For example, bevacizumab causes serious side effects including gastrointestinal, cardiovascular and renal toxicity.[15] One important element of predictive biomarker-driven cancer therapy will be the reduction in unnecessary treatment and adverse effects.

The changing pharmacoeconomic environment and the escalating cost of drugs has led regulatory bodies to emphasize the importance of predictive biomarkers, and the benefits that a predictive biomarker assay provides to patients and payer's budgets. Both the European Medicines Agency (EMA) and FDA are encouraging drug developers to identify predictive biomarkers as a companion diagnostic, which is widely accepted to become a more-common guideline and general practice.[16] In general, there is an increasing demand for predictive tools to allow patients with responsive disease to be identified and treated accordingly.

The expansion of our knowledge of cancer biology has created many different options for new types of biomarkers and there is now the possibility of achieving selective and specific personalized cancer treatments. However, there are consequences that result from tumor subtyping and personalizing cancer treatments. Older classes of drugs, such as nonspecific cytotoxic chemotherapeutic agents, which target general mechanisms shared between many different types of tumors, are usually used on a very broad spectrum of tumor types and hence are often widely employed. Usually, these agents are off patent and, therefore, generic and of significantly lower cost compared with the molecularly targeted drugs.[17] The commercial incentive to develop companion diagnostics for generic drugs is not compelling, which contrasts with the argument for therapeutic benefit. Conversely, highly targeted therapies that are aimed at discrete molecular aberrations will have their application restricted to a subset of patients whose tumors display the required biomarker. These newer targeted agents are frequently of high cost, and subject to economic scrutiny by regulatory authorities when the manufacturer seeks marketing approval.[17] In such cases, there is clear justification (reflecting commercial, clinical and regulatory arguments) to develop companion diagnostic support.

We have highlighted some of the predictive biomarker and companion diagnostic tests that are gaining increasing acceptance in the cancer clinic. Where appropriate we have detailed the pitfalls and the historical bottlenecks that were experienced in developing the current gamut of tests.

Predictive biomarkers in use

HER2 and breast cancer

Breast cancer is the most frequently occurring cancer in women.[18] The *HER2* gene is amplified and overexpressed in about 25% of tumors, and patients with HER2-positive tumors have a poorer prognosis than other types of breast cancer; for example, approximately 80% of patients with invasive ductal carcinomas show *HER2* amplification.[19–21] Trastuzumab is a recombinant humanized monoclonal antibody that targets the HER2 protein (Figure 1), and was developed on the basis that tumors overexpressing this target would respond favorably.[22] Trastuzumab was the first targeted therapy approved by the FDA for metastatic breast cancer in combination with adjuvant treatment regimens (doxorubicin, cyclophosphamide and paclitaxel) for node-positive, HER2-overexpressing tumors.[23]

In several large clinical studies, trastuzumab had a major impact on HER2-positive metastatic breast cancer, and in combination with chemotherapy was suggested to increase both survival and response rate compared with trastuzumab alone.[21,24,25] However, about 70% of HER2-positive patients do not respond to the drug, and resistance to the treatment develops rapidly (within a year of treatment) in virtually all patients.[22,26,27] Resistance mechanisms are an active area of investigation.[28] In addition, a number of multicenter randomized studies have reported significant benefit from the addition of trastuzumab to adjuvant therapy with up to 50% reduction in the relapse of breast cancer.[22]

Table 1 | Examples of predictive biomarkers for drug response

Biomarker	Cancer type	Drug therapy	Drug target
HER2 (gene amplification)	Breast	Trastuzumab	HER2
Estrogen receptor (protein expression)	Breast	Tamoxifen	Estrogen receptor
BCR–ABL (gene translocation)	CML	Imatinib, dasatinib, nilotinib	BCR–ABL
EGFR ± *KRAS* (*KRAS* mutation)	CRC	Cetuximab, panitumumab	EGFR
EGFR (kinase domain mutation)	NSCLC	Erlotinib, gefitinib	EGFR
PML–RAR (gene translocation)	APL	*All trans* retinoic acid	PML–RAR
BRCA1/2 (mutation)	Breast	Olaparib, veliparib	PARP
BRAF V600E (mutation)	Melanoma	Vemurafenib	BRAF
ALK (rearrangements)	NSCLC	Crizotinib	ALK

Abbreviations: APL, acute promyelocytic leukemia; CML, chronic myeloid leukemia; CRC, colorectal cancer; NSCLC, non-small-cell lung cancer.

The need for accurate detection of HER2 status as a predictive biomarker is important as therapeutic decisions have been increasingly influenced by the level of HER2 expression. The ability to reliably identify patients who might benefit from trastuzumab is not only important for clinical reasons (a significant proportion of grade 3 or 4 cardiotoxicity is associated with treatment[27]) but also for economic ones (trastuzumab costs about €42,000 per treatment course[17]). The most commonly used companion diagnostic tests are the HercepTest™ (Dako, Glostrup, Denmark) and Ventana Pathway (Ventana Medical Systems, Tucson, AZ), both representing standardized tests that measure HER2 protein expression levels by immunohistochemistry (IHC) in tumor biopsies.[29] However, the ability of HER2 expression status to predict the benefit of trastuzumab is a subject of much debate, as it seems to be modest at best with a positive predictive value (PPV) usually in the region of 25–40%.[30] IHC has numerous limitations—technical and interpretative—that impact on reproducibility and accuracy.

The increasing number of tests available for measuring HER2 levels has complicated this area. For example, a recent study indicated that one in five HER2 tests gave the incorrect result,[31] and more generally a large proportion of patients treated with trastuzumab are never tested for HER2 expression.[30–32] Currently, the debate on how best to select patients that respond favorably to trastuzumab also favors fluorescence *in situ* hybridization (FISH) to measure *HER2* amplification.[31,32] Thus, more than a decade after trastuzumab was approved there remain many obstacles in the practice of identifying responder patients and delivering personalized care, a situation that epitomizes the personalized medicine paradigm. With new opportunities for tests being developed,[29] and trials underway with trastuzumab in patients with earlier disease stage, it is likely that more biomarker tests will be added to the armamentarium to assist in guiding the personalization of breast cancer therapy.

CML and imatinib

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by high proliferation of myeloid cells. Most CML cases are associated with a specific chromosomal translocation between chromosome 9 and 22 resulting in the characteristic Philadelphia chromosome, creating a fusion protein, BCR–ABL, which acts as a constitutively active tyrosine kinase.[33] A selective inhibitor of BCR–ABL, imatinib,[34] is highly effective against CML, and is now an established first-line therapy.[35] However, resistance to imatinib occurs in about 10–15% of patients, which frequently is caused by mutations in the gene encoding the catalytic domain that prevent inhibition by imatinib, although BCR–ABL independent mechanisms can also occur.[36] It is estimated that 30–50% of patients with secondary resistance to imatinib have a catalytic domain mutation, and over 100 different mutations have been identified.[36] Consequently, inhibitors that act on imatinib-resistant mutants have been developed, and include drugs such as dasatinib and nilotinib (Table 1). Dasatinib is active against most imatinib-resistant BCR–ABL-positive tumors, and inhibits proliferation of BCR–ABL progenitor cells from patients with imatinib-resistant disease.[36] This drug was approved for treatment of CML patients with resistance to imatinib.[37–39] Several other BCR–ABL tyrosine kinase inhibitors are either approved or under development, such as nilotinib, a more-potent inhibitor than imatinib.[38–40] Both dasatinib and nilotinib have activity against other kinases, which might contribute to their activity in imatinib-resistant disease.[40–42] The level of resistance to imatinib, nilotinib and dasatinib depends on the mutation identified[43] as some mutations that result in amino acid substitutions, such as Tyr315Ile (which occur at the contact site between the P-loop and kinase domain), impart resistance to all three agents.[41]

Biomarkers for hematological malignancies have traditionally been assessed through cytogenetic analysis, such as identification of the Philadelphia chromosome in CML.[40,42] More sophisticated molecular approaches are now being combined with conventional analysis,[44] and catalytic domain mutation screening for imatinib resistance has added another level of diagnostic complexity.[45] Mutation in the catalytic domain of BCR–ABL that confers resistance to imatinib is used as a predictive biomarker for identifying patients that should be treated with dasatinib or nilotinib, and several companion diagnostics are available that enable these patients to be identified to receive the most appropriate treatment.[45,46]

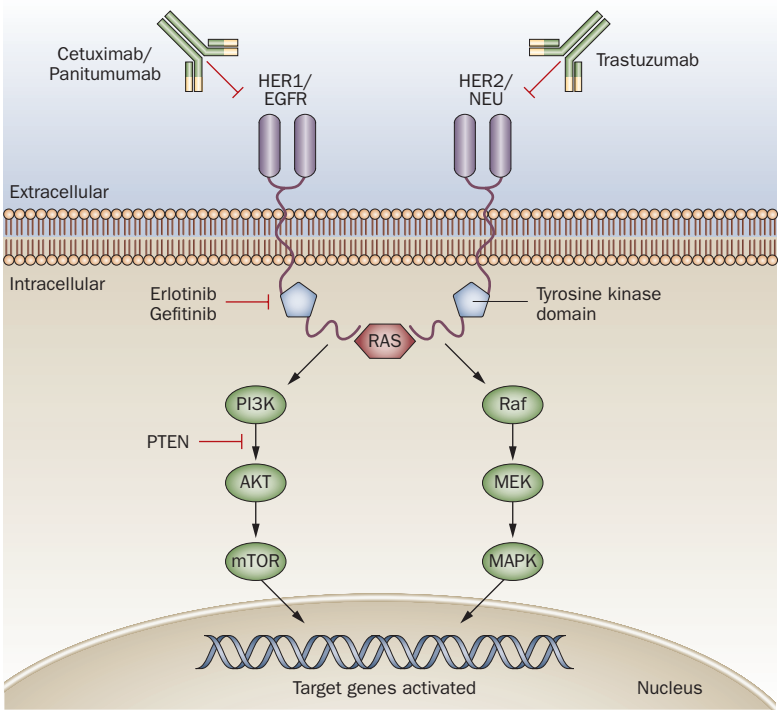

Figure 1 | Action of drugs targeting EGFR and HER2. HER family receptors consist of four members, HER1/EGFR, HER2/NEU, HER3 and HER4. The EGF ligand binds the extracellular domain of the EGFR or HER2 receptors, and the intracellular domain has intrinsic tyrosine kinase activity. Upon ligand binding, receptor dimerization occurs with activation of the tyrosine kinase leading to intracellular signaling (nodal points in the signaling pathway are indicated). AKT is activated downstream of PI3K, and has multiple targets including mTOR. Cetuximab and panitumumab are monoclonal antibodies that block EGFR activity. Trastuzumab is a humanized monoclonal antibody that blocks HER2 activity. Erlotinib and gefitinib inhibit the tyrosine kinase portion of EGFR and thereby prevent the receptor-mediated signaling pathway from being activated.

Imatinib also inhibits the receptor tyrosine kinase KIT, which led to clinical trials in patients with gastrointestinal stromal tumors (GIST), where antitumor activity has been observed in this poorly responsive disease.[47,48] Activating mutations in *KIT* correlate with drug response indicating that *KIT* mutations might be useful as a response-specific biomarker.[49]

EGFR and other biomarkers

EGFR is a member of the HER/ERB family of receptor kinases (Figure 1). It represents an important therapeutic target because it is frequently overexpressed and mutated in a number of cancers, including colorectal cancers (CRC) and non-small-cell lung cancer (NSCLC), affecting some 30% of carcinomas.[50] Both monoclonal antibodies and small-molecule tyrosine kinase inhibitors that target EGFR have become accepted therapies for both of these solid cancers.[50] Cetuximab was the first anti-EGFR monoclonal antibody to be approved for metastatic CRC, closely followed by panitumumab, which has demonstrated activity in metastatic chemorefractory CRC.[51,52] Both antibodies have similar efficacy and provide modest but clinically meaningful response rates of approximately 10% when used as monotherapy.[50]

In the initial clinical trials, the protein expression of EGFR was evaluated as a possible predictive biomarker at trial entry based on the assumption that EGFR expression would correlate with sensitivity to EGFR inhibitors.[50,53,54] However, a number of studies have shown that EGFR protein levels are poorly correlated with response in the clinical setting;[50] objective responses occur in patients with low or no EGFR expression as well as high EGFR expression.[50,54] The uncertainties surrounding the clinical value of EGFR expression led to the search for alternative biomarkers to identify patients that respond favorably to EGFR-targeted therapies.

Oncogenic activation of pathways downstream of EGFR involves a well-defined cascade that engages a variety of signaling proteins, including KRAS, RAF and PI3K (Figure 1). Mutation in *KRAS* results in continuous activation of MAPK or PI3K signaling, independently of EGFR activation;[55] *KRAS* is the most common gene mutation in the pathway (occurring in 35–45% of CRCs). Through retrospective analysis, the status of *KRAS* was found to be an important predictive biomarker for poor response to cetuximab and panitumumab.[14,56] Patients with tumors harboring *KRAS* mutations did not respond favorably or experience any survival benefit; progression-free survival was approximately half that of patients expressing wild-type *KRAS*.[57,58] *KRAS* is considered a predictive drug-response-specific biomarker, and the use of *KRAS* mutation as a diagnostic biomarker is increasingly being used to select patients who are unlikely to respond to these agents. In Europe, both panitumumab and cetuximab are indicated for patients with *KRAS* wild-type CRC, which is supported by a companion diagnostic (*KRAS* mutation detection); in the USA, a recent label update indicates that cetuximab and panitumumab treatment is no longer recommended for patients with *KRAS* mutations.[59,60]

NSCLC is the leading cause of cancer mortality, with *EGFR* activating mutations occurring in about 10% of cases.[50] As a consequence, two EGFR tyrosine kinase inhibitors were developed and subsequently approved for advanced-stage NSCLC, namely gefitinib and erlotinib.[50,61] Erlotinib and gefitinib show modest activity in NSCLC patients, although activating mutations that occur in *EGFR* correlate with a higher response rate.[62] Studies have shown that patients with tumors with exon 19 deletion *EGFR* mutations have longer survival following treatment.[63–66] *KRAS* mutations, which occur in 15–30% lung adenocarcinomas, have also been found to be associated with reduced response to these inhibitors.[50,67] Thus, *KRAS* mutation may similarly provide a response biomarker for patients that will not benefit, in contrast to *EGFR* activating mutations, which provide a predictive biomarker for a positive response.

Oncogenic rearrangements of the *ALK* gene have also been described in NSCLC, which occurs in about 3–5% patients and are mutually exclusive with *EGFR* mutations.[68] Crizotinib is a targeted therapy against ALK that has shown encouraging response rates in patients with a rearranged ALK gene in a predictive biomarker-driven clinical trial.[69]

In glioblastoma, *EGFR* is overexpressed in 40–90% of cases, usually due to gene amplification.[70] Clinical trials have suggested that the response to erlotinib or gefitinib is independent of *EGFR* amplification.[71] Response is associated with the co-expression of *PTEN*, which is frequently mutated in glioblastoma leading to activation of the PI3K/AKT signaling pathway[71,72] (Figure 1). Whilst studies on the value of PTEN as a predictive biomarker in glioblastoma are ongoing,[72] this finding does potentially provide an important means to subgroup patients into a responder population.

APL and *all trans* retinoic acid

Acute promyelocytic leukemia (APL) accounts for 10% of all acute myeloid leukemias. More than 99% of APL cases harbor a translocation between chromosome 15 and 17, which fuses the retinoic acid receptor (RAR)α gene on chromosome 17 with the PML gene on chromosome 15, resulting in a PML–RARα fusion protein.[73] Detection of the PML–RARα t(15:17) translocation is regarded as a diagnostic biomarker for APL, and used to define the most appropriate treatment regimen.[73,74]

We now have an understanding of how the PML–RARα fusion impacts on clinical response. The PML–RARα fusion protein binds to retinoic acid response elements in the promoters of target genes which, together with its heterodimeric partner protein RXR, recruits co-repressors, such as SMRT and N-CoR, and histone deacetylase (HDAC), with subsequent repression of retinoic acid responsive target genes.[74] The PML–RARα fusion protein binds co-repressors more avidly than the natural RARα protein, so physiological levels of *all trans* retinoic acid (ATRA) cannot overcome transcriptional repression mediated by the fusion protein.[75] Consequently, understanding the mechanism of action of the PML–RARα fusion protein led to disease remission in 90% of newly diagnosed patients.[76] ATRA therapy results in the differentiation of APL blast cells at pharmacological concentrations (10^{-6} M), but not physiological concentrations (10^{-9} M), and thus the higher dose became the relevant treatment regimen.[76] However, relapse frequently occurs within months following treatment,[76,77] which is now routinely followed by a chemotherapy combination.[78] Relapsed patients respond favorably to a combination therapy with arsenic trioxide, which has been suggested to target the PML–RARα fusion protein for degradation.[78] HDAC inhibitors also enhance ATRA-induced differentiation in NB4 cells, through a mechanism thought to reflect inhibition of HDAC and dissociation of the N-CoR/SMRT complex from PML–RARα.[79,80] Overall, the PML–RARα translocation correlates with response to ATRA and arsenic trioxide.[78] The detection of the translocation event has provided an important historical approach and a widely used predictive biomarker for diagnosis and treatment.

PARP inhibitors and *BRCA* deficiency

The breast cancer susceptibility genes *BRCA1* and *BRCA2* encode proteins that are components of the homologous recombination (HR) DNA-repair pathway, and mutation in either gene enhances cancer susceptibility

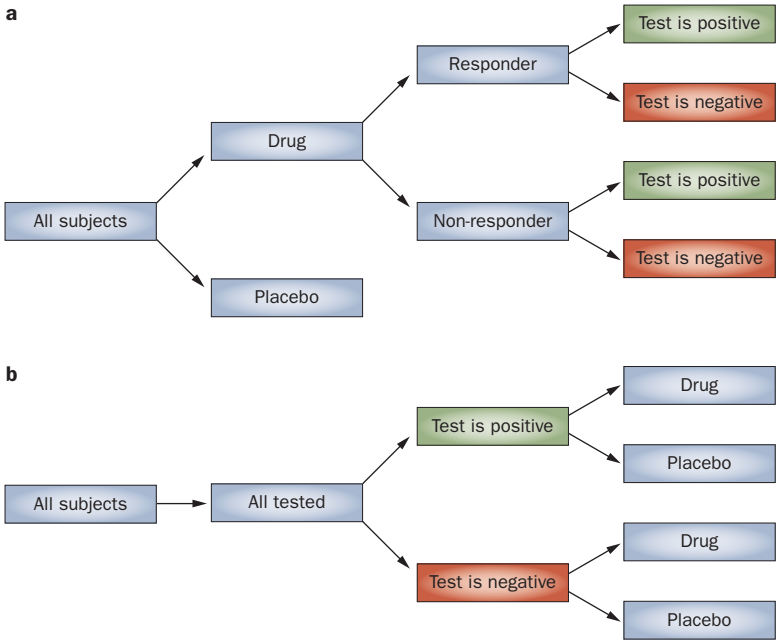

Figure 2 | Design of biomarker testing clinical trials. In **a** | a biomarker is assessed using historical data collected from previously conducted clinical trials where archived tissue from treated patients is compared for the expression of the biomarker in a retrospective analysis. In **b** | the biomarker status of all patients is used as a stratification factor in a prospective trial where each subgroup is subsequently randomized for treatment. The sample size is specified separately within each biomarker-based subgroup.

resulting in a 60% chance of developing breast cancer by the age of 90 years.[81] The fact that many pathways in mammalian cells are redundant has been exploited in the context of *BRCA1/2* deficiency and targeted therapy. The therapeutic strategy is based on the rationale that an aberration in one pathway, for example HR, renders cells more sensitive to specific inhibition of the compensatory pathway which, otherwise, would be non-essential.[82] This phenomenon of 'synthetic lethality' provides a potentially powerful approach to cancer treatment, and is illustrated well by the clinical strategy and anticipated utility of inhibitors of poly-ADP(ribose) polymerase (PARP) in *BRCA1/2* deficient tumors.[83]

PARP is a DNA-damage-sensing nuclear enzyme involved in DNA repair.[84] In most cell lines, treatment with PARP inhibitors has minimal effect on proliferation. However, breast cancer cells defective in *BRCA1* or *BRCA2* are highly sensitive to PARP inhibition, because of the role that each protein plays in the HR pathway, and the absence of redundant pathways.[85] Thus, in *BRCA1* and *BRCA2* defective cells, DNA damage normally repaired by the HR pathway remains unrepaired, and inhibition of PARP increases the prevalence of the damage, ultimately providing a signal for apoptosis.[83,85] Importantly, PARP inhibition is not critical for normal cells, most likely reflecting intact redundant pathways, so PARP inhibitors have the potential to impair tumor growth without damaging normal cells.[85] To date, clinical trials have shown promising results in *BRCA1* and *BRCA2* defective tumors, and in a range of tumors

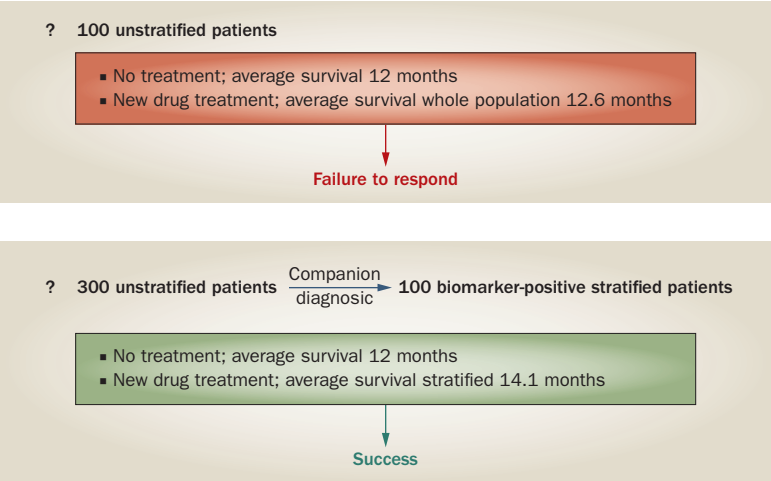

Figure 3 | Response-specific biomarkers in cancer clinical trials. In a clinical trial of a novel cancer drug with a typical response rate of 20% and increased survival of 3 months in an unselected patient population, the average survival in the overall population (12.6 months versus 12 months) is unlikely to represent a statistically significant difference and, therefore, provides insufficient justification to support further clinical activity. A patient population tested for a predictive biomarker with a 70% PPV for response in which a stratified subgroup of 100 patients is subsequently treated with a novel cancer drug will result in an average survival of 14.1 months. This increased survival would be significantly different enough compared to the unselected population to justify further clinical development. *This example assumes a 20% response rate in an unselected population and a companion PPV diagnostic value of 70%. Survival of non-responders is 12 months and of survivors 15 months. Abbreviation: PPV, positive predictive value.

PARP inhibitors enhance the cytotoxicity of a variety of agents.[86,87]

BRCA1/2 associated cancers account for only about 5% of breast cancer cases; however, they share a number of morphologic and molecular features with basaloid, triple-negative breast cancer, a subgroup that is notoriously difficult to treat.[88,89] O'Shaughnessy *et al.*[89] conducted a randomized phase II trial in patients with triple-negative breast cancer, to compare carboplatin plus gemcitabine with or without the PARP inhibitor iniparib. There was a greater degree of durable tumor stabilization in the PARP inhibitor arm (54% versus 34%) and median overall survival was improved by 5 months. Although this study raises questions about the relative contribution of PARP inhibition compared to an unknown mechanism of action, and that patient selection is based on triple-negative status rather than by mutational analysis, we await the results of the phase III study to see if these intriguing findings can be reproduced. A recent update has indicated that a phase III trial of iniparib combined with gemcitabine and carboplatin in patients with triple-negative metastatic breast cancer has failed to meet prespecified criteria for significance for the co-primary end points of overall survival and progression-free survival.[90]

The future clinical application of PARP inhibitors has yet to be determined. It is likely, however, to be dependent on identifying patients with *BRCA1* or *BRCA2* deficiency as a basis for personalized care. Myriad Genetics have developed a BRAC*Analysis*® test, which currently acts as a companion diagnostic to stratify patients in phase II clinical trials of PARP inhibitors (olaparib and veliparib).[91] The companion diagnostic BRAC*Analysis*®, which predicts the response to PARP inhibitors, might eventually be included on the drugs label.

Melanoma and *BRAF* V600E inhibitors

Melanoma accounts for about 80% of deaths from skin cancer, with a 5-year survival rate of 15%.[92] BRAF is a serine–threonine kinase that activates MAP/ERK kinase signaling (Figure 1). Approximately 60% of melanomas harbor activating mutations in *BRAF*,[92] and most commonly valine 600 is replaced by a glutamate residue (V600E). The specific BRAF inhibitor vemurafenib demonstrated an impressive antitumor response rate in patients identified according to the $BRAF^{V600E}$ mutation.[93] Patients were selected based on presence of $BRAF^{V600E}$, and in the phase I trial an 81% response rate was observed in patients with metastatic melanoma.[93] The presence of the $BRAF^{V600E}$ mutation acts as a predictive response-specific biomarker for vemurafenib, allowing patients to be subgrouped for treatment. Significantly, drug resistance frequently developed after initial responses sometimes occurring with low-grade squamous carcinomas, which was shown to reflect receptor tyrosine kinase-mediated activation or activated NRAS mediated reactivation of the MAPK pathway.[94] Importantly, the reactiviation of MAPK/ERK signaling predicts sensitivity to MEK inhibitors, and an additional therapeutic strategy. Upregulation of PDGFRβ or mutations in the *NRAS* gene are potential biomarkers for melanoma resistant to vemurafenib.[95]

Development of predictive biomarkers

The examples discussed previously provide evidence of the increasing importance of predictive biomarkers as companion diagnostics to assist the clinical application of cancer medicines. To date, the handful of biomarkers that have successfully reached the clinic and gained utility as companion diagnostics were identified mostly through retrospective analysis of clinical trial data and coincidental *ad hoc* genetic analysis. The historical knowledge of mutations and associated molecular heterogeneity that was available, before drug development, has rarely featured as an integral component of the prospective trial design. The challenge now is to exploit current techniques that enable predictive biomarkers to be identified in a systematic prospectively-driven fashion, allowing drug development to progress hand-in-hand with the associated biomarker, and thereby open up a new more hypothesis-based approach to developing personalized cancer therapy.

A variety of different high-throughput approaches have been applied to biomarker identification, including large-scale DNA sequencing, single-nucleotide polymorphism analysis, and transcript profiling by microarray and proteomics.[4] In general, these techniques produce correlative data that can identify genes and proteins that coincide with disease state or therapeutic response, but may be difficult to integrate with the mechanisms involved in

attaining the tumor phenotype or action of the drug.[13] Therefore, research has focused on developing platforms that allow functionally relevant biomarkers to be identified, which can then be rationalized in the context of the mechanism of tumor cell killing by the drug, and used to support and refine its clinical development.

At a theoretical level, it is possible to imagine two types of predictive biomarker that could find clinical utility; biomarkers that inform on drug resistance (such as *KRAS* mutation in CRC) and, conversely, those that identify drug-responsive tumors (such as *BRCA* mutation in breast cancer). The advent of genetic screens, performed either at the genome-wide level or on selected populations of genes by RNA interference, has facilitated the identification of both classes of biomarkers.[96] In a study seeking to identify genes that influence breast cancer sensitivity to trastuzumab, *PTEN* was identified as a modulator of drug sensitivity.[18] PTEN acts as a negative regulator of the PI3K pathway and inhibits proliferation (Figure 1).[97] Low levels of *PTEN* or oncogenic mutations in *PIK3CA* conferred resistance to trastuzumab and, in breast cancer patients, low *PTEN* levels or oncogenic *PIK3CA* mutations were associated with poor prognosis in response to trastuzumab.[18] In another study, RNA interference was used as a screen to identify genes that impact on tamoxifen sensitivity, and *CDK10* was identified as a determinant of resistance.[98] At a mechanistic level, *CDK10* depletion enhanced MAPK signaling through enhanced transcription of *RAF*, leading to a reduced reliance on estrogen-receptor signaling.[98] Significantly, low expression of *CDK10* was associated with poor clinical response to tamoxifen.[98]

A similar approach was taken to identify genes that modulate drugs that target epigenetic mechanisms. A genome-wide loss-of-function screen identified HR23B, a dual purpose protein involved in DNA repair and protein targeting to the proteasome, as a determinant of sensitivity to HDAC inhibitors. The proteasome targeting activity of HR23B is responsible for its role as a sensitivity determinant, which highlighted proteasome inhibition as part of the mechanism that contributes to the antitumor effect of HDAC inhibitors.[99] An analysis of HR23B as a predictive biomarker in patients treated with HDAC inhibitors found a favorable correlation between HR23B levels and clinical outcome.[100] Notably, the deregulation of proteasome activity implied that a combined HDAC inhibitor and proteasome inhibitor therapy regimen might be clinically beneficial. Several studies evaluating HDAC inhibitors in combination with proteasome inhibitors have suggested encouraging clinical activity.[101,102]

In another example of the synthetic lethal screening approach, genes that influenced the sensitivity of tumor cells to paclitaxel were identified as potential predictive biomarkers.[103] The genes included those encoding a number of proteasome subunits, and depleting proteasome subunits was shown to enhance sensitivity to paclitaxel.[103] Again, paclitaxel and bortezomib have been shown to be a favorable clinical combination.[104] In a study to identify kinases that impact on gemcitabine therapy in pancreatic cancer, CHK1 was identified as a sensitivity determinant, suggesting further that CHK1 might serve as a biomarker and possible therapeutic target for enhancing the activity of gemcitabine in pancreatic cancer.[3]

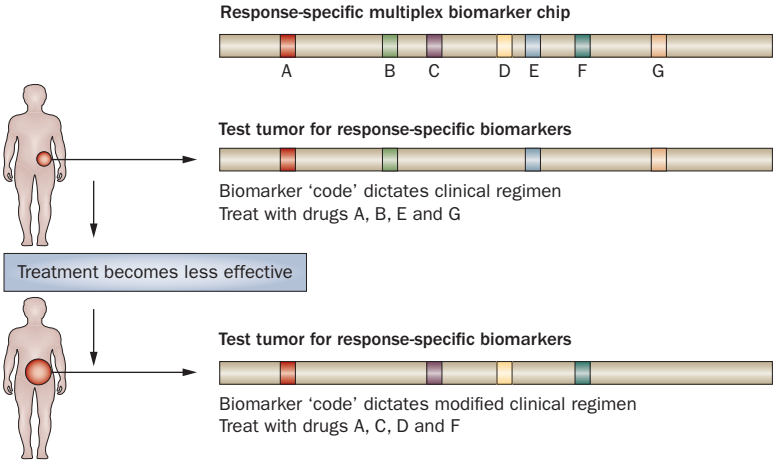

Figure 4 | A theoretical approach to personalized cancer therapy. It is envisaged that a multiplex chip, representing biomarkers that inform on the clinical activity of an array of cancer drugs, detects a biomarker profile (the biomarker 'code') in a tumor. The biomarker 'code' present in the tumor thereafter dictates the treatment regimen (in the example a combined treatment of drugs A, B, E and G). Should the treatment become less effective, a new biomarker 'code' might dictate a modified treatment regimen. This approach of establishing a biomarker 'code' with disease progression in theory can be repeatedly used.

These examples illustrate how functional genome-wide screening approaches can be employed to address the pathways affected by a drug, and assist in identifying relevant biomarkers for drug response. The challenge is to use these technologies and apply the information in the best possible way to facilitate drug development and consequent clinical benefit.

The shifting paradigm

HER2 amplification occurs in breast cancer, and its expression is associated with poor prognosis; therefore, the clinical development of trastuzumab focused on patients whose tumors expressed high levels of HER2.[22] Early studies in patients with metastatic breast cancer and HER2-positive tumors showed a significant survival benefit,[22] which subsequently led to the approval of trastuzumab for metastatic breast cancer.[26] As a consequence, it has been argued that incorporating predictive biomarkers in clinical development should result in faster, cost effective and more successful drug development.[4,13] Some biomarker-led clinical studies have not been as successful.

The development of cetuximab and panitumumab provide a case in point. The clinical development strategy was largely focused on patients exhibiting high EGFR expression.[14] A number of large randomized phase III studies in EGFR-positive patients with metastatic CRC concluded that there was clinical benefit,[53,54] indicating that EGFR levels act as a predictive biomarker for clinical response, although subsequent studies questioned this tenet. For example, one study found that the

clinical benefit of cetuximab was not confined to high EGFR expressing tumors, and another study showed that the response coincided with wild-type *KRAS* rather than mutated *KRAS*.[56–58] The hypothesis that EGFR expression correlates with clinical response was questioned and led to a retrospective search for other informative biomarkers.

Predictive biomarkers were identified late in the development of erlotinib and gefitinib. Clinical trials in advanced-stage NSCLC cancer suggested that responses occurred with tumors harboring *EGFR* mutations, although disease-free survival correlations have not yet been determined.[63–65] Despite these uncertainties, it is clear that the predictive biomarkers developed for EGFR targeted therapies have been mostly identified through retrospective analysis. Retrospective identification of predictive biomarkers is likely to be an important strategy well after approval of the drug. Once identified, the predictive power of the biomarker can then be tested in the clinical setting in a hypothesis-driven prospective trial, where patients are selected and stratified on the basis of biomarker expression (Figure 2).

Such retrospective correlative studies need to be appropriately statistically powered to be considered reliable and although the literature is littered with small and unconvincing reports there is a movement to embrace a more-standard format for publication of predictive marker studies and recognition that the sample size for such studies is about fourfold greater than for prognostic markers.[105] Once the marker has been validated in a well-designed retrospective study, logical progression to a prospective, stratified trial (Figure 2) in which the therapeutic agent would be expected to perform better in the biomarker-selected patient group in a phase II setting, would increase the likelihood of success and path to registration (Figure 3).

Conclusions

A new era of personalized cancer medicine is upon us, with the unprecedented opportunity to personalize virtually any new or existing cancer drug. The remarkably powerful and effective technologies currently available allow predictive response-specific biomarkers to be defined and validated in the laboratory, and thereafter tested in a hypothesis-driven fashion in the context of the clinical disease. Biomarkers identified based on rigorous scientific studies and tested in focused well-designed clinical trials will allow more efficient clinical development, with an associated reduced drug-attrition rate. For the cancer patient, the benefits will be enormous, reflecting the approval of more-efficacious and less-toxic therapies. It now is a realistic possibility to correlate biomarker expression with disease progression, identify a biomarker 'code' and thereafter tailor the treatment on a continuous basis to maximize patient benefit (Figure 4). In effect, the long sought after goal of managing cancer as a chronic disease, where therapeutic choice is guided by an informative predictive biomarker 'code' is finally a reality. A coordinated large-scale effort aimed at delivering biomarkers that inform on drug response, subsequently deployed in the cancer clinic as robust companion diagnostics, provides us with the unique and unprecedented opportunity to deliver personalized cancer therapy on an ongoing and rational basis.

Review criteria

The PubMed database was searched for articles published before January 2011. Electronic early-release publications were also included. Only articles published in English were considered. The search terms used included "companion diagnostics", "cancer biomarkers", and "targeted therapy".

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Author contributions

Both authors researched data for inclusion in the article. N. B. La Thangue contributed to the writing, editing and reviewing the manuscript before submission and during the reviewing process.