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REVIEW PAPER

Somatic variation and cancer: therapies lost in the mix

Andrew V. Biankin · Thomas J. Hudson

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Abstract Cancer arises as a consequence of mutations in genomes of cancer cells, which over time allow them to proliferate and spread to distant sites. Large-scale sequencing of cancer genomes is revealing an increasing number of potential driver mutations that may allow specific targeting of cancer genes, proteins, and pathways. Comprehensive views of cancer genomes are also revealing enormous heterogeneity of mutation profiles, even among tumours derived from the same organs and having similar pathological characteristics. There are now many examples where mutation profiles observed in tumours have been shown to correlate with clinical features of disease, drug response, and patient outcomes. When ignored, molecular heterogeneity can lead to failures in drug development, as drugs that may have efficacy in subgroups of patients with specific molecular phenotypes may show marginal response when tested in large groups of unselected patients. This article explores issues relevant to the clinical translation of sequence-based mutation profiles in the clinical development of targeted therapies and in the future management of cancer patients.

A. V. Biankin
Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, Sydney, NSW 2010, Australia

T. J. Hudson (✉)
Ontario Institute for Cancer Research, 101 College Street, Toronto, ON M5G 0A3, Canada
e-mail: tom.hudson@oicr.on.ca

Introduction

The development of therapies that target specific molecules or pathways involved in tumour growth and progression has improved outcomes in several cancer types and ushered in a new era in cancer therapy (Majewski and Bernards 2011). Benefits from these therapies usually correlate to the presence of a biomarker of drug responsiveness and/or resistance in the tumour of the patient (Wistuba et al. 2011). Historically, the most effective and successful biomarker of drug responsiveness is the oestrogen receptor (ER), which predicts response to the anti-cancer agent Tamoxifen which targets oestrogen signalling and improves survival of breast cancer patients. The most recent overview of randomised trials of early breast cancer conclude that 5 years of adjuvant tamoxifen therapy in ER+ disease reduces annual risk of death from breast cancer by 31% (EBCTCG 2005). This, and more recent examples of biologically targeted therapies, e.g., Herceptin® in c-erbB2/HER-2-amplified breast cancer (Harries and Smith 2002) and Erlotinib® and EGFR mutations in lung cancer (Chin et al. 2011), have led to a growing consensus that the effective management of cancer requires a stratified, and ultimately individualised approach based on the use of therapies guided by biomarkers that can be measured in the patient or the tumour prior to therapy.

One can imagine a day where an individual's germline and cancer genomes will be deeply characterised to guide the clinician to the right therapeutic strategy for that person. This article explores the opportunities and challenges created through advances in genomic sequencing technologies in advancing such "personalised medicine" strategies for cancer treatment. Given several recent reviews describing the exponential growth in knowledge regarding cancer genomes (Chin et al. 2011; Farrell 2011; Green and

Guyer 2011; Macconaill and Garraway 2010; McDermott et al. 2011; Stratton et al. 2009), this article will only briefly describe the emerging concepts in cancer genomics, before expanding into issues relevant to clinical translation.

The cancer genome

Cancer arises as a consequence of acquired changes in the structure and DNA sequence of the genomes of cancer cells. Progressive accumulations of aberrations that provide cells with specific advantages allow them to survive and proliferate in their microenvironment, and subsequently spread beyond the confines of their tissue and organ to invade other structures and metastasise to distant sites.

There are several types of distinct somatic mutations that occur in the DNA sequence of a cancer cell genome. These may include single nucleotide substitutions where a single base is exchanged for another; insertions or deletions where one or more nucleotides of DNA fragments are added or deleted; inversions, where the orientation of a chromosomal segment is reversed; translocations, where DNA segments from non-homologous chromosomes recombine; and gains/amplifications, where the copy number of a gene or DNA segment increases from the usual two copies (to greater than a thousand copies in some cases). In addition, a cancer cell may acquire DNA from an external source such as a virus (e.g., HPV). These somatic, as distinct from inherited (germline), alterations in DNA sequence are acquired by a cancer cell during its lifetime, both before and after neoplastic transformation (Stratton et al. 2009). It is probable that only a minority of aberrations seen in the DNA sequence of a cancer cell result in an alteration in phenotype (driver mutations). Differentiating "driver" mutations from "passenger" mutations to identify therapeutic targets is a current challenge in cancer genomics (Chin et al. 2011; Chin and Gray 2008). In addition, epigenetic modifications also play an important role (Rodríguez-Paredes and Esteller 2011). To further add to this complexity, alterations that did play a significant role in early neoplastic transformation and cancer progression may cease to be important later in the disease process (Stratton et al. 2009). Moreover, the microenvironment and treatment may also alter the phenotype of cancer cells by changing selection pressures such that metastases from a single primary may have significantly different genetic aberrations, with potentially differential sensitivities to therapy. Whilst experimental models have demonstrated that cells can be transformed by as few as 4 or 5 key driver mutations, more recent high throughput, large-scale genome analyses have demonstrated that the mutational landscape of cancer is complex (Beerenwinkel et al. 2007). These studies have suggested that cancers evolve by accumulating driver mutations in their genomes, of as many as 20, and perhaps hundreds, of cancer-associated genes. Defining key driver mutations, the resulting aberrations in molecular mechanisms and the subsequent phenotype underpins the contemporary development of novel therapeutic strategies (Croce 2008). Advances in sequencing technology have initiated several coordinated national and international efforts including The Cancer Genome Atlas (TCGA 2008) and the International Cancer Genome Consortium (ICGC 2010), to generate comprehensive catalogues of genomic, transcriptomic, and epigenomic changes in multiple different tumour types and will pave the way for sequencing to become an integral component of clinical trials and clinical practice.

Large-scale studies of cancer genomes from many cancer types suggest that distinct molecular phenotypes are numerous and in many cases each phenotype accounts for 10% or less of a particular cancer type. This growing evidence regarding the high molecular heterogeneity of cancer leads many to postulate that the clinical heterogeneity observed in patients, particularly for phenotypes which vary in outcome and therapeutic responsiveness, is a consequence of the molecular heterogeneity observed in cancer genomes (2011a). This has implications in regards to current clinical practices and the development of novel therapeutic strategies (Chin et al. 2011; Hall et al. 2010).

Cancer heterogeneity and therapeutic development

Difficulties in developing cancer therapeutics over the last 50 years (Ocana and Tannock 2011; Seruga et al. 2010) may potentially be due to a failure in addressing issues of cancer heterogeneity (Arrowsmith 2011; Bates 2010). It is even possible that potentially useful drugs for subsets of patients were deemed failures because clinical trials were searching for a "wonder drug" to capture all cancers of a specific organ, e.g., lung or pancreatic cancer. Drugs that would have been useful in subgroups of patients with specific molecular subtypes of cancer tested would not have shown efficacy in larger groups of patients with disparate molecular phenotypes. Such candidate therapeutics that were "lost in the mix" could potentially be reassessed, revived and used effectively if responsive subgroups could be identified prior to, or early in therapy.

As we better appreciate the biological heterogeneity of cancer, and that of each individual's germline, research is focusing on defining responders and either targeting them directly, or enriching for them using biomarkers. This approach significantly impacts on therapeutic development. Clinical trials in the development of Trastuzumab (Herceptin®), the therapeutic monoclonal antibody against

HER2/*neu* recruited only those patients with over-expression/amplification of the target molecule, which substantially decreased the number of patients required to demonstrate efficacy, accelerating its implementation and subsequent extension to gastric cancer (Bang et al. 2010). Despite these experiences and others such as resistance to Cetuximab® therapy in KRAS mutant colon cancer and EGFR mutation and Erlotinib sensitivity in lung cancer, significant investment in large scale clinical trials in unselected patients persists (LoRusso et al. 2010; Tursz et al. 2011). Although some regulatory authorities have released guidelines concerning approvals for targeted therapies with companion diagnostics (2011b), there remains no consensus, and regulatory approval is still heavily dependent on traditional definitive large scale non-selective clinical trials (Hutchinson 2011; McClellan et al. 2011; Tursz et al. 2011).

Developing biomarkers

Key to implementing personalised medicine is the development of reliable biomarkers that inform the physician of the underlying risk or disease characteristic. Biomarkers that predict responsiveness and/or resistance to existing therapies are obviously of high importance to clinicians. Biomarkers have also become instrumental in the development of novel therapeutics, and in many cases are developed as companion tests to new targeted therapies (Harris 2010; Schilsky 2010).

Although the term biomarker can be defined to encompass indicators of physiological and pathogenic processes such as blood pressure or heart rate, biomarker is more commonly used to define molecular assays such as prostate-specific antigen and liver function tests. Oncology biomarkers used to guide therapies analyse a range of DNA mutations including copy number alterations, gene expression, DNA methylation, and protein expression. A companion article in this issue of Human Genetics by Pajic et al. (2011) provides a more detailed background on biomarker development strategies. It is worth noting that in general, biomarkers for genetic lesions are more predictive than overexpression [e.g., FISH for amplified HER2 vs. immunohistochemistry (IHC) for ERBB2 as biomarkers of response to Herceptin] (Kovacs and Stenman 2010).

Biomarkers for existing therapies

The methods to evaluate any biomarker diagnostic test have long been underdeveloped compared with methods to evaluate therapeutics (Andre et al. 2011). This is a problem that is increasing in complexity as biomarkers move from single-entity to higher throughput and multiplex assays. It is clear that the use of biomarkers has been compromised by both insufficient validation and lack of evidence to support clinical value over conventional clinico-pathological indicators. Even for commercialised FDA-approved cancer biomarker assays (e.g: OncotypeDX and MammaPrint), there remain unanswered questions as to the real clinical utility of these in vitro diagnostics. These concerns regarding classifiers already in clinical use, serve to highlight that translation of candidate biomarkers to implementation in the clinic is complex (Hood and Friend 2011; Mendelsohn et al. 2011). This is exemplified by the fact that even though myriad candidate biomarkers of prognosis and therapeutic responsiveness have been assessed, very few have made it through to routine clinical use that improves patient management and outcome (Andre et al. 2011). Knowledge of the target and the underlying molecular mechanisms of action of a therapeutic significantly enhance the development of biomarkers, and has led to the development of several biomarkers for established therapies (e.g., *KRAS* mutation and Cetuximab® responsiveness in colon cancer; Karapetis et al. 2008). For more traditional cytotoxic therapies that target more generic mechanisms and constitute over 80% of current therapies, there have been very few successes. Furthermore, there is little commercial incentive to develop such strategies, even though the impact on the community may be substantial. This lack of significant investment in co-ordinated efforts to develop such tools has resulted in insufficient or inappropriate infrastructure to successfully identify, validate, test and implement clinically useful biomarkers. Currently, the majority of such efforts have relied on disparate patient cohorts of convenience which are accessed individually without broad-based co-ordination of appropriate test populations.

Novel therapeutic/companion biomarker development

Increasingly, the development of a novel targeted therapy involves defining drug-diagnostic combinations where the presence of a molecular marker identifies patients who are most likely to respond to the new treatment. This model of developing treatment and diagnostic/companion biomarker combinations in order to target patient populations with a greater chance of benefiting from treatment as exemplified by the earliest targeted therapies, i.e: ER/Tamoxifen and HER2/Trastuzumab. This strategy is facilitating significant advances in some cancer types, with recent examples being the successful use of the BRAF inhibitor PLX4032, which targets a specific mutation (V600E) in melanoma (Nathanson 2010), and the targeting of the EML4-ALK fusion in NSCLC (Gerber and Minna 2010) (Fig. 1).

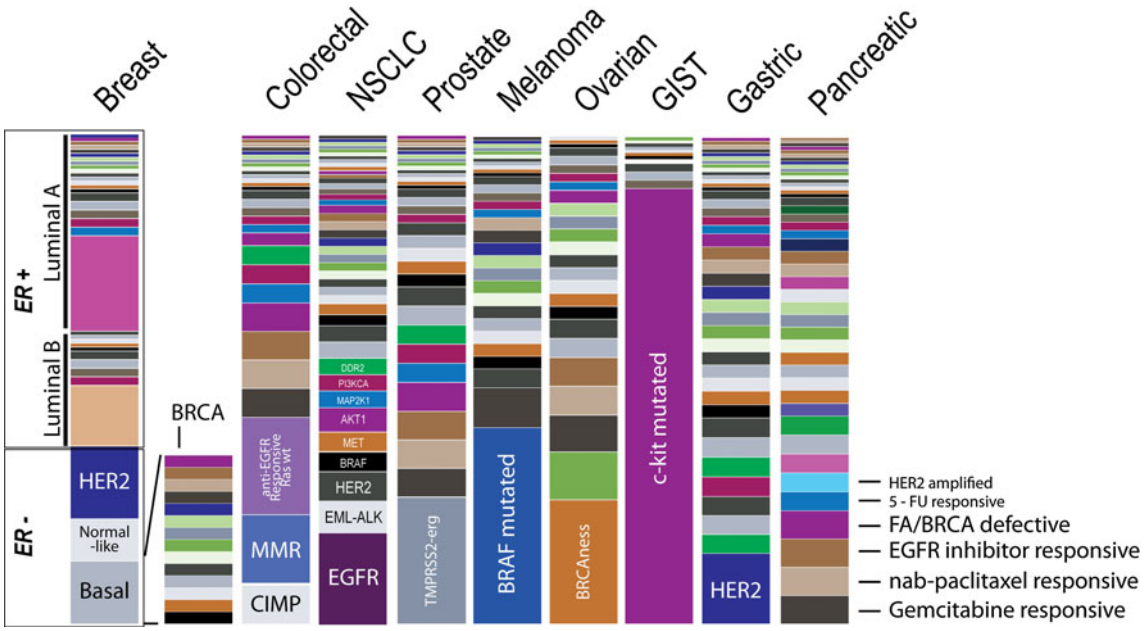

Fig. 1 Emerging molecular taxonomy of several solid organ cancers that inform clinical decisions with respect to prognosis, treatment and the design of clinical trials. Defining efficacy in less prevalent phenotypes is below the level of sensitivity of conventional clinical trial approaches, and may contribute to the lack of current effective therapies for cancer phenotypes that occur at low frequency such as subtypes of basal breast cancer and potentially pancreatic cancer. The degree of fractionation (stratification) is likely to increase over time as knowledge concerning molecular phenotypes and the development of appropriate therapies grows. Note the phenotypic "tails" of multiple variants are long, and that even those cancers where dominant phenotypes are known to exist, the residual subtypes may be very heterogeneous, and as a consequence, difficult to treat, e.g., basal breast cancer

The road to implementation

The National Cancer Institute (NCI) has defined a strategy for the development of biomarkers through to clinical application, which is represented by four phases (Cordon-Cardo 2004; Freidlin et al. 2010; Khleif et al. 2010) (Fig. 2):

Phase I *Assay development and pilot studies.* In the initial phase, appropriate robust and reproducible assays are developed. These are assessed in relatively small cohorts of patients, often referred to as training sets, to define biomarkers with potential clinical utility.

Phase II *Retrospective clinical analysis*. Those biomarkers that are informative in phase I are tested in larger independent, well-characterised cohorts of patients. These include retrospective analysis of material from prospective clinical trials.

Phase III *Prospective confirmatory analysis*. Candidate biomarkers with supportive data from phases I and II are assessed prospectively, but not as a definitive trial. Approaches used include: (a) prospective sample acquisition in a trial where there is a therapeutic hypothesis and biomarker assessment is a secondary objective, and (b) prospective biomarker assessment that is underpowered to definitively address biomarker utility, but provides additional supportive data to justify the significant investment of further testing.

Phase IV *Multi-institutional validation trials*. These are high-powered prospective randomised controlled trials designed specifically to test the clinical utility of the candidate biomarker and provide level 1 evidence for implementation.

Clinical trials of biomarkers (phases III and IV)

Optimal design of clinical trials assessing biomarker utility will vary according to the potential clinical use. Factors influencing the design of the trial will include the existing level of evidence, statistical considerations, ethical implications and feasibility. In general, randomised controlled trials for the evaluation of biomarkers broadly fit into four designs (Lee et al. 2009; Mandrekar and Sargent 2009; Sargent et al. 2005) (Fig. 3).

1. *Biomarker analysis within existing clinical trials* (Fig. 3a). A relatively cost-effective method of adding to the body of evidence to support a significant investment in prospectively testing a promising biomarker is assessment in a previously conducted or

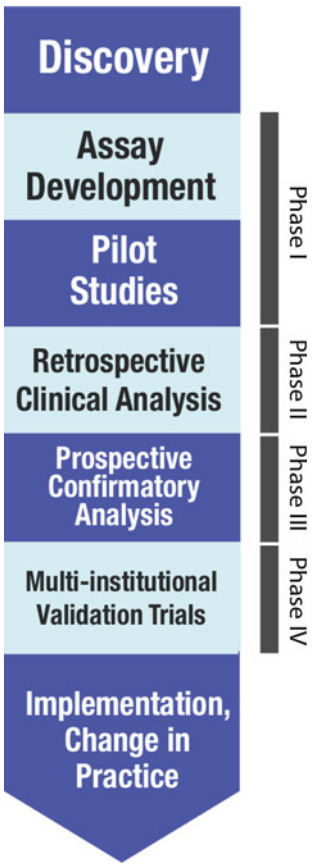

Fig. 2 The NCI strategy for biomarker discovery and development

currently active clinical trial designed to address a therapeutic question. An example of this is the current validation of the Oncotype DX® Colon Cancer Assay in QUASAR, which examined the benefit of adjuvant 5-Fluorouracil in stage II colon cancer. Although these studies can be informative, they were designed to test a therapeutic, not a biomarker, so that patient selection and statistical power to adequately assess the biomarker is unlikely to be optimal. In addition, the variable availability of usable samples further influences the statistics and may introduce further bias.

2. *Non-targeted randomised controlled trial* (Fig. 3b). Amongst several reasons for utilising non-targeted trial designs, the most common is insufficient evidence to support altering current practice even in a clinical trial setting. In this trial design, treatment decisions are not based on results of biomarker analysis, but sample collection and assessment is performed prospectively within a trial that is statistically appropriate to address the biomarker hypothesis.
3. *Targeted* (*enrichment design*) *randomised controlled trial* (Fig. 3c). This is the most efficient design since only patients where the signature result is likely to alter outcomes with alternative treatment are recruited, thus minimising the overall number of patients required. This trial design is most appropriate when there is sufficient evidence either from previous studies, such as from 1 and 2 above, or biological evidence to justify significant alterations in clinical practice in the setting of a trial. An example is the testing of trastuzumab only in HER2 amplified breast, and subsequently gastric cancers.
4. *Classical randomised controlled trial* (Fig. 3d). In this trial design, a novel therapeutic strategy is assessed against current standard practice. Biomarker-guided therapy in addition to conventional tests is compared to conventional tests only. This design is not efficient, since the subgroup of patients where the signature may actually influence outcomes usually constitutes the minority of recruited individuals

Emerging clinical trial strategies

There are recent examples of dramatic successes where therapeutics that target specific mechanisms have progressed rapidly to implementation, most notably that of the EML4-ALK translocation in NSCLC and response to the ALK inhibitor crizotinib (Gerber and Minna 2010). In this example, alignment of several key factors resulted in dramatic efficacy, which was rapidly identified in a clinical trial. The degree of alignment of these factors ultimately determines not only the applicability of the drug but also the speed and ease it is translated to the clinic. These factors include: (1) correct clinical indication, (2) drug efficacy in the appropriate phenotype, (3) correlation of target (biomarker) and response, (4) accuracy and reproducibility of the assay in detecting the target. Figure 4 presents the various challenges within the spectrum of clinical trial designs in defining efficacious therapeutic strategies. Although unselected patients in phase III clinical trials reduce the risk of missing potential responders, the higher proportion of non-responders would result in a reduced ability to detect efficacy. The incorrect indication and indirect enrichment for potential responders such as performance status may occur inadvertently in phase II clinical trials, or be part of inclusion/exclusion criteria in phase III trials, but may also miss the true responders.

Even when the drug is developed to a specific target, the presence of the target does not necessarily directly reflect response due to off-target effects, as well as a number of mechanisms of resistance that are inherent to the tumour (such as decreased cellular drug uptake or mutation of target genes) or extrinsic (variability in angiogenic, inflammatory or stromal response) (Mellinghoff and Sawyers 2002; Shekhar 2011). Recent studies of acquired

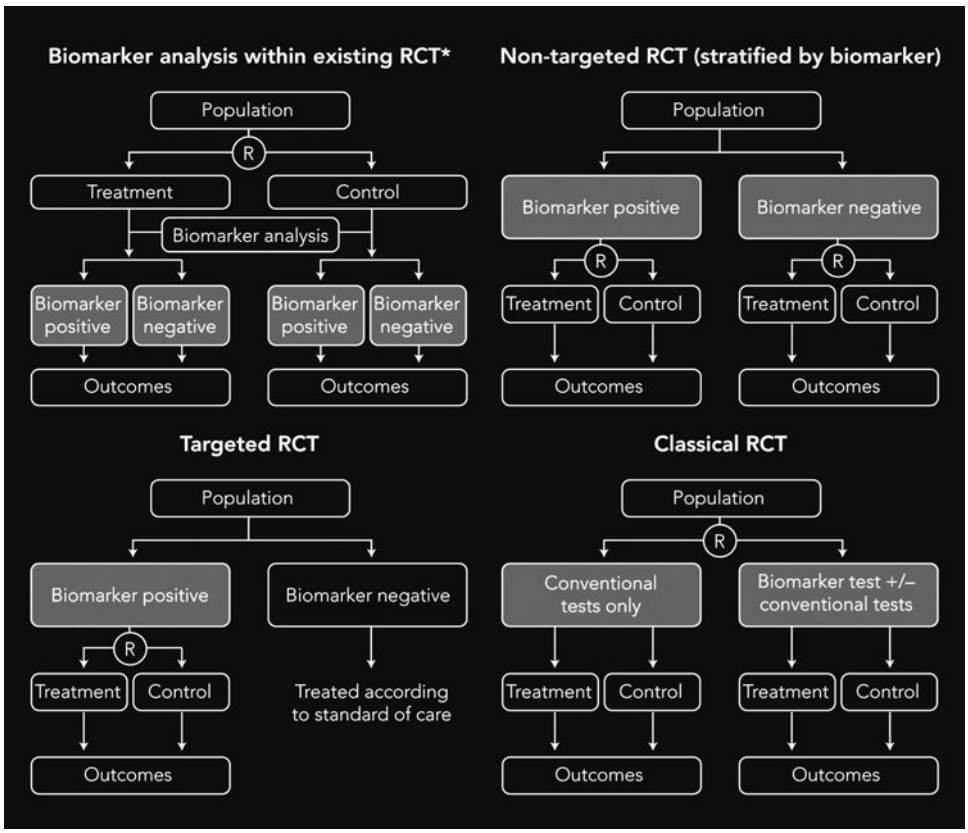

Fig. 3 Schema for randomised controlled trials used to assess biomarkers. From Lee et al. (2009) © Copyright 2009. *The Medical Journal of Australia*—reproduced with permission. *RCT* randomised controlled trial, ® randomisation

resistance to the RAF inhibitor PLX4032 suggest three new possible mechanisms of resistance (Johannessen et al. 2010; Nazarian et al. 2010), although the relative importance of each needs to be further explored (Solit and Rosen 2011).

The accuracy of the assay in detecting the target, or responders adds another layer of complexity. As a consequence, inadequacy in any of these criteria is likely to lead to failure to accurately detect true responders in a targeted clinical trial, and may not be a reflection of inadequate functional biology that underpins the therapeutic strategy. When there is a good alignment of all these factors, as with EML4-ALK translocations and treatment with crizotinib, or BCR-ABL and treatment with Imatinib, then the effect is clearer, such that clinical trial results are more interpretable. Less direct correlation between the presence of the target and response and the accuracy of the assay are evident in the implementation of Herceptin for HER2-amplified cancer (breast and gastric), where assays may lack sensitivity, and a significant proportion of tumours with the target are intrinsically resistant (Esteva et al. 2010). This is also a factor with the use of cetuximab in colon cancer and the role of mutant KRAS (Allegra et al. 2009), and EGFR mutations and lung cancer (Wheeler et al. 2010). Fortunately, initial studies for these indications were able to be modified or redesigned to identify and adjust target populations and assays. Although these drugs display efficacy in these subgroups, there is also potential to refine them further over time. The central challenge is to define principles and strategies where responders, the target and assays do not align perfectly, so that they can be modified as the trial progresses to better define responders (Nelson 2010).

Whilst comparative efficacy testing for all-comers is well established, biomarker-driven trials still need to be developed to their full potential in order to fulfill the demands of contemporary cancer care (Fig. 5). Pre-clinical models may prove to be beneficial in resolving some of these issues more efficiently (Caponigro and Sellers 2011), and an article addresses this strategy later in this issue (Pajic et al. 2011). One of the challenges in clinical trials of novel targeted agents is that no single, or suite of trial designs suits all; however, the principle is primarily concerned with the ability to adjust the target population (Rosenberger and Lachin 1993). Novel clinical trial strategies are evolving that build in designs that are adaptable and respond to findings as the trial is running. An example of this is the BATTLE trial for lung cancer (Kim et al. 2011) (Biomarkers-integrated approaches of targeted therapy for lung cancer elimination), where initial experience with the strategy enabled the refinement of the target population for each drug. This adaptive phase II trial of 255 patients with advanced NSCLC assessed if specific drugs were effective in molecular phenotypes defined using

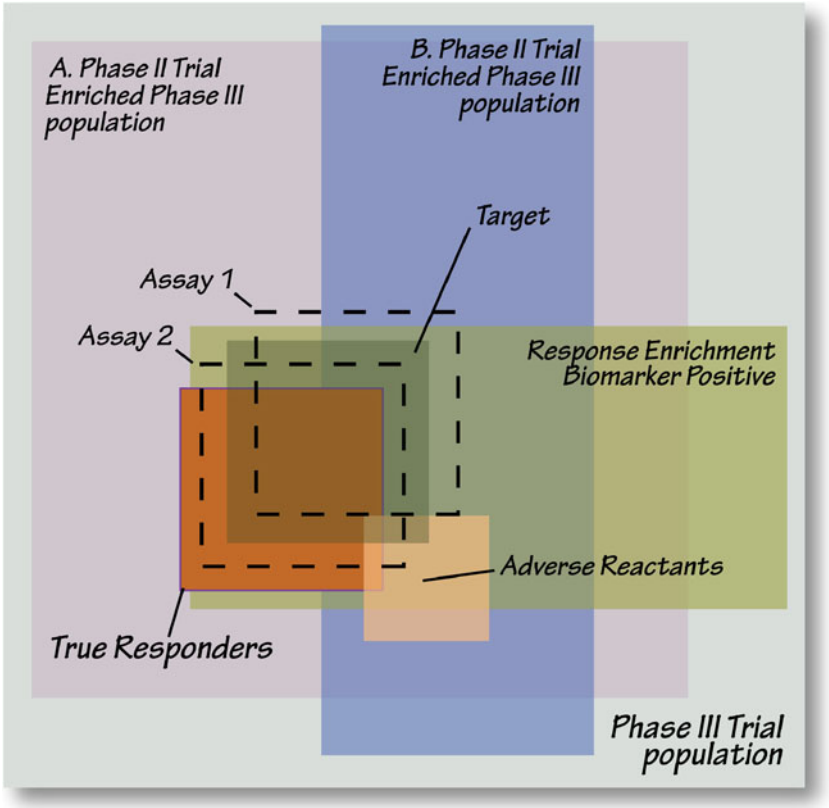

Fig. 4 The impact of the heterogeneity of cancer on clinical trials of novel therapeutic strategies. True responders constitute 5% of the overall phase III trial population. If evenly distributed between treatment and control arms, a trial of more than a thousand patients will be required to detect a difference. Uneven distribution may increase, or decrease the ability to detect efficacy, depending which arm the responders were more prevalent. This would also be dependent on the inherent biology of the responders, and be enhanced if they were a poor prognostic group. The clinical indication and indirect enrichment using traditional criteria (performance status, tumour burden, etc.) may enhance the likelihood of detecting efficacy if it captures proportionally more true responders than non-responders (*A*), but may be detrimental (*B*). Inherent and acquired resistance, and off-target efficacy means that the presence of the target does not directly match with responders. Another level of complexity emerges based on the assays used to detect the target. Surrogate biomarkers, or less stringent target detection assays can improve the sensitivity to detect true responders, but at the cost of specificity and is a more direct form of enrichment. Those individuals that are susceptible to toxicity and adverse reaction are also captured differently with each strategy, and may have associations with true responders. With this level of complexity, it is little wonder that it is challenging to translate discoveries into the clinic. In addition, it suggests how many drugs, although promising in phase II, fail in phase III, even in enriched phase III studies if the incorrect population is selected. Targeted trials using biomarkers are dependent on using the correct indication, the correct marker, the best assay, and the inherent efficacy of the drug. Lack of accuracy in any of these, or slight inaccuracies which compound in each can lead to a false result

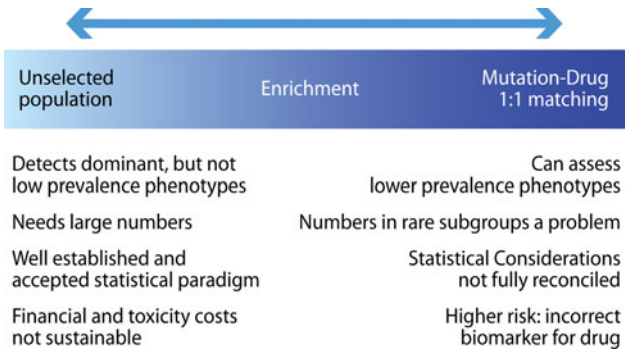

Fig. 5 The heterogeneity of cancer leads to complex challenges in therapeutic development as clinical trials of unselected patients move across a spectrum through enrichment to target–drug matching

specific biomarkers informed through an understanding of the molecular mechanism of action of the therapeutic. Fresh core biopsies were analysed for four biomarkers: epidermal growth factor receptor (EGFR), KRAS/BRAF, vascular endothelial growth factor (VEGF), and cyclin D1/RXR.

Initially, 40% of patients were randomised to one of four treatments: (1) erlotinib (EGFR inhibitor), (2) vandetanib (VEGF inhibitor), (3) sorafenib (multikinase inhibitor) and (4) erlotinib–bexarotene (RXR receptor activator). In the adaptive phase, the remaining 60% of patients were given therapies that were effective based on the molecular subtype in the initial phase. The trial demonstrated that patients with both EGFR mutations and high expression of

EGFR were 100% responsive to erlotinib and 100% non-responsive to sorafenib. Conversely, patients with KRAS mutations responded best to sorafenib. In addition, the erlotinib–bexarotene combination showed benefit in patients with tumours with increased cyclin D1 expression. Overall, patients in the adaptive phase did better than those in the initial phase (Editorial 2010; Kim et al. 2011).

Recently, Von Hoff and colleagues described an approach where patients with cancer of any organ were tested for multiple biomarkers and their treatment selected based on those analyses (Doroshow 2010; Von Hoff et al. 2010). The study was in patients with metastatic end-stage disease who had failed conventional therapy. They demonstrated that such a strategy is feasible, and suggested that it was associated with improved survival. Currently, numerous direct to consumer testing companies offer tests that claim to increase the chance of getting the most effective treatment. Despite low-level evidence to support efficacy of this approach, the industry is growing rapidly.

Next generation sequencing and therapeutic strategy development

A significant hurdle in advancing biomarker-guided therapeutic strategies is the requirement for robust reproducible assays that can test the spectrum of mutation classes observed in cancer (simple variants, gains, losses, translocations, inversions, etc.). Next generation sequencing provides the ability to do this on a single instrument. Furthermore, it is the technology that is fuelling the rapidly increasing knowledge of somatic and germline mutations by TCGA, ICGC, and other cancer genome projects. Despite the rapidly increasing use of next generation sequencing for targeted exome or whole genome sequencing of tumours in research projects, there are only a handful of reported cases to date where the technology has been used in a clinical setting (Link et al. 2011). Clinical laboratories that are using multiplex methods to screen for many somatic mutations are focussing largely on "actionable" cancer gene mutations that are known to correlate with resistance or response to targeted therapies and can reliably be tested in both frozen and FFPE tumour tissues. Genotyping, as opposed to sequencing, is currently used for this purpose. For example, multiple mutation profiling using multiplex genotyping of $\sim$400 mutations in 33 known cancer genes has been adapted for clinical decision making (MacConaill et al. 2009). Sequencing the complete genes (as opposed to specific exons known to harbour recurrent mutations) will provide opportunities to detect additional mutations in these genes, but the interpretation of the clinical consequences of entirely new mutations will be more problematic. As multiplex mutation profiling expands to hundreds and thousands of genes, and ultimately whole genomes sequencing, there will be additional challenges in regards to implementing high throughput technologies in clinical laboratories, data management, costs, interpretation of results, reporting or results to clinicians, and ultimately patients. Already, a number of leading cancer research institutes are implementing these approaches in the clinical trials setting, in order to learn and develop "best practices", and further improve the ability to identify specific correlations between genetic alterations, treatments, and outcomes.

A potential trial design for pancreatic cancer is presented below, where current systemic therapies are largely ineffective and overall survival has not improved for almost 50 years.

Genotype-guided therapeutic strategies for pancreatic cancer using next generation sequencing

Pancreatic cancer (PC) is the fourth leading cause of cancer death in Western societies with an overall 5-year survival rate of less than 5% (Michalski et al. 2007). Although the use of adjuvant therapy has improved the 5-year survival rate from 10% with surgery alone, to 20–25% with 5-fluorouracil (5-FU) (Neoptolemos et al. 2001) or gemcitabine (Oettle et al. 2007), little progress has been made in the treatment of the advanced form of the disease. As a consequence, there is a great need to develop novel therapeutic strategies (Philip et al. 2009).

Gemcitabine has been considered the standard treatment for patients with advanced PC since 1997 (Burris et al. 1997). A multitude of established systemic chemotherapeutics that have been assessed in combination with gemcitabine have failed to demonstrate improved efficacy in phase III clinical trials (Tabernero and Macarulla 2009). Apart from gemcitabine, the only other systemic chemotherapeutic to show any detectable efficacy in phase III clinical trials is the tyrosine kinase inhibitor Erlotinib (Tarceva®), which has marginal benefit overall, but is effective in a subgroup of patients who develop a cutaneous rash with therapy (Moore et al. 2007). This subgroup is not well defined genetically, and although no candidate markers of response were identified when assessed retrospectively in the trial (da Cunha Santos et al. 2010), a response signature has been proposed based on preclinical studies using primary xenograft models (Jimeno et al. 2008). There are two further opportunities where genetic aberrations are known to occur at low frequency, and can potentially be targeted: Fanconi Anaemia/BRCA Homologous Recombination Pathway defects and HER2 amplification. These individual targetable phenotypes (homologous recombination defects; HER2 amplified; anti-EGFR responsive) occur at low frequency, but collectively

they are present in about 15% of pancreatic cancer (Showalter et al. 2010). Effectively detecting these aberrations to define responsive phenotypes and applying the most appropriate treatment in each case may dramatically alter the outcomes of patients with PC, who currently have a less than 5% overall survival, and a 2-year survival of 10% or less for metastatic disease. A major hurdle has been the ability to efficiently test for molecular aberrations, particularly if a druggable phenotype is defined through many potential aberrations that cannot be easily tested.

Homologous recombination defects

Perhaps the most striking cellular hallmark of tumours with mutations in the Fanconi anaemia (FA)/BRCA homologous recombination pathway is their hypersensitivity to DNA damaging (cross-linking) agents, such as mitomycin C, platinum or the novel targeted PARP inhibitors (Byrski et al. 2009; Xia et al. 2007). Platinum agents and PARP inhibitors are currently in clinical trials for the treatment of hereditary breast and ovarian cancers and initial results are encouraging (Byrski et al. 2009; Clark-Knowles et al. 2010; Polyak and Garber 2011). Around 7% of sporadic PC patients carry mutations in the BRCA2 tumour suppressor gene (Liede et al. 2004), and the prevalence increases to 12% if there is a family history of pancreatic cancer, and to 17% in those with a strong family history (van der Heijden et al. 2004).

Evidence for the potential use of DNA cross-linking agents in PCs that harbour these defects is mounting (Burch et al. 2000; Isacoff et al. 2007; Showalter et al. 2010; van der Heijden et al. 2005; van der Heijden et al. 2004). Case reports of pancreatic cancers responding well to similar therapies in the presence of defects in other components of the Fanconi Anaemia (FA) pathway including defects in the Partner and Localiser of BRCA2 (*PALB2*) are showing dramatic efficacy. In this case, the patient responded dramatically to mitomycin C after progressing on gemcitabine, to still be alive almost 4 years from the time the therapy was changed (Villarroel et al. 2011). Responders in phase 2 trials of platinum agents in PC may be those that harbour homologous recombination defects. Retrospective assessment of tissue from these trials may address this question.

HER2 amplified

HER2 amplification occurs in about 15% of breast cancers, and Herceptin® treatment is currently approved and reimbursed for patients who have demonstrable HER2 amplification in their cancer. More recently, a phase III randomised controlled trial demonstrated efficacy of Herceptin® in HER2-amplified Gastric Cancer (ToGA trial) suggesting that targeting HER2 amplification may be effective in other cancer types apart from breast cancer (Bang et al. 2010). Although early studies identified HER2 overexpression using IHC in up to 40% of PCs (Safran et al. 2004), more recent studies show that true HER2 amplification using FISH, a more accurate measure of Herceptin® responsiveness, only occurs in about 3% of pancreatic cancers. Trastuzumab suppresses tumour growth in orthotopic xenograft mouse models of HER2-amplified PC (Buchler et al. 2005; Kimura et al. 2006); however, phase II studies of trastuzumab have not been successful. This may not have been due to drug activity, but the testing strategy, and the appropriateness of assays which would have significantly influenced patient selection in these trials.

Anti-EGFR therapy responsive

Pre-clinical studies using primary xenografts of pancreatic cancer have identified and validated an anti-EGFR response signature (Jimeno et al. 2008); however, no anti-EGFR response signature assay has been developed for clinical use. Whilst HER2 amplification has a well established assay, there are also limitations in assays available for FA/BRCA pathway members, and currently only exist for BRCA1 and BRCA2 for germline testing. The influence of low epithelial content of tumours, a feature of pancreatic cancer where on average 75% of the tumour mass is desmoplastic stroma, is likely to influence these standardised assays.

Next generation sequencing can assess all the relevant genomic aberrations that are likely to characterise these response markers. A potential adaptive clinical trial design would screen patients for these strategies and any other druggable mutations that have existing therapies in cancers of other organs. Those with mutations would be randomised between standard therapy, and a personalised approach. Figure 6 shows the suggested trial schema. In a BATTLE-like strategy, at the completion of an initial phase, initial less accurate criteria for phenotype selection can be refined based on response data within each of the phenotypes (adaptive phase). The trial can be iterative and expanded to include other druggable targets.

Could a molecular classification of cancer facilitate translation and implementation of therapeutic strategies?

The number of pharmaceuticals that directly target molecular abnormalities has been, and is continuing to grow dramatically. In the USA alone, there are hundreds of small molecule inhibitors in development. The number of target–drug combinations with efficacy is growing, and some are suggesting that the era of personal genomic cancer therapy is already here (Harris 2010; Schilsky

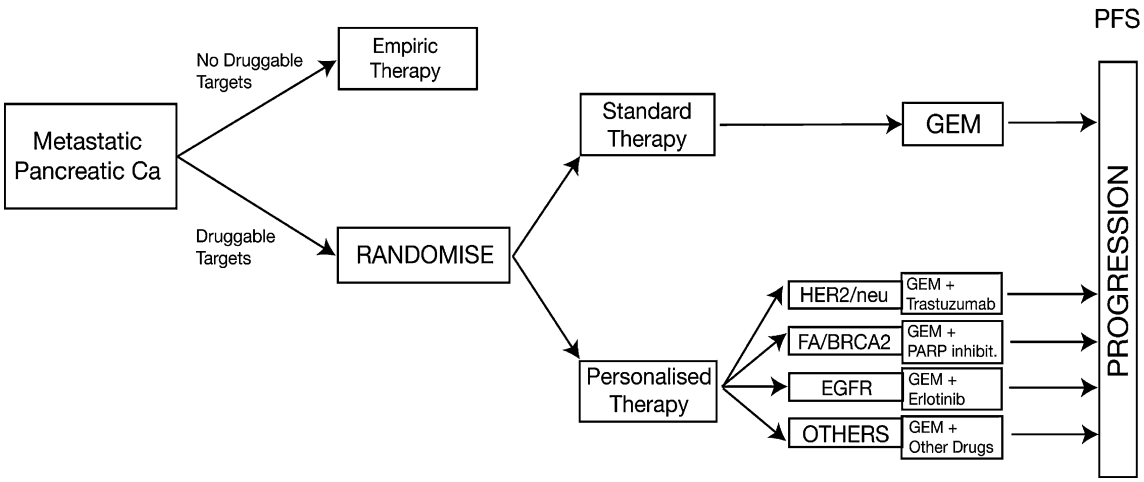

Fig. 6 Schema of a potential trial design to test genotype-guided (NGS) therapies for pancreatic cancer

2010). Notwithstanding, some clear instances where molecular tests are used to select cancer therapies, most patients are not yet deriving benefits because the heterogeneity and complexity at the molecular level does not allow accurate predictions of therapeutic response. Large-scale sequencing efforts in glioblastoma and pancreatic cancer suggest that apart from a few dominant genetic aberrations, many of which have been well known for years, the majority of novel mutations occur at a frequency of less than 10%, although there is strong evidence that mutated genes are involved in common gene pathways and processes (Jones et al. 2008; Parsons et al. 2008; TCGA 2008). There are several examples, where drugs effectively target the same, similar, and even different genomic aberrations in different cancer types. One of the first tyrosine kinase inhibitors to be developed, Imatinib (Gleevec®) targets the BCR-ABL fusion protein in CML, but is also effective in other diseases such as gastrointestinal stromal tumours (GIST) that express mutant kit. The monoclonal antibody trastuzumab (Herceptin®), first developed for HER2-amplified breast cancer is also effective in HER2-amplified gastric cancer (Bang et al. 2010). Effective drugs may also be beneficial in cancers of other organs. This would require a screening strategy that identifies tumours with similar molecular phenotypes and consideration of the mechanism of action of the drug, such as trastuzumab and gastric cancer, as well as recruitment criteria for individual clinical trials that are based on a susceptible molecular phenotype rather than the organ from which the cancer arose.

Classification of cancers is mainly based on organ and morphology. This approach to classifying tumours is subjective, only represents a surrogate of the underlying molecular pathology, and may hamper the implementation of therapies that target molecular mechanisms (Cagle et al. 2011; Gruver et al. 2011; Palanisamy et al. 2010). A "Biotype" classification of cancer (Fig. 7) may be more appropriate in many circumstances, particularly since our ability to better resolve molecular aberrations will generate increasingly larger numbers of subtypes, reducing the incidence and prevalence of each to near orphan diseases. Trial designs based on this strategy would recruit based on molecular phenotype, and then adapt to exclude non-responsive subgroups, which may or may not co-segregate with the organ where the cancer arises, or with other factors that may be identified based on extensive characterisation of each tumour. In addition, this approach would also potentially uncover mechanisms of resistance that would form the basis for further investigation. Such a strategy may be more effective in metastatic disease where the primary site is unknown, or where there has been

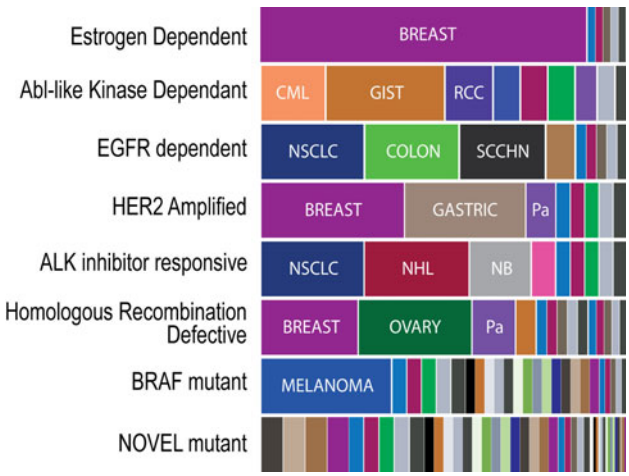

Fig. 7 A "Biotype" classification of cancer may facilitate clinical trials assessing efficacy of a targeted therapeutic. Individual phenotypes in a cancer from a particular organ may occur too infrequently to perform clinical trials; however, including them using such a classification will enlarge the potential indications for a specific therapeutic strategy

significant pretreatment, which is likely to have altered the original molecular phenotype of the disease.

Conclusions

The rise in the cost of healthcare associated with an ageing population in most Western countries is a major factor driving the need for innovations is cancer diagnostics and treatment. At the current rate, the cost of cancer treatment will rapidly outstrip our ability to provide it. Avoiding unnecessary ineffective therapies so that the right drug is given to the right patient without delay will substantially impact health costs and improve outcomes. However, in addition to the many technological challenges of delivering biomarker assays in the clinic, there are significant issues surrounding the return on investment for stakeholders in the delivery of complex diagnostics above and beyond traditional approaches to clinical decision-making. Significant debate amongst the pharmaceutical industry, consumers, healthcare insurers and providers is ongoing regarding the implications of providing a personalised approach to cancer treatment. In particular, the significant cost of manufacturing and providing diagnostic multi-gene tests and targeted therapies and the impact of these costs on access for patients. Our ability to accurately and inexpensively globally analyse genomic aberrations will facilitate this process, but the requisite infrastructure and framework will need to be in place to facilitate rapid translation into the clinic.

References

(2011a) Cancer drugs: remedy required. Nat Med 17:231

(2011b) Regulatory watch: FDA guidance on co-developing investigational drugs. Nat Rev Drug Discov 10:86. http://www.nature.com/nrd/journal/v10/n2/index.html

Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, McAllister PK, Morton RF, Schilsky RL (2009) American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 27:2091–2096

Andre F, McShane LM, Michiels S, Ransohoff DF, Altman DG, Reis-Filho JS, Hayes DF, Pusztai L (2011) Biomarker studies: a call for a comprehensive biomarker study registry. Nat Rev Clin Oncol 8:171–176

Arrowsmith J (2011) Trial watch: phase III and submission failures: 2007–2010. Nat Rev Drug Discov 10:87

Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Ruschoff J, Kang YK (2010) Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 376:687–697

Bates SE (2010) Striving to improve outcomes in oncology: unmet expectations in a complex disease. Clin Cancer Res 16:5950

Beerenwinkel N, Antal T, Dingli D, Traulsen A, Kinzler KW, Velculescu VE, Vogelstein B, Nowak MA (2007) Genetic progression and the waiting time to cancer. PLoS Comput Biol 3:e225

Buchler P, Reber HA, Eibl G, Roth MA, Buchler MW, Friess H, Isacoff WH, Hines OJ (2005) Combination therapy for advanced pancreatic cancer using Herceptin plus chemotherapy. Int J Oncol 27:1125–1130

Burch PA, Ghosh C, Schroeder G, Allmer C, Woodhouse CL, Goldberg RM, Addo F, Bernath AM, Tschetter LK, Windschitl HE, Cobau CD (2000) Phase II evaluation of continuous-infusion 5-fluorouracil, leucovorin, mitomycin-C, and oral dipyridamole in advanced measurable pancreatic cancer: a North Central Cancer Treatment Group Trial. Am J Clin Oncol 23:534–537

Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD (1997) Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15:2403–2413

Byrski T, Huzarski T, Dent R, Gronwald J, Zuziak D, Cybulski C, Kladny J, Gorski B, Lubinski J, Narod S (2009) Response to neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat 115:359–363

Cagle PT, Allen TC, Dacic S, Beasley MB, Borczuk AC, Chirieac LR, Laucirica R, Ro JY, Kerr KM (2011) Revolution in lung cancer: new challenges for the surgical pathologist. Arch Pathol Lab Med 135:110–116

Caponigro G, Sellers WR (2011) Advances in the preclinical testing of cancer therapeutic hypotheses. Nat Rev Drug Discov 10:179–187

Chin L, Gray JW (2008) Translating insights from the cancer genome into clinical practice. Nature 452:553–563

Chin L, Andersen JN, Futreal PA (2011) Cancer genomics: from discovery science to personalized medicine. Nat Med 17:297–303

Clark-Knowles KV, O'Brien AM, Weberpals JI (2010) BRCA1 as a therapeutic target in sporadic epithelial ovarian cancer. J Oncol 2010:891059

Cordon-Cardo C (2004) p53 and RB: simple interesting correlates or tumor markers of critical predictive nature? J Clin Oncol 22:975–977

Croce CM (2008) Oncogenes and cancer. N Engl J Med 358:502–511

da Cunha Santos G, Dhani N, Tu D, Chin K, Ludkovski O, Kamel-Reid S, Squire J, Parulekar W, Moore MJ, Tsao MS (2010) Molecular predictors of outcome in a phase 3 study of gemcitabine and erlotinib therapy in patients with advanced pancreatic cancer: National Cancer Institute of Canada Clinical Trials Group Study PA.3. Cancer 116:5599–5607

Doroshow JH (2010) Selecting systemic cancer therapy one patient at a time: is there a role for molecular profiling of individual patients with advanced solid tumors? J Clin Oncol 28:4869–4871

EBCTCG (2005) Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365:1687–1717

Editorial (2010) Trial watch: adaptive BATTLE trial uses biomarkers to guide lung cancer treatment. Nat Rev Drug Discov 9:423

Esteva FJ, Yu D, Hung MC, Hortobagyi GN (2010) Molecular predictors of response to trastuzumab and lapatinib in breast cancer. Nat Rev Clin Oncol 7:98–107

Farrell A (2011) A close look at cancer. Nat Med 17:262–265

Freidlin B, McShane LM, Korn EL (2010) Randomized clinical trials with biomarkers: design issues. J Natl Cancer Inst 102:152–160

Gerber DE, Minna JD (2010) ALK inhibition for non-small cell lung cancer: from discovery to therapy in record time. Cancer Cell 18:548–551

Green ED, Guyer MS (2011) Charting a course for genomic medicine from base pairs to bedside. Nature 470:204–213

Gruver AM, Portier BP, Tubbs RR (2011) Molecular pathology of breast cancer: the journey from traditional practice toward embracing the complexity of a molecular classification. Arch Pathol Lab Med 135:544–557

Hall J, Dennler P, Haller S, Pratsinis A, Sauberli K, Towbin H, Walthe K, Woytschak J (2010) Genomics drugs in clinical trials. Nat Rev Drug Discov 9:988

Harries M, Smith I (2002) The development and clinical use of trastuzumab (Herceptin). Endocr Relat Cancer 9:75–85

Harris T (2010) Gene and drug matrix for personalized cancer therapy. Nat Rev Drug Discov 9:660

Hood L, Friend SH (2011) Predictive, personalized, preventive, participatory (P4) cancer medicine. Nat Rev Clin Oncol 8:184–187

Hutchinson L (2011) Personalized cancer medicine: era of promise and progress. Nat Rev Clin Oncol 8:121

ICGC (2010) International network of cancer genome projects. Nature 464:993–998

Isacoff WH, Bendetti JK, Barstis JJ, Jazieh AR, Macdonald JS, Philip PA (2007) Phase II trial of infusional fluorouracil, leucovorin, mitomycin, and dipyridamole in locally advanced unresectable pancreatic adenocarcinoma: SWOG S9700. J Clin Oncol 25:1665–1669

Jimeno A, Tan AC, Coffa J, Rajeshkumar NV, Kulesza P, Rubio-Viqueira B, Wheelhouse J, Diosdado B, Messersmith WA, Iacobuzio-Donahue C, Maitra A, Varella-Garcia M, Hirsch FR, Meijer GA, Hidalgo M (2008) Coordinated epidermal growth factor receptor pathway gene overexpression predicts epidermal growth factor receptor inhibitor sensitivity in pancreatic cancer. Cancer Res 68:2841–2849

Johannessen CM, Boehm JS, Kim SY, Thomas SR, Wardwell L, Johnson LA, Emery CM, Stransky N, Cogdill AP, Barretina J, Caponigro G, Hieronymus H, Murray RR, Salehi-Ashtiani K, Hill DE, Vidal M, Zhao JJ, Yang X, Alkan O, Kim S, Harris JL, Wilson CJ, Myer VE, Finan PM, Root DE, Roberts TM, Golub T, Flaherty KT, Dummer R, Weber BL, Sellers WR, Schlegel R, Wargo JA, Hahn WC, Garraway LA (2010) COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature 468:968–972

Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321:1801–1806

Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR (2008) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359:1757–1765

Khleif SN, Doroshow JH, Hait WN (2010) AACR-FDA-NCI Cancer Biomarkers Collaborative consensus report: advancing the use of biomarkers in cancer drug development. Clin Cancer Res 16:3299–3318

Kim ES, Herbst1 RS et al. (2011) The BATTLE Trial: personalizing therapy for lung cancer. Cancer Discov 1

Kimura K, Sawada T, Komatsu M, Inoue M, Muguruma K, Nishihara T, Yamashita Y, Yamada N, Ohira M, Hirakawa K (2006) Antitumor effect of trastuzumab for pancreatic cancer with high HER-2 expression and enhancement of effect by combined therapy with gemcitabine. Clin Cancer Res 12:4925–4932

Kovacs A, Stenman G (2010) HER2-testing in 538 consecutive breast cancer cases using FISH and immunohistochemistry. Pathol Res Pract 206:39–42

Lee CK, Lord SJ, Coates AS, Simes RJ (2009) Molecular biomarkers to individualise treatment: assessing the evidence. Med J Aust 190:631–636

Liede A, Karlan BY, Narod SA (2004) Cancer risks for male carriers of germline mutations in BRCA1 or BRCA2: a review of the literature. J Clin Oncol 22:735–742

Link DC, Schuettpelz LG, Shen D, Wang J, Walter MJ, Kulkarni S, Payton JE, Ivanovich J, Goodfellow PJ, Le Beau M, Koboldt DC, Dooling DJ, Fulton RS, Bender RH, Fulton LL, Delehaunty KD, Fronick CC, Appelbaum EL, Schmidt H, Abbott R, O'Laughlin M, Chen K, McLellan MD, Varghese N, Nagarajan R, Heath S, Graubert TA, Ding L, Ley TJ, Zambetti GP, Wilson RK, Mardis ER (2011) Identification of a novel TP53 cancer susceptibility mutation through whole-genome sequencing of a patient with therapy-related AML. JAMA 305:1568–1576

LoRusso PM, Schnipper LE, Stewart DJ, Boerner SA, Averbuch SD, Wolf W (2010) Translating clinical trials into meaningful outcomes. Clin Cancer Res 16:5951–5955

Macconaill LE, Garraway LA (2010) Clinical implications of the cancer genome. J Clin Oncol 28:5219–5228

MacConaill LE, Campbell CD, Kehoe SM, Bass AJ, Hatton C, Niu L, Davis M, Yao K, Hanna M, Mondal C, Luongo L, Emery CM, Baker AC, Philips J, Goff DJ, Fiorentino M, Rubin MA, Polyak K, Chan J, Wang Y, Fletcher JA, Santagata S, Corso G, Roviello F, Shivdasani R, Kieran MW, Ligon KL, Stiles CD, Hahn WC, Meyerson ML, Garraway LA (2009) Profiling critical cancer gene mutations in clinical tumor samples. PLoS One 4:e7887

Majewski IJ, Bernards R (2011) Taming the dragon: genomic biomarkers to individualize the treatment of cancer. Nat Med 17:304–312

Mandrekar SJ, Sargent DJ (2009) Clinical trial designs for predictive biomarker validation: theoretical considerations and practical challenges. J Clin Oncol 27:4027–4034

McClellan M, Benner J, Schilsky R, Epstein D, Woosley R, Friend S, Sidransky D, Geoghegan C, Kessler D (2011) An accelerated pathway for targeted cancer therapies. Nat Rev Drug Discov 10:79–80

McDermott U, Downing JR, Stratton MR (2011) Genomics and the continuum of cancer care. N Engl J Med 364:340–350

Mellinghoff IK, Sawyers CL (2002) The emergence of resistance to targeted cancer therapeutics. Pharmacogenomics 3:603–623

Mendelsohn J, Tursz T, Schilsky RL, Lazar V (2011) WIN Consortium-challenges and advances. Nat Rev Clin Oncol 8:133–134

Michalski CW, Weitz J, Buchler MW (2007) Surgery insight: surgical management of pancreatic cancer. Nat Clin Pract Oncol 4:526–535

Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W (2007) Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 25:1960–1966

Nathanson KL (2010) Using genetics and genomics strategies to personalize therapy for cancer: focus on melanoma. Biochem Pharmacol 80:755–761

Nazarian R, Shi H, Wang Q, Kong X, Koya RC, Lee H, Chen Z, Lee MK, Attar N, Sazegar H, Chodon T, Nelson SF, McArthur G, Sosman JA, Ribas A, Lo RS (2010) Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature 468:973–977

Nelson NJ (2010) Adaptive clinical trial design: has its time come? J Natl Cancer Inst 102:1217–1218

Neoptolemos JP, Dunn JA, Stocken DD, Almond J, Link K, Beger H, Bassi C, Falconi M, Pederzoli P, Dervenis C, Fernandez-Cruz L, Lacaine F, Pap A, Spooner D, Kerr DJ, Friess H, Buchler MW (2001) Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. Lancet 358:1576–1585

Ocana A, Tannock IF (2011) When are "positive" clinical trials in oncology truly positive? J Natl Cancer Inst 103:16–20

Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H (2007) Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA 297:267–277

Pajic M, Scarlett CJ, Chang DK, Sutherland RL, Biankin AV (2011) Preclinical strategies to define predictive biomarkers for therapeutically relevant cancer subtypes. Hum Genet. doi:10.1007/s00439-011-0990-0

Palanisamy N, Ateeq B, Kalyana-Sundaram S, Pflueger D, Ramnarayanan K, Shankar S, Han B, Cao Q, Cao X, Suleman K, Kumar-Sinha C, Dhanasekaran SM, Chen YB, Esgueva R, Banerjee S, LaFargue CJ, Siddiqui J, Demichelis F, Moeller P, Bismar TA, Kuefer R, Fullen DR, Johnson TM, Greenson JK, Giordano TJ, Tan P, Tomlins SA, Varambally S, Rubin MA, Maher CA, Chinnaiyan AM (2010) Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. Nat Med 16:793–798

Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW (2008) An integrated genomic analysis of human glioblastoma multiforme. Science 321:1807–1812

Philip PA, Mooney M, Jaffe D, Eckhardt G, Moore M, Meropol N, Emens L, O'Reilly E, Korc M, Ellis L, Benedetti J, Rothenberg M, Willett C, Tempero M, Lowy A, Abbruzzese J, Simeone D, Hingorani S, Berlin J, Tepper J (2009) Consensus report of the national cancer institute clinical trials planning meeting on pancreas cancer treatment. J Clin Oncol 27:5660–5669

Polyak K, Garber J (2011) Targeting the missing links for cancer therapy. Nat Med 17:283–284

Rodríguez-Paredes M, Esteller M (2011) Cancer epigenetics reaches mainstream oncology. Nat Med 17:330–339

Rosenberger WF, Lachin JM (1993) The use of response-adaptive designs in clinical trials. Control Clin Trials 14:471–484

Safran H, Iannitti D, Ramanathan R, Schwartz JD, Steinhoff M, Nauman C, Hesketh P, Rathore R, Wolff R, Tantravahi U, Hughes TM, Maia C, Pasquariello T, Goldstein L, King T, Tsai JY, Kennedy T (2004) Herceptin and gemcitabine for metastatic pancreatic cancers that overexpress HER-2/neu. Cancer Invest 22:706–712

Sargent DJ, Conley BA, Allegra C, Collette L (2005) Clinical trial designs for predictive marker validation in cancer treatment trials. J Clin Oncol 23:2020–2027

Schilsky RL (2010) Personalized medicine in oncology: the future is now. Nat Rev Drug Discov 9:363–366

Seruga B, Hertz PC, Wang L, Booth CM, Cescon DW, Krzyzanowska M, Tannock IF (2010) Absolute benefits of medical therapies in phase III clinical trials for breast and colorectal cancer. Ann Oncol 21:1411–1418

Shekhar MP (2011) Drug resistance: challenges to effective therapy. Curr Cancer Drug Targets 11:613–623

Showalter SL, Charles S, Belin J, Cozzitorto J, Einstein P, Richards NG, Sauter PK, Kennedy EP, Witkiewicz A, Brody JR, Yeo CJ (2010) Identifying pancreatic cancer patients for targeted treatment: the challenges and limitations of the current selection process and vision for the future. Expert Opin Drug Deliv 7:273–284

Solit DB, Rosen N (2011) Resistance to BRAF inhibition in melanomas. N Engl J Med 364:772–774

Stratton MR, Campbell PJ, Futreal PA (2009) The cancer genome. Nature 458:719–724

Tabernero J, Macarulla T (2009) Changing the paradigm in conducting randomized clinical studies in advanced pancreatic cancer: an opportunity for better clinical development. J Clin Oncol 27:5487–5491

TCGA CGARN (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455:1061–1068

TCGA (2008b) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455:1061–1068

Tursz T, Andre F, Lazar V, Lacroix L, Soria JC (2011) Implications of personalized medicine-perspective from a cancer center. Nat Rev Clin Oncol 8:177–183

van der Heijden MS, Brody JR, Gallmeier E, Cunningham SC, Dezentje DA, Shen D, Hruban RH, Kern SE (2004) Functional defects in the Fanconi anemia pathway in pancreatic cancer cells. Am J Pathol 165:651–657

van der Heijden MS, Brody JR, Dezentje DA, Gallmeier E, Cunningham SC, Swartz MJ, DeMarzo AM, Offerhaus GJ, Isacoff WH, Hruban RH, Kern SE (2005) In vivo therapeutic responses contingent on Fanconi anemia/BRCA2 status of the tumor. Clin Cancer Res 11:7508–7515

Villarroel MC, Rajeshkumar NV, Garrido-Laguna I, De Jesus-Acosta A, Jones S, Maitra A, Hruban RH, Eshleman JR, Klein A, Laheru D, Donehower R, Hidalgo M (2011) Personalizing cancer treatment in the age of global genomic analyses: PALB2 gene mutations and the response to DNA damaging agents in pancreatic cancer. Mol Cancer Ther 10:3–8

Von Hoff DD, Stephenson JJ Jr, Rosen P, Loesch DM, Borad MJ, Anthony S, Jameson G, Brown S, Cantafio N, Richards DA, Fitch TR, Wasserman E, Fernandez C, Green S, Sutherland W, Bittner M, Alarcon A, Mallery D, Penny R (2010) Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. J Clin Oncol 28:4877–4883

Wheeler DL, Dunn EF, Harari PM (2010) Understanding resistance to EGFR inhibitors-impact on future treatment strategies. Nat Rev Clin Oncol 7:493–507

Wistuba II, Gelovani JG, Jacoby JJ, Davis SE, Herbst RS (2011) Methodological and practical challenges for personalized cancer therapies. Nat Rev Clin Oncol 8:135–141

Xia B, Dorsman JC, Ameziane N, de Vries Y, Rooimans MA, Sheng Q, Pals G, Errami A, Gluckman E, Llera J, Wang W, Livingston DM, Joenje H, de Winter JP (2007) Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. Nat Genet 39:159–161