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Review

Predictive biomarkers for personalised anti-cancer drug use: Discovery to clinical implementation

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ABSTRACT

A priority translational research objective in cancer medicine is the discovery of novel therapeutic targets for solid tumours. Ideally, co-discovery of predictive biomarkers occurs in parallel to facilitate clinical development of agents and ultimately personalise clinical use. However, the identification of clinically useful predictive biomarkers for solid tumours has proven challenging with many initially promising biomarkers failing to translate into clinically useful applications. In particular, the 'failure' of a predictive biomarker has often only become apparent at a relatively late stage in investigation. Recently, the field has recognised the need to develop a robust clinical biomarker development methodology to facilitate the process. This review discusses the recent progress in this area focusing on the key stages in the biomarker development process: discovery, validation, qualification and implementation. Concentrating on predictive biomarkers for selecting systemic therapies for individual patients in the clinic, the advances and progress in each of these stages in biomarker development are outlined and the key remaining challenges are discussed. Specific examples are discussed to illustrate the challenges identified and how they have been addressed. Overall, we find that significant progress has been made towards a formalised biomarker developmental process. This holds considerable promise for facilitating the translation of predictive biomarkers from discovery to clinical implementation. Further enhancements could eventually be found through alignment with regulatory processes.

1. Introduction

The advent of targeted anti-cancer therapies has highlighted the need to develop biomarkers to optimise drug development and clinical use.[1] It is possible to adopt a generic definition of biomarkers as characteristics that can be objectively measured as indicators of a biological or pathological process or pharmacological response to a therapeutic intervention[2] and

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in doing so identify biomarkers as of potential utility across the whole translational cancer research process. Accordingly, in drug development, biomarkers are being utilised in 'go/no go' decision making during the discovery stages, and in assessing the performance of drugs in pre-clinical and early clinical studies, collectively these can be referred to as pharmacological biomarkers.[1,3,4] In the clinic, biomarkers can be used to facilitate precision and decrease invasiveness in cancer diagnosis; for patient selection for treatment based upon estimation of natural history of disease (prognostic biomarkers) or estimation of probability of response to a particular agent (predictive biomarker); or for detecting toxicity.[5,6] Such clinically useful biomarkers may also have potential value as surrogate end-points in clinical trials.

While there has been some success in the identification and development of diagnostic, prognostic and to a lesser extent toxicity and pharmacological biomarkers, there has been little progress in identifying clinically useful predictive biomarkers for solid tumours.[7] Predictive biomarkers are potentially the most useful for clinical decision-making. Predictive biomarkers are distinct from prognostic biomarkers in that the latter provide an estimation of the natural history of a patient's cancer independent of therapy, while the former produce an estimation of probability of response to therapy. In practice, many biomarkers have both predictive and prognostic impacts.

Biomarkers can also be classified according to modality of assessment, and this has implications for how particular biomarkers might be developed. The most common types of biomarkers according to this classification are provided in Table 1.

Herein we review the 'state of the art' and progress in the development process for predictive biomarkers and identify key challenges, problems and future directions. In particular the need for formulation of robust methodology for the biomarker development process from discovery to clinical implementation is identified and addressed.

2. Overview of the biomarker development process

The biomarker development process begins with discovery. Subsequent development of leads from discovery involves two key processes: the process of establishing a fit-for-purpose assay to objectively measure the biomarker, this can be defined as biomarker validation; and the evidentiary process of establishing a causal or correlative relationship between the biomarker and the clinical end-point or other biological or pathological end-point, this can be defined as biomarker qualification.[8–10] These definitions usefully delineate the processes that must be undertaken in biomarker development. Unfortunately this terminology has not been consistently used in the literature which has hindered biomarker development, for example, the terms investigation, determination and validation have been frequently and interchangeably used to refer to qualification. The final stage of biomarker development involves the clinical implementation of the appropriately qualified biomarker using the validated assay. Fig. 1 provides a schematic for the biomarker development process and each of these stages of biomarker discovery is now discussed.

3. Biomarker discovery

Biomarker discovery involves the comprehensive molecular characterisation of the clinical outcome of interest as

Table 1 – Biomarker classification according to modality. Examples included in this table are for predictive biomarkers. This is an illustrative not an exhaustive list.

Biomarker type	Subtypes	Example	Stage in development
Genetic	Gene mutation	*EGFR* mutation for erlotinib in non-small cell lung cancer	In clinical use
	Gene copy number	*HER2* amplification for trastuzumab in breast cancer	In clinical use
	Translocations	*BCR-abl* translocation for imatinib in chronic myeloid leukaemia	In clinical use
Genomic	Gene expression profiles	Mamma Print™70-gene gene expression profile for neoadjuvant chemotherapy response in breast cancer	Late stage clinical qualification
Protein	Serum protein assays	CK18 cell death assays for response to therapy	Early clinical qualification
Proteomic	Proteomic analysis of serum or tumour	Various	Preclinical and early clinical validation and qualification
Pathological	Immunohistochemistry (IHC)	HER2 IHC for trastuzumab in breast cancer	Routine clinical use
	Histopathology	Non-squamous NSCLC and response to pemetrexed	Late stage clinical qualification
Imaging	PET	FDG PET for metabolic response to chemotherapy in oesophagogastric cancer	Late stage clinical qualification
Other	–	Circulating tumour cells (CTC)	Early clinical validation and qualification

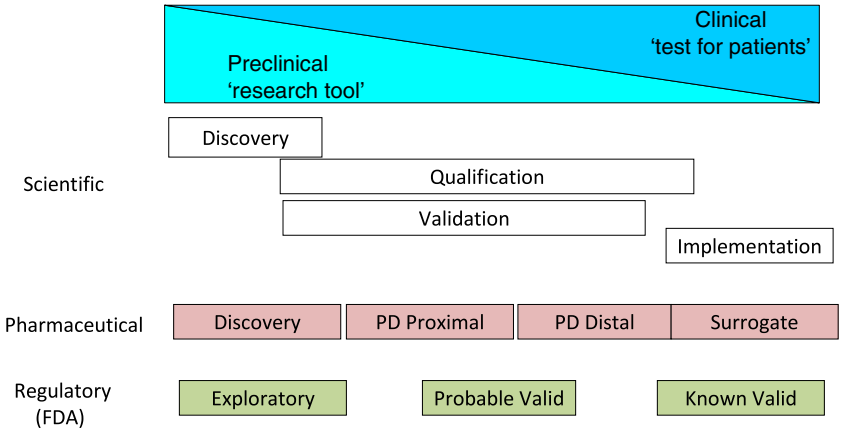

Fig. 1 – Schematic of biomarker development process, illustrating the key stages of discovery, qualification, validation and clinical implementation (discussed in detail in the text). Also illustrates alternative views on the biomarker development process from the position of different stakeholders and how this might interact with the scientific process (PD = pharmacodynamic).

assessed by an established clinical end-point.[7,11] For predictive biomarkers, the clinical end-point of interest is improved overall survival following treatment with the drug. Ideally, a causal mechanistic relationship between a particular molecular pathway and the clinical outcome in individual patients should be established. Due to molecular complexity and heterogeneity, establishment of such causal mechanistic relationships in solid tumour oncology is difficult and it is easier to establish a correlative relationship – this may still form the basis for a useful predictive biomarker, however caution must be applied in this case due to unanticipated interaction between the biomarker and therapy which may have no relationship with clinical outcome.

Discovery can be optimised by combining clinical correlation studies involving the analysis of bio-specimens from patients with investigation in preclinical model systems (both *in vitro* and *in vivo*).[11] Ideally, molecular characterisation of model systems to the same extent as clinical bio-specimens should be undertaken, since this facilitates a rapid testing of clinically generated hypotheses and vice-versa. It is imperative to use multiple preclinical model systems in discovery before identifying leads for further development, however the acceptance criteria for lead identification are not universally agreed and at present are largely based upon 'in house' criteria which have been experience driven.

A key decision in biomarker discovery is what to biomark – aspects of disease biology e.g. cell death or a specific molecule. The decision is complex but is dependent upon the *a priori* understanding of the underlying disease biology and the molecular characterisation of the clinical end-point. In solid tumour oncology for the majority of disease types, there is a relatively inadequate understanding of the biology underlying disease pathogenesis or clinical outcome and accordingly hypothesis-generating approaches to discover biomarkers using 'omic' platforms are often utilised and justified.[11–16]

4. Biomarker validation

Validation of a biomarker involves a systematic evaluation to assure that the technique used to assay the biomarker is reliable to perform its task.[9,17] Biomarker validation is guided by the established principles of bio-analytical method validation.[18,19] However, as discussed below, not all these principles are directly transferable either in the context of 'fit-for-purpose' biomarker validation as already discussed or due to specific analytical challenges specific to biomarkers.[9] In the UK,

Table 2 – Definitions of some of the key terms in biomarker assay validation (see also references[9,10] and[24] for further definitions of terms and discussions.

Key term	Definition
Calibration curve	The relationship between the analyte concentration in the standards (calibrators) and the measurement. The calibration curve is used to estimate the analyte concentration in test samples
Selectivity	The extent to which an assay can identify an analyte within a mixture without interference from components of the mixture
Specificity	The ability to unequivocally measure the analyte in the presence of other components that may be present in the biological specimen, including impurities, metabolites and endogenous biological molecules similar to the analyte itself
Precision	The degree to which repeated measurements made by the test (unchanged conditions) show the same result (also known as reproducibility)
Accuracy	The degree of closeness of measurements to its actual value

guidance is provided by the British Association for Research into Quality Assurance (BARQA) in the guidelines for Good Clinical laboratory Practice, although this has no regulatory basis as yet.[20]

The principles of bio-analytical method validation have been termed Good Laboratory Practice (GLP), but as already stated these are not always applicable in validating biomarkers.[10,21,22] Specifically, there are a number of issues that prevent full application of GLP to biomarker assay validation.[9,10,21] For example, a calibration curve (defined in Table 2) for studying a biomarker is very difficult to construct due to the existence of the biomarker within a biological matrix, where other components might interfere. Moreover, biomarkers are typically endogenous macromolecules that are accordingly often present in all samples, which make it difficult to create standards that do not contain the analyte. Therefore, researchers have to use non-certified standards and a variety of surrogate matrices in order to construct a calibration curve from which unknowns can be read and this leads to methodological challenges and difficulties that must be overcome to formulate a valid assay.[10,23]

4.1. *'Fit-for-purpose' biomarker validation*

Addressing these issues, 'Fit-for-purpose' biomarker validation proposed by Lee at al.[9] provides a standard for the field and establishes the validation required for biomarkers used in drug development and those used in clinical decision-making. The key aspect is that the biomarker assay is required to be 'fit-for-purpose' namely, a biomarker that is used as a research tool in a translational laboratory or in a drug development process needs to be less rigorously validated than an assay that might be used to influence a clinical decision, such as a predictive biomarker.[9]

4.2. *Process of biomarker validation*

The processes used for biomarker validation include developing a method for validation, pre-study method validation and in-study validation.[10] The first stage aims to perform feasibility studies and assess reagent availability; the outcome is the generation of a validation plan. This plan is then put into effect in the second stage (pre-study validation). In this stage, controls (from patient samples or suitable surrogates) that contain known concentrations of the biomarker being studied are used. The goals of this step are to assure that the assay meets acceptable standards of performance to produce an analytical report and to construct a standard operating procedure that can be then used for patient sample analysis. The performance of the assay is tested in the second stage by assessing different parameters including selectivity, sensitivity, different choice of controls, different analyte recovery methods, precision, accuracy and reproducibility[9,10,24] (simple definitions of these key terms are provided in Table 2). Testing the stability of the analyte in controls and patient samples is also performed at this stage.[25] There is still some uncertainty in the field with regard to acceptance criteria for these parameters of biomarker assays, for example, there might be different parameters for different types of biomarker assays or for assays with different purposes.[10] The last step, which is the in-study validation, is to test the assay on real patient samples using the controls from stage two to confirm consistent performance of the assay. The aim in this stage is to identify any issues that may occur when analysing real patient samples. The three stages are summarised in Table 3.

5. Qualification of biomarkers

5.1. *General principles of qualification*

Biomarkers must be qualified for a specified purpose prior to clinical implementation.[26] The aim in the qualification of a biomarker is to define its sensitivity and specificity for clinical end-point determination and to prove its clinical utility.[27] Use of unqualified biomarkers can lead to incorrect treatment decisions, which will impact adversely on patient health outcomes. Therefore, clinical studies must be conducted to properly assess the clinical utility of a biomarker.[28]

Although qualification of a biomarker to demonstrate clinical utility can proceed without full validation (valid patient assay – see previous section), the closer a biomarker comes to clinical implementation the more important the validation becomes. Therefore, validation must proceed in parallel with qualification and be 'fit-for-purpose' at each stage.[9,10] It is also important to acknowledge that qualification may be hindered by the use of an inadequately validated biomarker assay.

The clinical utility of biomarkers has been traditionally settled by debate, consensus and time. This process is very slow that a consensus on a qualification can take several years to be reached. The US Food and Drug Administration (FDA) are seeking ways by which this process can be shortened.[29] However, the qualification of biomarkers remains a challenging process for investigators and overall remains relatively underdeveloped, for example, in comparison with the process for novel anti-cancer agent development into the clinic. In addition to clinical utility, the criteria for acceptance of biomarker qualification also include demonstration of cost-effectiveness, which is mainly important to industry and regulatory authorities.[29]

The US FDA has adopted a process for qualifying biomarkers which forms a regulatory process as well. This process starts with an early submission of a request to qualify a

Table 3 – The stages of biomarker assay validation. Modified from Cummings et al.[10].

Stage	Description	Main purpose	Result
1	Method development	Develop method and perform preliminary validation	Validation plan
2	Pre-study validation	Assure meeting acceptable standards of performance	Validation report; and standard operating procedure for the assay
3	In-study validation	Real patient sample analysis	Valid patient data

biomarker to a panel of experts created by the FDA. These experts will then assess the biomarker context and evaluate the qualification study strategy. During this step, a consensus between the FDA and the developer of the biomarker should be reached with regards to the design of the study. Finally, the experts will review the qualification study results and provide a recommendation as to accept or reject the biomarker for the suggested use.[30]

5.2. *Clinical trial design for qualification*

There is considerable debate regarding the optimal clinical study design and strategy for qualification of a biomarker.[31] Formal guidelines for clinical trial design in this area do not exist. A common suggestion is that clinical studies for biomarker qualification start by retrospectively analysing material from prior well-controlled studies from which high quality sample material as well as clinical outcome regarding treatment efficacy is available.[28] An example of this type of study is the association between Epidermal Growth Factor Receptor (EGFR) mutation and responsiveness of erlotinib in Non-Small Cell Lung Cancer (NSCLC) or K-ras mutations for cetuximab and panitumumab in colorectal cancer.[32,50] While analysis might be retrospective, collection of tissues should be prospectively determined and performed. Otherwise, there will be a marked attrition in the proportion of samples collected and there is a real danger that the bio-specimen collection will not be representative of either the study population or more importantly the general disease population.[33] In this regard, there are several key criteria that might be applied to define the quality of retrospective predictive biomarker qualification data and we will return to this important aspect later in this review when specific examples of predictive biomarkers in qualification are discussed.

An alternative to this is the utilisation of materials from cancer bio-banks. There has been a rapid proliferation in the number and size of bio-banks; both in academia and in industry and these provide a valuable resource. However, they are potentially more useful for biomarker discovery rather than qualification.[34] This is because they are, generally speaking, less robust as compared to bio-specimen collection from clinical trials in terms of clinical annotation (especially with regard to therapy, toxicity and accuracy of clinical outcome measurement). Furthermore, they might not be as robust in terms of standard operating procedures applied for collection and storage of specimens, for example, over a long period of time in the bio-bank.

Following the retrospective studies, a prospective clinical study for the biomarker can be designed as an adjunct to a clinical trial of which the primary objective is to test the efficacy of a drug. As already highlighted, sample collection in this type of studies should be prospective and must be undertaken according to predetermined standard operating procedures and other clinical trial regulatory guidance.[18,20] An example of these studies is the FOCUS trial that was designed, in part, to assess the predictability of a number of biomarkers for irinotecan and oxaliplatin in colorectal cancer with the primary end-points of the trial being progression-free survival and overall survival. The investigators prospectively studied the interaction between these biomarkers and the benefit from the two drugs. This study concluded that Topo1 immunohistochemistry (IHC) identified patients who benefited or who did not benefit from irinotecan or oxaliplatin and suggested further evaluation of the biomarker.[35,36]

The final step in the clinical qualification is a well-dimensioned prospective study of which the primary objective should be evaluation of the predictive power of the biomarker.[28] Three approaches have been proposed for clinical

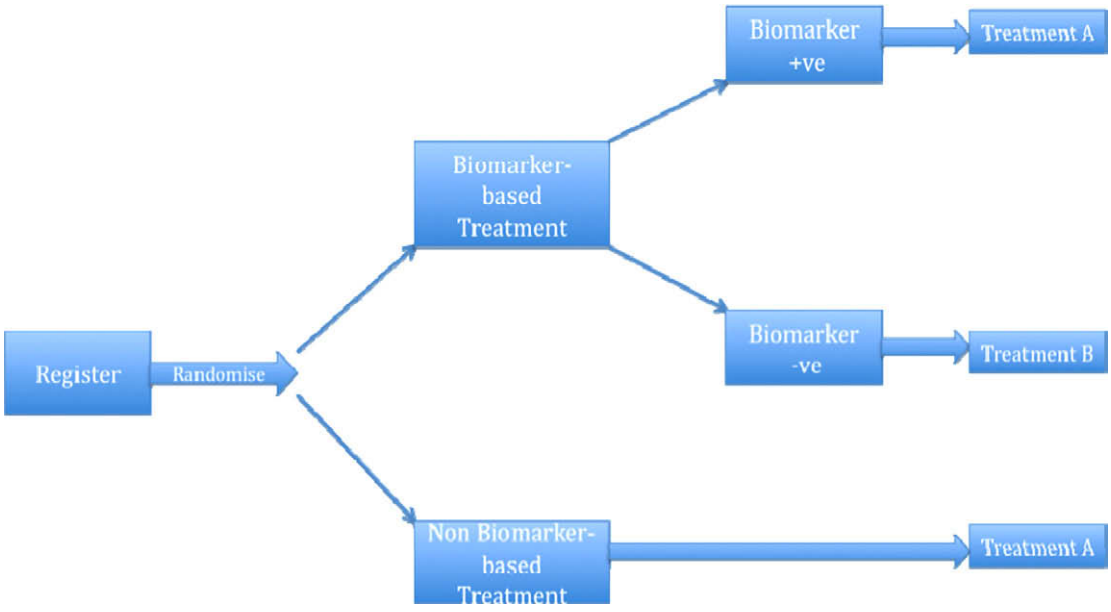

Fig. 2 – This design randomises patients to biomarker-based and non-biomarker-based treatments first then assigns biomarker-positive patients to treatment A and biomarker-negative patients to treatment B. Non-biomarker-based treatment assigns patients to treatment A only. Modified from Sargent et al.

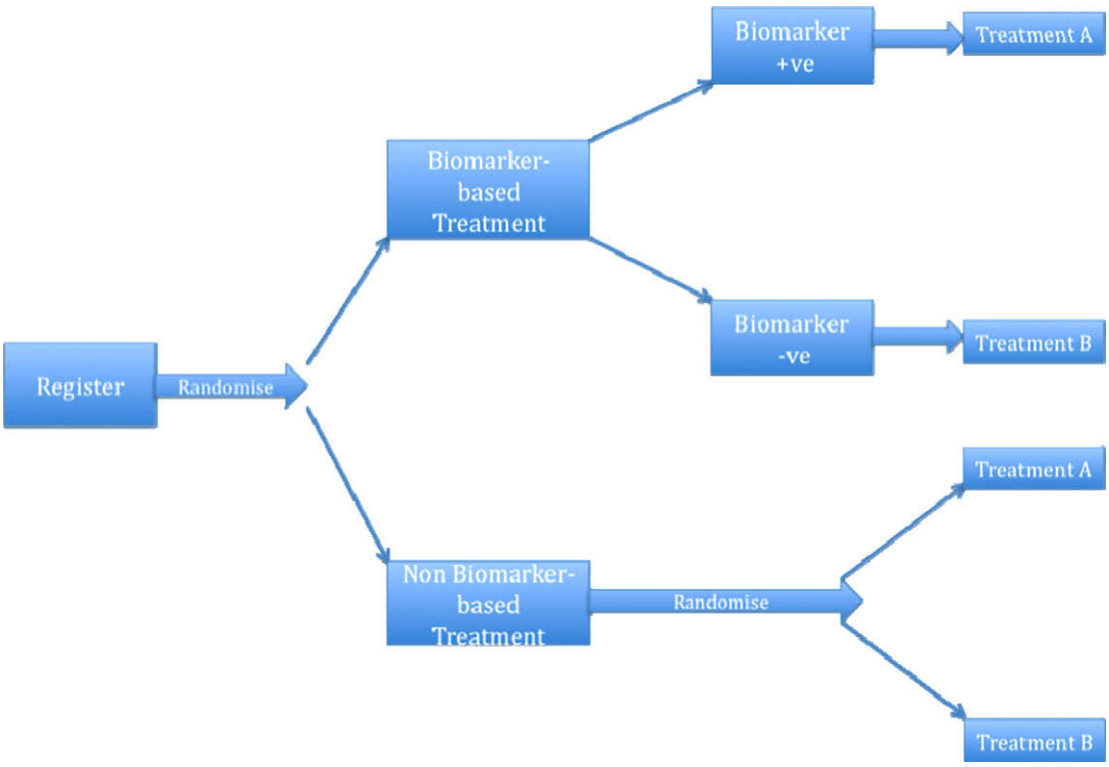

Fig. 3 – This design randomises patients to biomarker-based and non-biomarker-based treatments first then assigns biomarker-positive patients to treatment A and biomarker-negative patients to treatment B. Non-biomarker-based strategy randomises patients to both treatments. Modified from Sargent et al.

trial design for the qualification of a biomarker.[27] The first one (see Fig. 2) involves randomising patients to receive biomarker-based, or non-biomarker-based treatments, and then assigning biomarker-positive patients to one treatment and biomarker-negative patients to the other. The non-biomarker-based treatment is unified. In this approach, the predictive value of the biomarker is assessed by comparing the outcomes of patients in the biomarker-based treatment arm to those of all the patients in the non-biomarker-based treatment arm.

The second approach (see Fig. 3) is similar to the first one except that non-biomarker-based strategy is to randomise biomarker-negative patients to receive both treatments. This design allows the interaction between a biomarker and the treatment to be examined. This is important, for example, if one treatment is better than the other in both biomarker-positive and biomarker-negative patient subgroups. It may also allow a retrospective assessment of an alternative classification for the biomarker. A major disadvantage of these two designs is that they are relatively inefficient due to many patients in each arm being assigned to the same treatment and therefore they require a very large sample size.

The third approach (see Fig. 4), which is much simpler than the previous two, is to divide patients into biomarker-positive and biomarker-negative groups and then randomise each group to the different treatments. This approach requires much less sample size than the previous approaches. It undertakes two independent studies for all treatments, one in each patient group defined by biomarker status.[27] This again allows a test for interaction and also allows some assessment of the 'therapy independent' prognostic impact of a biomarker. However, it does not directly examine the predictive impact of the biomarker if it is used to make a clinical decision. A major drawback of this approach is that it cannot be applied if there is more than one biomarker to be tested, if there are more than two treatments, or if other outcomes in addition to efficacy should be explored.

5.3. *Guidelines for qualification*

Poor study design and inadequate reporting of studies have been identified as a major obstacle to progress in the field of biomarkers.[6,16,17,27,29] In response, the Statistics Subcommittee of the National Cancer Institute-European Organization for Research and Treatment of Cancer (NCI-EORTC) Working Group on Cancer Diagnosis developed tumour biomarker study reporting guidelines: REporting recommendations for tumour MARKer prognostic studies (REMARK guidelines).[37] These guidelines address the reporting of study design, hypotheses, patient and bio-specimen characteristics, assay methods and statistical analysis methods used in the studies of prognostic biomarkers. They also provide useful recommendations on the presentation of data and key points to be addressed in the discussion sections of papers. The REMARK guidelines were created primarily for reporting prognostic biomarkers studies, and while applicable to predictive biomarkers there is a clear need for the development of similar guidelines or agreed modification of these existing

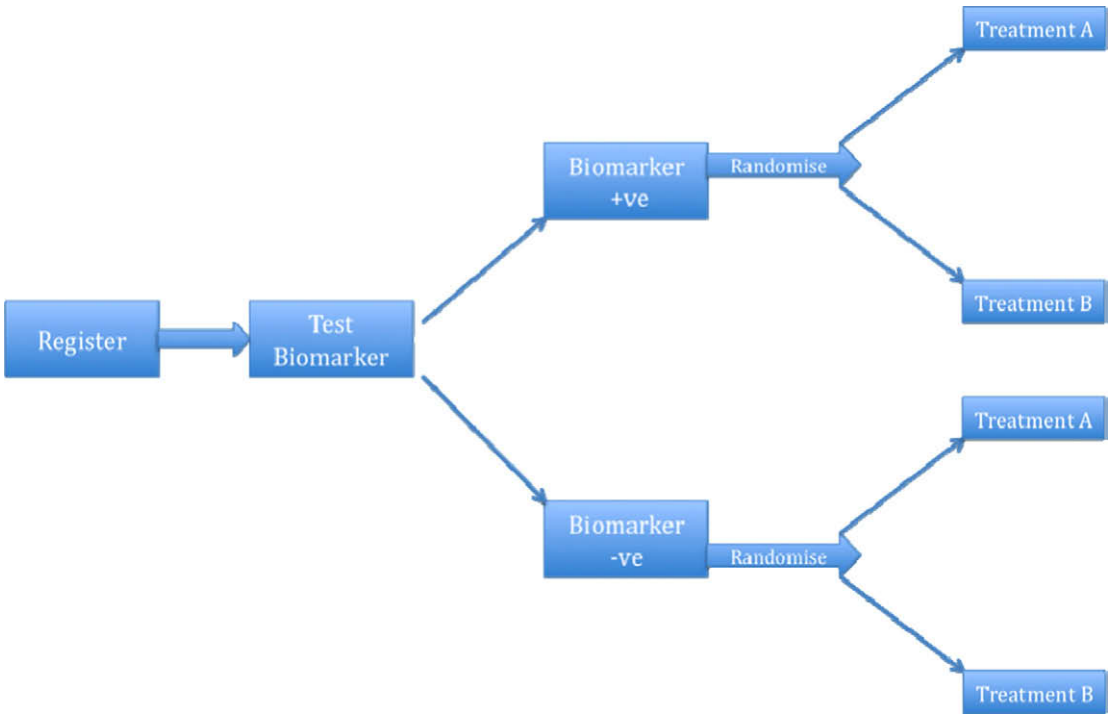

Fig. 4 – This design divides patients initially according to biomarker status and then randomises each group to both treatments. Modified from Sargent et al.

guidelines specifically for cancer predictive biomarker study reporting.

As long ago as 1996, an approach to evaluate the clinical usefulness of biomarkers was suggested by Hayes and colleagues.[38] Their Tumour Marker Utility Grading System (TMUGS) was designed to assess the evidence associated with a biomarker in order to conclude on its impact for improved patient outcomes. Applying the TMUGS allows a score of the biomarker to be allocated, on a scale from 0 to +++; 0 confirming inapplicability of the biomarker confirmed by adequate evaluation; and +++ strongly recommending the use of the biomarker as the sole criterion for clinical decision-making. The system, also, provides assessment of the evidence associated with a biomarker. It grants "I" (as the highest level) to strong evidence from studies conducted specifically to assess the biomarker, and "V" (as the lowest level) to evidence from small pilot studies that were not designed specifically to test for the biomarker utility.[38] This grading system could provide a useful framework for the evidence-based evaluation and implementation of predictive biomarkers in cancer medicine.

6. Clinical Implementation of biomarkers

There are three key issues associated with implementing a biomarker test in the clinic. The first is the approval of the test by regulatory authorities, the second is the acceptability of the test by physicians and patients and the third is the impact of the biomarker test on the cost-effectiveness of anticancer treatments. Addressing these issues is essential when trying to implement a biomarker in the clinic.

6.1. *Approval of regulatory authorities*

The US FDA has acknowledged the importance of biomarkers in its Critical Path Opportunities report published in March 2006 and a regulatory framework to be applied in biomarker development is outlined. This framework divides biomarkers into exploratory, probable valid and known valid, each of which can be applied in defined situations during drug development or in the clinic.[29] There is also a well-established medical test and device regulatory process established in the USA, which can be applied to biomarkers. At present there is no regulatory framework for premarketing regulation of biomarkers in the EU and UK and regulation occurs largely in post-marketing space.

6.2. *Acceptability of biomarkers*

Any biomarker test will not be fully utilised unless it is accepted by the physician and the patient. Efforts and resources might be wasted on developing a biomarker test that does not adequately or precisely address the area of clinical need, or is too expensive or too invasive to be accepted by stakeholders. It is noteworthy that this aspect of biomarker implementation has not been investigated to any great extent particularly from the perspective of cancer patients.

6.3. *Impact of biomarkers on cost-effectiveness of anticancer treatment*

Evaluation of cost-effectiveness is a critical component in the approval for the use of novel agents in many healthcare

systems. Successful predictive biomarkers would be expected to result in avoidance of cost-ineffective treatment for those who are unlikely to benefit from the drug. Therefore, assessment of the impact of the biomarker test on the cost-effectiveness of treatments prior to implementing the test is of central importance and may facilitate the use of targeted agents in clinical practice. A full discussion of this issue is outside the scope of this review. However, in recognition of the importance of this aspect of biomarker development there is an increasing literature base regarding the cost-effectiveness impact of predictive biomarkers, which will provide an increasingly robust and reliable evidence base to undertake this type of analysis.[39–41]

7. Examples of successful predictive biomarkers

To illustrate some of the issues raised in this review, some specific examples from the published literature and clinical practice are now discussed. There has been a considerable increase in the number of published papers about predictive biomarkers since 2000 (see Fig. 5). Accordingly, the number of cancer predictive biomarkers being investigated or in development is rapidly increasing. There is significant attrition during predictive biomarker development, but no specific published data regarding the magnitude of this. In particular, there is a significant attrition from discovery to early qualification which involves failed attempts to qualify the biomarker in an adequately sized independent set of clinical specimens retrospectively (often called a clinical reference set) ideally in an independent laboratory. Therefore, the examples discussed are predictive biomarkers that have been implemented in the clinic or those in the later stages of qualification, and excluding those in the discovery phase of development. For each example, we will review the validation, qualification and clinical implementation processes and highlight specifically the challenges identified in this review.

7.1. *Human Epidermal Growth Factor Receptor 2 (HER2)*

HER2 is the target of the anti-cancer drug trastuzumab used for the treatment of breast cancer. HER2 protein is over-expressed in about 25% of patients (as detected by IHC).[33] This over-expression is used as a predictive biomarker for patient selection for treatment with trastuzumab. HER2 IHC as a biomarker has been properly qualified for its intended use through retrospective and prospective clinical trials.[33] However, serious reproducibility issues were associated with the studies that bio-analytically validated the IHC testing of HER2.[42] This necessitated new guidelines for HER2 testing and illustrates the importance of 'fit-for-purpose' biomarker assay validation for optimal clinical implementation even for a well-qualified biomarker. The recent ToGA trial demonstrating a benefit from the addition of trastuzumab

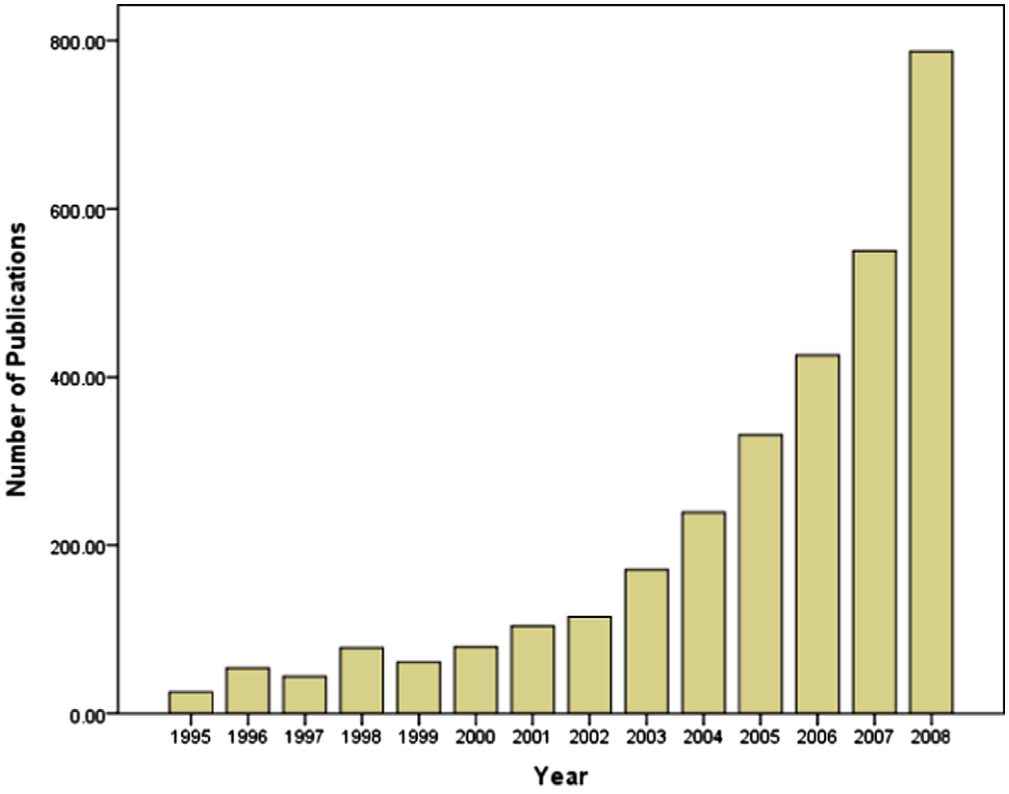

Fig. 5 – The trend of cancer predictive biomarkers published literature: The publications were searched for in Medline, Embase and SCOPUS using all forms and synonyms of the search terms 'biomarker', 'prediction' and 'cancer' and duplicates were removed. There appears to be a considerable increase in the number of cancer predictive biomarkers published papers since the year 2000.

to chemotherapy in oesophagogastric adenocarcinoma was greatly facilitated by the ability to use fully validated immunohistochemistry and FISH assays for HER2 as a predictive biomarker to select patients.[43] In this way many of the problems encountered in breast cancer associated with inadequate validation of HER2 assay (that was not fit for purpose) have been avoided.

7.2. *Epidermal Growth Factor Receptor (EGFR)*

EGFR mutations, gene copy number and EGFR protein expression in NSCLC have been investigated as possible predictive biomarkers for the EGFR tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib. *EGFR* mutations and gene copy number are correlated with clinical benefit from both the drugs however results have been conflicting.[32,44] Interpretation of data has, to a certain extent, been complicated by individual study designs which have not always allowed qualification of the distinction between potential prognostic and/or predictive value of EGFR testing.[32,44] This illustrates the importance of study design for predictive biomarkers qualification as discussed earlier in this review. Some studies have relied on retrospective analysis of samples collected from clinical trials conducted on the related drugs and, as discussed in this review, this is likely to explain some of the conflicting data. Nevertheless, prospective data from Mok and colleagues[45] demonstrate that non-smoker EGFR mutation-positive adenocarcinoma NSCLC patients have worse survivals when treated with chemotherapy as opposed to gefitinib (an EGFR TKI) whereas mutation-negative patients have worse survivals with gefitinib and do better with chemotherapy. As a result of this and similar studies, the European Medicines Agency (EMEA) has granted a marketing authority for gefitinib in any line of treatment of EGFR mutation-positive NSCLC patients. However, the possibility that perhaps a clinically significant subgroup of EGFR mutation-negative NSCLC patients may benefit from EGFR TKIs is not excluded by this or other data. Therefore while fully validated assays exist, EGFR 'testing' is not yet fully or optimally qualified for clinical implementation as a predictive biomarker for EGFR TKIs in NSCLC. Recognition of this is demonstrated by the recent instigation of further prospective clinical trials aimed at qualification of the role of EGFR testing as a predictive biomarker; for example, the MARVEL study in North America, which will determine the predictive utility of *EGFR* copy number testing by FISH for use of erlotinib in the second line setting in advanced NSCLC. Overall, the clinical trial data for EGFR testing in NSCLC illustrate a number of key issues of critical importance for effective and efficient predictive biomarker development, namely; the limitations of retrospective qualification, the importance of clinical trial design for prospective predictive biomarker qualification to distinguish predictive versus prognostic impact; the potential impact on predictive accuracy of type of (fully validated)assay performed for the biomarker molecule; the source of the tumour material used for biomarker assay, surgical resection, biopsy, FNA; and the potential impact of clinical (for example, NSCLC in never smokers) and histopathological disease heterogeniety on the predictive impact of a biomarker. The data illustrate that ideally these issues should be appreciated a priori, as far as possible and thus accounted for in preclinical and clinical study design and interpretation in predictive biomarker development.

7.3. *K-ras mutations*

K-ras is a small G-protein that has a significant role in the signal transduction of EGFR, which is expressed in, and has a pathogenic role in, colorectal cancer (CRC). Activating mutations in *K-ras* may isolate the pathway from the requirement for EGFR signalling rendering anti-EGFR targeting drugs, such as cetuximab and panitumumab ineffective.[46] Several studies have demonstrated that benefit from the anti-EGFR drugs cetuximab and panitumumab is restricted to CRCs with wild-type *K-ras* and there is no benefit in patients whose tumours have mutant k-ras.[46–50] Despite this data being derived from retrospective analysis of samples from therapeutic-driven randomised (as opposed to biomarker-driven) clinical trials, the consistency and strength of the data have led to both the US FDA and UK National Institute for Health and Clinical Excellence (NICE) to approve testing for *K-ras* mutation, using DNA sequencing or quantitative PCR, to guide patient selection.[47–50] This represents the first example of the implantation of a predictive biomarker in oncology without a specifically designed prospective trial and in this respect offers a 'proof of principle' for this type of approach based on the retrospective evaluation of samples from a randomised controlled trial. In the context of the potential pitfalls for retrospective biomarker analysis, the key points are that the retrospective analysis is from more than one independent, well-designed phase III-randomised controlled trial; samples were available from a large majority of patients; there is a well validated and accepted assay for the biomarker; the biomarker hypothesis was prospectively stated; and prospective sample size and power calculations performed to justify the analysis on the samples were available. These criteria are met for K-ras mutation testing as a predictive biomarker in CRC and accordingly may provide useful guidance for the application of retrospective qualification in other settings. This would provide a more feasible and timely option, although a specifically designed biomarker-driven prospective randomised clinical trail will remain the gold standard and may be necessary and indeed the most appropriate option in many clinical situations for qualification and /or optimal clinical implementation, for example, where there are competing assays or biomarkers.

8. Conclusions

While progress has been limited to date the potential benefits of predictive biomarkers to direct the use of systemic anticancer agents in individual patients are well recognised. Accordingly, there is significant motivation within the field to improve the biomarker development process. A key aspect would be the formulation of robust clinical biomarker development methodology with defined processes and acceptance criteria for ongoing development. Increasingly this is occurring and alongside this the key challenges at each stage of biomarker development are being identified and

investigational approaches to overcome them being defined. An important emerging aspect here for clinical researchers involves assessing the utility and quality of retrospective qualification data and useful opinion and guidance are developing to address this. While the biomarker development process remains relatively 'underdeveloped' at present, current prioritisation of research addressing this amongst stakeholders and parallel alignment of funding and regulation is likely to facilitate the development of clinically useful predictive biomarker assays through the formulation of a robust developmental process. The appearance of guidance in the form of 'roadmaps' from national and multinational review groups for biomarker development will be the next key step and will represent a realistic and practical starting point for establishing robust methodology for predictive biomarker development from discovery to clinical implementation.

Conflict of interest statement

None declared

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REFERENCES

1. Collins I, Workman P. New approaches to molecular cancer therapeutics. *Nat Chem Biol* 2006;**2**(12):689–700.
2. NIH, Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Toxicol* 2001;**69**(3):2020–27.
3. Kelloff GJ, Sigman CC. New science-based endpoints to accelerate oncology drug development. *Eur J Cancer* 2005;**41**(4):491–501.
4. Sarker D, Workman P. Pharmacodynamic biomarkers for molecular cancer therapeutics. *Adv Cancer Res* 2006;**96**:213–68 [ate of Pubaton].
5. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer* 2005;**5**(11):845–56.
6. Maruvada P, Srivastava S. Joint National Cancer Institute-Food and Drug Administration workshop on research strategies, study designs, and statistical approaches to biomarker validation for cancer diagnosis and detection. *Cancer Epidemiol Biomarkers Prev* 2006;**15**(6):1078–82.
7. Sawyers CL. The cancer biomarker problem. *Nature* 2008;**452**(7187):548–52.
8. Chau CH, Rixe O, McLeod H, et al. Validation of analytic methods for biomarkers used in drug development. *Clin Cancer Res* 2008;**14**(19):5967–76.
9. Lee JW, Devanarayan V, Barrett YC, et al. Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res* 2006;**23**(2):312–28.
10. Cummings J, Ward TH, Greystoke A, et al. Biomarker method validation in anticancer drug development. *Br J Pharmacol* 2008;**153**(4):646–56.
11. Chin L, Gray JW. Translating insights from the cancer genome into clinical practice. *Nature* 2008;**452**(7187):553–63.
12. Petty RD, Nicolson MC, Kerr KM, et al. Gene expression profiling in non-small cell lung cancer: from molecular mechanisms to clinical application. *Clin Cancer Res* 2004;**10**(10):3237–48.
13. Petty RD, Kerr KM, Murray GI, et al. Tumor transcriptome reveals the predictive and prognostic impact of lysosomal protease inhibitors in non-small-cell lung cancer. *J Clin Oncol* 2006;**24**(11):1729–44.
14. van't Veer LJ, Bernards R. Enabling personalized cancer medicine through analysis of gene-expression patterns. *Nature* 2008;**452**(7187):564–70.
15. Debucquoy A, Haustermans K, Daemen A, et al. Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer. *J Clin Oncol* 2009;(March) [ePublished Ahead of Print].
16. Martinkova J, Gadher SJ, Hajduch M, et al. Challenges in cancer research and multifaceted approaches for cancer biomarker quest. *FEBS Lett* 2009;**583**(11):1772–84.
17. Shah VP. The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. *AAPS Journal* 2007;**9**(1) [Arte Number: 5. ate of Pubaton].
18. Fontaine N, Rosengren B. Directive 2001/20/EC of the european parliament and of the council: on the approximation of the laws, regulations and administrative provisions of the members states relating to the implementation of good clinical practice in the conduct of clinical trails on medicinal products for human use. *OJEC* 4-4-2001.
19. FDA. Guidance for industry: bioanalytical method validation. 2001.
20. Stiles T, Grant V, Mawbey N. *Good clinical laboratory practice (GCLP): a quality system for laboratories that undertake the analysis of samples from clinical trials*. Suffolk, UK: British Association of Research Quality Assurance (BARQA); 2003.
21. Lee JW, Weiner RS, Sailstad JM, et al. Method validation and measurement of biomarkers in nonclinical and clinical samples in drug development: a conference report. *Pharm Res* 2005;**22**(4):499–511.
22. Korfmacher WA. Principles and applications of LC–MS in new drug discovery. *Drug Discov Today* 2005;**10**(20):1357–67.
23. Smolec J, DeSilva B, Smith W, et al. Bioanalytical method validation for macromolecules in support of pharmacokinetic studies. *Pharm Res* 2005;**22**(9):1425–31.
24. Shah VP, Midha KK, Findlay JWA, et al. Bioanalytical method validation – a revisit with a decade of progress. *Pharm Res* 2000;**17**(12):1551–7.
25. Nowatzke W, Wood E. Best practices during bioanalytical method validation for the characterisation of assay reagents and the evaluation of analyte stability in assay standards quality controls and study samples. *AAPS J* 2007;**9**:E117–22.
26. Amur S, Frueh F, Lesko LJ, et al. Integration and use of biomarkers in drug development, regulations and clinical practice: a US regulatory perspective. *Biomarkers Med* 2008;**2**(3):305–11.
27. Sargent DJ, Conley BA, Allegra C, et al. Clinical trial designs for predictive marker validation in cancer treatment trials. *J Clin Oncol* 2005;**23**(9):2020–7.
28. Brunner N, Vang Nielsen K, Offenberg H, et al. Biomarkers for therapeutic efficacy. *Eur J Cancer* 2007;**5**(5 Suppl.):129–42.
29. Goodsaid F, Frueh F. Biomarker qualification pilot process at the US Food and Drug Administration. *AAPS J* 2007;**9**(1):E105–8.

30. Goodsaid F, Frueh F. Process map proposal for the validation of genomic biomarkers. *Pharmacogenomics* 2006;**7**(5):773–82.
31. Burckart GJ, Amur S, Goodsaid FM, et al. Qualification of biomarkers for drug development in organ transplantation. *Am J Transplant* 2008;**8**(2):267–70.
32. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer – molecular and clinical predictors of outcome. *N Engl J Med* 2005;**353**(2):133–44.
33. Waring PM. Matching patients with drugs: triumphs and challenges. *Personalized Med* 2006;**3**(3):335–44.
34. Moretti F, Belardelli F. Needs and challenges in translational medicine: filling the gap between basic research and clinical applications. *ISTISAN Congr* 2008;**08**(C7).
35. Seymour MT, Maughan TS, Ledermann JA, et al. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. *Lancet* 2007;**370**(9582):143–52.
36. Braun MS, Richman SD, Quirke P, et al. Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. *J Clin Oncol* 2008;**26**(16):2690–8.
37. McShane LM, Altman DG, Sauerbrei W, et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Eur J Cancer* 2005;**41**(12):1690–6.
38. Hayes DF, Bast RC, Desch CE, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. *J Natl Cancer Inst* 1996;**88**(20):1456–66 [see comment].
39. Williams SA, Slavin DE, Wagner JA, et al. A cost-effectiveness approach to the qualification and acceptance of biomarkers. *Nat Rev Drug Discovery* 2006;**5**(11):897–902.
40. Wagner JA, Williams SA, Webster CJ. Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. *Clin Pharmacol Ther* 2007;**81**(1):104–7.
41. Chatterjee SK, Zetter BR. Cancer biomarkers: knowing the present and predicting the future. *Future Oncol* 2005;**1**(1):37–50.
42. Gown AM. Current issues in ER and HER2 testing by IHC in breast cancer. *Mod Pathol* 2008;**21**:S8–S15.
43. Van Cutsem E, Kang Y, Chung H, et al. Efficacy results from the ToGA trial: a phase III study of trastuzumab added to standard chemotherapy (CT) in first-line human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer (GC). *J Clin Oncol* 2009;**27**(18S):LBA4509 [Meeting Abstracts].
44. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;**304**(5676):1497–500.
45. Mok TS, Wu Y, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;**361**(10):947–57.
46. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008;**359**(17):1757–65.
47. De Roock W, Piessevaux H, De Schutter J, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008;**19**(3):508–15.
48. Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. *Br J Cancer* 2007;**96**(8):1166–9.
49. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;**66**(8):3992–5.
50. Linardou H, Dahabreh IJ, Kanaloupiti D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. *Lancet Oncol* 2008;**9**(10):962–72.