

Companion Biomarkers: Paving the Pathway to Personalized Treatment for Cancer

Michael J. Duffy^{1,2*} and John Crown³

BACKGROUND: Companion biomarkers are biomarkers that are used in combination with specific therapies and that prospectively help predict likely response or severe toxicity. In this article we review the role of companion biomarkers in guiding treatment in patients with cancer.

CONTENT: In addition to the established companion biomarkers such as estrogen receptors and HER2 (human epidermal growth factor receptor 2) in breast cancer, several new companion biomarkers have become available in recent years. These include v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations for the selection of patients with advanced colorectal cancer who are unlikely to benefit from anti-epidermal growth factor receptor antibodies (cetuximab or panitumumab), epidermal growth factor receptor (*EGFR*) mutations for selecting patients with advanced non-small cell lung cancer (NSCLC) for treatment with tyrosine kinase inhibitors (gefitinib or erlotinib), v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) mutations for selecting patients with advanced melanoma for treatment with anti-BRAF agents (vemurafenib and dabrafenib), and anaplastic lymphoma receptor tyrosine kinase (*ALK*) translocations for identifying patients with NSCLC likely to benefit from crizotinib.

SUMMARY: The availability of companion biomarkers should improve drug efficacy, decrease toxicity, and lead to a more individualized approach to cancer treatment.

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Most patients with cancer currently receive systemic therapy such as chemotherapy, hormone therapy, biological therapy, or various combinations of these therapies. Until now, the factors considered in the selection of the most appropriate systemic therapy have in-

cluded tumor anatomical origin, stage, and histological grade and patient age and performance status. Although these criteria will continue to be used, they are increasingly being reinforced by the availability of biomarkers, known as companion biomarkers. Companion biomarkers may be defined as biomarkers that are used in combination with therapy to prospectively help predict likely response or resistance. Companion biomarkers may also help in the selection of the therapeutic dose as well as the likely development of severe toxicity.

Although companion biomarkers may be used in guiding treatment for different diseases, they are particularly important in cancer because of the following factors (1):

- Life expectancy in cancer patients may be short, especially with advanced disease.
- Many cancer treatments, especially the newer biological therapies, have efficacy in only a minority of treated patients.
- The risk of toxicity from anticancer treatments is high.
- Certain anticancer treatments, particularly some of the newer targeted agents, are expensive.

In recent years, a multiplicity of companion biomarkers have become available that can be used to predict responses to a range of therapies in different cancer types. Our aim in this article was to review these companion biomarkers. In addition, we discuss methods for the validation of new companion biomarkers. Markers for predicting severe toxicity from anticancer agents are not discussed.

Estrogen Receptor as a Companion Biomarker for Hormone Treatment in Breast Cancer

The estrogen receptor (ER)⁴ was one of the first companion biomarker in oncology. Research carried out in the 1960s and 1970s showed that surgical removal of the ovaries and/or adrenal glands induced objective tu-

¹ UCD School of Medicine and Medical Science, Conway Institute, University College Dublin, Ireland; ² Clinical Research Centre and ³ Department of Medical Oncology, St Vincent's University Hospital, Dublin, Ireland.

* Address correspondence to this author at: UCD Clinical Research Centre, St Vincent's University Hospital, Dublin 4, Ireland. Fax +353-1-269-6018; e-mail michael.j.duffy@ucd.ie.

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⁴ Nonstandard abbreviations: ER, estrogen receptor; IHC, immunohistochemistry; ASCO, American Society for Clinical Oncology; CAP, College of American Pathologists; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; FISH, fluorescent in situ hybridization; EGFR, epidermal growth factor receptor; CRC, colorectal cancer; K-RAS, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; FDA, US Food and Drug Administration; ALK, anaplastic lymphoma kinase.

mor response in ER-positive patients with advanced breast cancer (2, 3). In contrast, tumor regression following these surgical ablations rarely occurred in women with ER-negative cancers. Subsequently, endocrine ablation was replaced with drugs such as the antiestrogen agent tamoxifen. Administration of adjuvant tamoxifen for 5 years to patients with ER-positive early breast cancer has been shown to reduce recurrence rates by almost 50% (4, 5). In addition, mortality from ER-positive breast cancer during the first 15 years after treatment is reduced by about one third (4, 5).

Despite the success of tamoxifen, its use in postmenopausal patients with breast cancer has been largely superseded, at least as first-line hormone therapy, by a group of drugs known as aromatase inhibitors. Aromatase inhibitors such as anastrozole, letrozole, and exemestane act by preventing the formation of estrogen from its precursors (6). The end result of treatment with an aromatase inhibitor is thus similar to that of tamoxifen, which prevents estrogen from binding to ER and stimulating breast cancer cell growth, i.e., both drugs block estrogen from mediating its biological effects.

Several trials have shown that aromatase inhibitors are superior to tamoxifen, at least for progression-free survival, in postmenopausal patients with early breast cancer [for review, see (6)]. In head-to-head comparisons, however, aromatase inhibitors have not yet been shown to be superior to tamoxifen in extending overall survival. It remains to be shown if a significant difference with respect to overall survival will emerge with further patient follow-up. Unlike tamoxifen, aromatase inhibitors cannot be used in premenopausal women. On the basis of the above findings, most expert panels currently recommend the use of an aromatase inhibitor, at least as part of an adjuvant therapy regime, for postmenopausal patients with ER-positive breast cancer (7–10).

Currently, the standard assay method for determining ER is immunohistochemistry (IHC) with a validated antibody. Among the advantages of IHC over the older biochemical assays are that this method can be applied to formalin-fixed paraffin-embedded tissue, it is simple and cheap to use, and can be performed on small amounts of tissue, including fine needle aspirates and core needle biopsies (11). An additional advantage is that the normal breast epithelial cells in adjacent tissue provide an internal positive control. Unlike biochemical assays, it cannot provide quantitative data or information on the functionality of the receptor, i.e., its ability to bind ER. Furthermore, the interpretation of IHC is subjective and difficult to standardize (11).

Detailed guidelines for performing IHC for ER have been published by both the American Society for Clinical Oncology (ASCO)/College of American Pa-

thologists (CAP) (12) and the National Academy of Clinical Biochemistry (11). The key points in the ASCO/CAP guidelines are as follows (12):

- ER should be measured on all invasive breast cancers.
- Measurement should be performed with a validated assay.
- The length of time from tumor acquisition to fixation should be as short as possible.
- Samples should be fixed in 10% neutral buffered formalin for 6 to 72 h.
- Receptor positivity should be defined as $\geq 1\%$ of tumor cells staining positive.
- The percentage or proportion of tumor cells staining positively should be recorded and reported.
- The intensity of staining should also be recorded and reported as weak, moderate, or strong.
- Assay results should be reported as receptor positive, receptor negative, or receptor uninterpretable.
- Participation in external quality assurance (proficiency testing) programs with at least 2 testing events per year is mandatory.

HER2 as a Companion Biomarker for Anti-HER2 Therapy in Breast Cancer

Another routinely measured companion biomarker in breast cancer is human epidermal growth factor receptor 2 (HER2), which is mandatory in selecting patients for treatment with anti-HER2 therapy. The HER2 gene [v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) (*ERBB2*; also known as *HER2*)]⁵ is amplified and overexpressed in 15%–20% of primary invasive tumors (13). In these amplified and overexpressed cancers, the *ERBB2* protooncogene appears to be a primary driver of cancer cell proliferation. Consequently, as with estrogen-dependent breast cancer discussed above, blocking HER2 might be expected to limit growth of HER2-dependent cancers.

The consequence of HER2 gene (*ERBB2*) amplification and overexpression in breast cancer is that instead of having only 2 copies of the HER2 gene per cell, breast cancer cells may have >50 copies. As a result, the number of HER2 protein molecules per cell can in-

⁵ Human genes: *ERBB2*, v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) (also known as *HER2*); *EGFR*, epidermal growth factor receptor; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *ALK*, anaplastic lymphoma receptor tyrosine kinase; *EML4*, echinoderm microtubule associated protein; *NPM1*, nucleophosmin (nucleolar phosphoprotein B23, numatrin); *VCL*, vinculin; *TPM3*, tropomyosin 3; *TPM4*, tropomyosin 4; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset.

crease from 20 000–50 000 to approximately 2 million (14). This large differential in concentration of HER2 proteins allows selective targeting of HER2-amplified breast cancer cells vis-à-vis normal breast cells as well as other normal cells throughout the body.

The first anti-HER2 therapy approved for clinical use was the humanized monoclonal antibody, trastuzumab (Herceptin). Trastuzumab binds to the extracellular region of HER2 (domain IV) and appears to inhibit growth of HER2-overexpressing breast cancer through multiple mechanisms, including disruption of ligand-independent HER2–HER3 interaction, prevention of downstream HER2 signaling, blocking cleavage of the HER2 extracellular domain, increasing DNA repair, and induction of antibody-dependent cell cytotoxicity (15).

In the pivotal trial evaluating trastuzumab in patients with advanced HER2-positive breast cancer, Slamon et al. (16) found that the addition of trastuzumab to chemotherapy resulted in a longer time to disease progression (median, 7.4 vs 4.6 months; $P < 0.001$), higher response rates (50% vs 32%, $P < 0.001$), longer duration of response (median, 9.1 vs 6.1 months; $P < 0.001$), and longer survival (median survival, 25.1 vs 20.3 months; $P = 0.01$).

It is important to state that if this trial had been performed without the preselection of patients on the basis of HER2 overexpression, the beneficial effect of trastuzumab could have been missed (17, 18). For example, in the above trial, the response rate for trastuzumab-treated patients was 50% and the mortality rate at 1 year was 22%. For the control arm, without trastuzumab, the corresponding values were 32% and 33%, respectively. In the absence of selection for trastuzumab on the basis of HER2 determination, the response rate would have been 37%, and the 1-year mortality rate would be 30%. In this situation, the difference in outcome between trastuzumab-treated and control patients would not have been statistically different (17). Indeed, to achieve a statistically significant outcome with trastuzumab in an all-comers phase III clinical trial, it has been calculated that in excess of 20 000 patients would be required (18). It is unlikely that such a trial would have been carried out, because it would have taken an excessively long time to complete and thus would be prohibitively expensive. Thus, the therapeutic benefit observed with trastuzumab might not have been detected without the upfront measurement of HER2 (17).

Since the publication of the above results, at least 8 randomized clinical trials have compared combined HER2-targeted agents and standard therapies in HER2-positive patients with advanced breast cancer (19). Following a metaanalysis of these trials, Harris et al. (19) calculated that administration of combined

Table 1. Anti-HER2 drugs in clinical use or undergoing clinical trials.

Drug	Type of molecule	Phase of development
Trastuzumab	Monoclonal antibody	In clinical use
Lapatinib	EGFR/HER2 TKI	In clinical use
Pertuzumab	Monoclonal antibody	In clinical use
T-DM1 ^a	Antibody drug conjugate	In clinical use
Neratinib	Pan HER inhibitor	In clinical trials
Afatinib	Pan HER inhibitor	In clinical trials
Dacomitinib	Pan HER inhibitor	In clinical trials

^a T-DM1, trastuzumab-DM1/trastuzumab emtansine.

HER2-targeted agents and standard therapy improved overall survival [hazard ratio (HR), 0.78; 95% CI, 0.67–0.91], total time to progression (HR, 0.56; 95% CI 0.48–0.64), progression-free survival (HR, 0.63; 95% CI 0.53–0.74), and overall response rate (HR, 1.67; 95% CI, 1.46–1.9) vis-à-vis the standard therapy alone (19).

As in advanced breast cancer, trastuzumab is also effective in patients with HER2-positive early breast cancer. At least 6 randomized trials have shown that the addition of trastuzumab to chemotherapy reduced disease recurrence and the risk of death compared with chemotherapy alone (20). Following a metaanalysis of these trials, Yin et al. (20) calculated that the addition of trastuzumab to chemotherapy, compared with chemotherapy alone, significantly improved disease-free survival, overall survival, locoregional recurrence rates, and distant recurrences ($P < 0.001$ for all endpoints investigated). However, the addition of trastuzumab to adjuvant chemotherapy resulted in a higher number of central nervous system recurrences. A possible reason for the increased number of central nervous system recurrences is the increased survival as a result of being treated with trastuzumab. Alternatively, HER2-positive tumors may have a propensity to metastasize to the brain. Available data suggest that the benefit of concurrent adjuvant trastuzumab and chemotherapy is greater than that of chemotherapy followed by trastuzumab (21).

Although trastuzumab was the first approved anti-HER2 therapy for patients with breast cancer, several other agents targeting this protein have become available in recent years (22–30) (Table 1). Of the newer anti-HER2 therapies listed in Table 1, only lapatinib, pertuzumab, and trastuzumab-DM1 have been approved for clinical use. The available evidence suggests that, as with trastuzumab, HER2 gene amplification/

overexpression is necessary for these anti-HER2 drugs to demonstrate efficacy (24, 25).

Two main types of assay are used for detecting HER2 in breast tumors, i.e., IHC and fluorescent in situ hybridization (FISH) (11, 31). The advantages of IHC are its low costs, simplicity, and wide availability. Disadvantages include the subjective evaluation, difficulty in standardization, and the requirement for additional testing with borderline (level 2+) staining levels (11).

As with ER, detailed guidelines for performing HER2 assays have been published by ASCO and CAP (31). The main points in these guidelines are as follows:

- Positivity for HER2 using IHC is defined as either uniform intense membrane staining of >30% of invasive tumor cells or FISH-amplified [ratio of HER2 to CEP17 (chromosome 17 centromere) of >2.2] or average HER2 gene copy number >6 signals/nucleus for those test systems without an internal control probe.
- Interpretation of results should be based on the counting of at least 20 cells.
- A pathologist must confirm that staining is present in the invasive tumor component.
- Time from tissue acquisition to fixation should be as short as possible.
- Samples for testing should be fixed in neutral buffered formalin for 6–48 h.
- Sections should ideally not be used for HER2 measurement if cut >6 weeks earlier.
- Laboratories performing HER2 testing for clinical use should participate in external proficiency testing/external quality assurance programs with at least 2 series of tests per year.

KRAS Mutational Status as a Companion Biomarker for Anti-Epidermal Growth Factor Receptor Therapy in Colorectal Cancer

Cetuximab and panitumumab are monoclonal antibodies approved for use in selected patients with advanced colorectal cancer (CRC). Because both of these antibodies act by binding to the extracellular domain of epidermal growth factor receptor (EGFR), it was originally assumed that concentrations of this receptor would predict efficacy. Several studies however, failed to show a significant association between immunohistochemically determined concentrations of EGFR and response to anti-EGFR antibodies (32). In fact, despite the presence of immunoreactive EGFR in most CRCs investigated, only 10%–20% of patients with unselected advanced cancers benefited from treatment with cetuximab or panitumumab (32). Furthermore, some patients responded who lacked detectable immunoreactive EGFR (33).

EGFR, coded by the epidermal growth factor receptor (*EGFR*) gene, mediates growth proliferation, cell death inhibition, and promotion of invasion by signaling through the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) protein (32). Although EGFR concentrations were unrelated to benefit from anti-EGFR antibodies (33), retrospective analysis of several clinical trials showed that patients with specific mutations in the gene that codes for the *KRAS* protein [v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*)], especially in codon 12, rarely responded to cetuximab or panitumumab [for review, see (32)]. On the other hand, 30%–40% of patients with wild-type *KRAS* experienced tumor regression when treated with these antibodies alone or combined with chemotherapy.

These findings were confirmed in a systematic review and metaanalysis of published studies relating the mutational status of the *KRAS* gene with response to anti-EGFR antibodies in patients with advanced CRC. In this high-level evidence study, Adelstein et al. (34) found that the addition of anti-EGFR antibodies to standard treatment resulted in a 20% reduction in the hazard for progression (HR, 0.8; 95% CI, 0.64–0.99) in the subgroup of patients with wild-type *KRAS*. In contrast, combined antibody and chemotherapy treatment failed to provide benefit in patients with specific activating mutations in *KRAS*. The combination of anti-EGFR antibodies and chemotherapy [e.g., oxaliplatin/5-FU (fluorouracil) based] appeared to be detrimental compared to chemotherapy alone in patients with *KRAS* mutations (34).

Because of the multiplicity of findings relating the presence of specific *KRAS* mutations with lack of benefit from anti-EGFR antibodies, several expert panels recommend determination of the *KRAS* mutation status before administration of cetuximab or panitumumab to patients with advanced CRC (35–37). Only patients lacking specific mutations, especially in codon 12, should be considered for administration of these antibodies. Thus, the mutation status of *KRAS* has become an established companion biomarker for guiding treatment with anti-EGFR antibodies in patients with advanced CRC.

Although codon 12 is the most frequently mutated *KRAS* site in CRC, mutations can also be found in codons 13 and 61. Some reports have shown that, unlike the codon 12 mutations, codon 13 mutations are not associated with resistance to anti-EGFR antibodies (38). These findings however, require confirmation. At this stage, little work has been carried out on the relationship between codon 61 mutations and response.

Several methods are available for determining the mutational status of *KRAS* in CRC, including Sanger sequencing, pyrosequencing, allele-specific PCR, high-

resolution melting analysis, and array/strip assays (39). The main advantages and disadvantages of these different methods have been reviewed by Shackelford et al. (39). At present there is, however, no recommended or best available assay for determining the mutational status of *KRAS*. The *KRAS* report, however, should list the specific mutations investigated and the specific methodology used for their detection.

***EGFR* Mutational Status as a Companion Biomarker for *EGFR* Tyrosine Kinase Inhibitors in Non–Small Cell Lung Cancer**

As in CRC, anti-*EGFR* treatment is also available for patients with advanced non–small cell lung cancer (NSCLC) (40). However, unlike the situation in CRC in which anti-*EGFR* antibodies are used, the best-validated anti-*EGFR* therapies for NSCLC patients involve administration of the tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib. Without patient preselection, however, response to the *EGFR* TKIs is rare, being generally 10% or less.

Although, in the absence of patient preselection, the response to *EGFR* TKIs is rare, several phase III trials have shown that approximately 70% of patients with activating mutations in the *EGFR* gene, especially in exons 18–21, respond to either gefitinib or erlotinib (40). Furthermore, these inhibitors improve median progression-free survival by 3–5 months compared to chemotherapy in patients with activating *EGFR* mutations (40). In contrast, patients lacking these activating mutations rarely benefit from anti-*EGFR* TKIs. On the basis of these findings, an ASCO Provisional Clinical Opinion stated that “patients with NSCLC who are being considered for first-line therapy with an *EGFR* TKI (patients who have not previously received chemotherapy or an *EGFR* TKI) should have their tumor tested for *EGFR* mutations to determine whether an *EGFR* TKI or chemotherapy is the appropriate first-line therapy” (41).

Although specific activating mutations in *EGFR* are associated with response to gefitinib and erlotinib, the T790M (threonine-to-methionine) mutation is associated with resistance to these agents. This mutation is detected in approximately 50% of NSCLC patients who develop resistance to either gefitinib or erlotinib (42). The T790M mutations appear to give rise to a conformation in the *EGFR* protein that precludes the binding of gefitinib and erlotinib. A new generation of TKIs, known as irreversible TKI, is currently undergoing clinical trials in NSCLC patients with the T790M mutation (42).

Methods for determining mutations in *EGFR* in NSCLC can be divided into 2 main types, screening methods that detect all mutations and targeted meth-

ods that identify specific and known mutations [for review, see (43)]. Screening methods such as direct sequencing are widely available and can potentially detect all mutations. These methods, however, are labor-intensive, can exhibit low analytical sensitivity, and may require enrichment of tumor cells by macro- or microdissection. Targeted methods, on the other hand, are generally faster and give higher analytical sensitivity but have the disadvantage that rare mutations are not detected (43).

***BRAF* MUTATIONAL STATUS AS A COMPANION BIOMARKER FOR ANTI-*BRAF* THERAPIES IN MELANOMA**

Serine/threonine-protein kinase B-Raf (*BRAF*) protein is a member of the RAF kinase family involved in downstream signaling from *EGFR*-RAS. Mutations in the v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) gene are present in approximately 50%–60% of skin melanomas. Approximately 80% of these involve substitution of a valine residue for a glutamic residue at amino acid 600 of *BRAF*, giving rise to the V600E mutation (44). Less frequently found mutations include V600K (valine to lysine) and V600D (valine to aspartate) (44).

Several inhibitors are now available that selectively bind to the *BRAF* mutated protein. The 2 most widely investigated in clinical trials are those for vemurafenib and dabrafenib. A phase I expansion clinical trial showed that the administration of vemurafenib to patients with advanced melanomas harboring V600E produced a response rate in 26/32 (81%) of those treated (45). The pivotal clinical trial involved randomizing 675 patients with advanced melanomas, possessing a V600E mutation, to either vemurafenib or the cytotoxic drug dicarbazine (46). Follow-up analysis showed that the overall survival rate at 6 months was 84% for those treated with vemurafenib vs 64% for those treated with dicarbazine. Progression-free survival was 5.3 months in the vemurafenib arm compared to 1.6 months in the dicarbazine arm.

The above results led the US Food and Drug Administration (FDA) to approve vemurafenib for the treatment of patients with advanced melanoma containing the V600E mutation. At the same time, the FDA approved a companion biomarker assay (cobas 4800 *BRAF* V600 Mutation Test) for selecting patients with advanced melanoma for treatment with vemurafenib. This was the first simultaneous approval of a therapeutic drug and companion biomarker by the FDA. It should be a model for the future development of companion biomarkers and targeted drugs.

The FDA-approved test for detecting *BRAF* mutations detects a single point mutation, i.e., V600E. However, as stated below, other mutations may occur in this gene that confer sensitivity to anti-*BRAF* agents, as dis-

cussed below. As pointed out by Menzies et al. (47), it is important that clinical tests for *BRAF* mutations have the ability to detect all of the V600 alterations. It has been suggested that failure to detect the V600K mutation may prevent 10%–15% of melanoma patients from receiving anti-*BRAF* therapy (47). Measurement of non-V600E mutations should thus be included in future trials evaluating anti-*BRAF* drugs in patients with melanoma.

Since the approval of vemurafenib, a second anti-*BRAF* kinase inhibitor, dabrafenib, has been shown in a phase I clinical trial to be effective in patients with *BRAF* mutation–positive advanced melanoma, including some patients with brain metastasis (48). In this study, dabrafenib induced regression not only in patients with the V600E mutation but also in some with the V600K mutation. Furthermore, response to dabrafenib was found in patients with *BRAF*-positive non-melanoma malignancies, such as papillary thyroid cancer, gastrointestinal stromal cancer, and NSCLC (48).

***EML4-ALK* Translocation as a Companion Marker for Crizotinib in NSCLC**

Fusion of the anaplastic lymphoma receptor tyrosine kinase (*ALK*) and echinoderm microtubule associated protein like 4 (*EML4*) genes, which is found in 3%–7% of patients with NSCLC, results from an inversion on chromosome 2 (49). This fusion leads to continuous activation of anaplastic lymphoma kinase (*ALK*) activity, which in turn gives rise to enhanced cell proliferation and decreased cell survival. Although *ALK* translocations are found in approximately 5% of all NSCLC cases, this positivity rate can be increased by selecting patients with adenocarcinoma histology, tumors negative for *EGFR* mutations, and tumors from patients who have never smoked (50).

Crizotinib, a tyrosine kinase inhibitor originally developed to block MET, was found to induce tumor regression and prolong survival in patients with advanced NSCLC harboring an *ALK* translocation (51, 52). On the basis of these results, the FDA gave accelerated approval to crizotinib for the treatment of patients with locally advanced or metastatic NSCLCs that are positive for the *EML4-ALK* translocation. A predictive marker test for identifying patients likely to be responsive to crizotinib was simultaneously approved by the FDA (Vysis *ALK* Break-Apart FISH probe kit). The National Comprehensive Cancer Network guidelines currently recommend treatment with crizotinib for patients with *EML4-ALK*-positive NSCLCs (42). Because *ALK* translocations are present in only approximately 5% of all NSCLC cases, the measurement of this biomarker was essential for the successful devel-

opment and approval of crizotinib for clinical use. However, >1500 NSCLC patients had to be tested for the *EML4-ALK* translocation to identify the 82 selected patients in the original phase I trial (51).

As with the evaluation of vemurafenib for *BRAF* mutation–positive melanoma, the above validation approach for crizotinib in *ALK*-positive NSCLC provides a good example of parallel development of a therapy and its companion biomarker. In both of these situations, the relevant biomarker was incorporated in clinical trials at stage I. Clearly, in both situations codevelopment resulted in more rapid testing and approval than might be expected from the traditional approach of the independent evaluation of the drug and biomarker. However, such an approach can be costly in the short term because it requires coordinated assay development and validation as well as the clinical testing of the new drug. It may also restrict the technology platform for measuring the biomarker and result in an assay that may exhibit suboptimum predictive potential. Thus, as mentioned earlier, specifically measuring V600E mutations in melanoma may miss patients with other mutations that could respond to anti-*BRAF* treatments.

Although *ALK* rearrangements are found in only approximately 5% of NSCLC, related gene rearrangements have been found in other types of malignancies [for review, see (53)]. Thus, *ALK* fusions with nucleophosmin (nucleolar phosphoprotein B23, numatrin) (*NPM1*) have been detected in anaplastic large cell lymphomas, fusion with vinculin (*VCL*) in renal medullary cancer, and fusion with tropomyosin 3 (*TPM3*) or tropomyosin 4 (*TPM4*) in inflammatory myofibroblastic tumors (53). In addition, in neuroblastoma and anaplastic thyroid cancers, *ALK* can be activated by missense mutations (53). These findings suggest that *ALK* inhibitors such as crizotinib may be effective in several different cancer types. Although the effectiveness of crizotinib in these cancers is not yet proven, the determination of the *ALK* gene activation status may also predict benefit from anti-*ALK* treatments in these tumors.

Other Companion Biomarkers

Other companion and emerging companion biomarkers for cancer therapeutics are listed in Table 2.

Validation of Companion Biomarkers

To progress to clinical use, candidate companion biomarkers must undergo rigorous analytical and clinical validation and show clinical utility (59, 60). Initial testing may be performed in preclinical systems such as cell lines and animal models. Although these systems are

Table 2. Predictive biomarkers currently available for selecting treatment in patients with different cancers.

Therapy	Cancer	Biomarker	Abnormality	Reference
Hormone	Breast	ER, PR ^a	Protein level	Jensen et al. (2); McGuire et al. (3); EBCTCG et al. (4)
Anti-HER2 (trastuzumab, lapatinib, pertuzumab)	Breast	<i>ERBB2</i>	Gene amplification or overexpression	Harris et al. (19); Yin et al. (20); Untch et al. (26); Baselga et al. (27); Ahn and Vogel (28); Baselga et al. (29); Verma et al. (30); Wolff et al. (31)
Anti-EGFR (cetuximab, panitumumab)	Colorectal	<i>KRAS</i>	Mutation	Bardelli and Siena (32); Chung et al. (33); Adelman et al. (34)
Anti-EGFR (gefitinib, erlotinib)	NSCLC	<i>EGFR</i>	Mutation	Soria et al. (40); Keedy et al. (41)
Anti-BRAF (vemurafenib, dabrafenib)	Melanoma	<i>BRAF</i>	Mutation	Cantwell-Dorris et al. (44); Flaherty et al. (45); Chapman et al. (46)
Anti-ALK (crizotinib)	NSCLC	<i>EML4-ALK</i>	Translocation	Kwak et al. (51); Shaw et al. (52)
Anti-HER2 (trastuzumab)	Gastric	<i>ERBB2</i>	Gene amplification or overexpression	Bang et al. (54)
Imatinib	GIST	<i>KIT</i> ^b	Mutation	Blay et al. (55); Reichardt et al. (56)
PARP inhibitors ^c (olaparib)	Breast/ovarian	<i>BRCA1/2</i>	Mutation	Fong et al. (57); Tutt et al. (58)

^a PR, progesterone receptor; GIST, gastrointestinal stromal tumor; PARP, poly (ADP-ribose) polymerase.
^b Human genes: *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; *BRCA1*, breast cancer 1, early onset; *BRCA2*, breast cancer 2, early onset.
^c Not currently in clinical use.

less than ideal models for human cancers (61), they may provide biological insights into the modes of action of the test drug as well as providing early data indicating if a candidate biomarker has therapy-predictive potential. At this early stage, it is also important to perform preliminary analytical validation of the candidate companion biomarker assay, especially with respect to reproducibility between assays (59, 60, 62).

CLINICAL VALIDATION AND DEMONSTRATION OF CLINICAL UTILITY

Following preclinical proof-of-principle studies, candidate companion biomarkers must undergo validation in clinical trials. Clinical validation in this context relates to the marker's ability to exhibit therapy-predictive value for the relevant drug. It is now widely recommended that candidate predictive biomarkers be incorporated into drug-related clinical trials at an early stage and undergo parallel evaluation with the test drug (18, 63–66). Ideally, the candidate predictive biomarker should have undergone preliminary validation by the end of a phase II clinical trial to provide a definitive validation in phase III (59, 63).

Ideally, clinical use should require validation in a phase III randomized clinical trial. Various trial designs have been proposed for the simultaneous validation of experimental therapeutic drugs and accompanying candidate companion biomarker (1, 63–67). These designs are divided into 2 main types, the all-

comers and enrichment designs (63, 67). In the all-comers design, patients with both high and low concentrations of candidate companion biomarker are randomized to 1 of 2 treatments, with the aim of showing a different treatment benefit in the 2 groups. Although this design provides the highest level of evidence, it requires large numbers of patients and disease events such as recurrences and deaths. The EORTC (European Tarceva vs Chemotherapy) trial, in which patients with advanced NSCLC had their tumor tested for *EGFR* mutations and then were randomized to receive erlotinib or chemotherapy, is an example of this design (68).

Although an adequately powered prospective randomized clinical trial in which the biomarker is the main objective of the trial remains the gold standard method for predictive biomarker validation (63, 67), such trials are time-consuming and costly. To accelerate validation and reduce costs, retrospective analysis of previously completed randomized drug trials carried out using an all-comers strategy has been suggested as an acceptable alternative approach (69).

Validation using this approach, however, should be planned in a detailed prospective manner (69). A specific and technically validated biomarker assay should be used and measurement carried out with a standard operating practice. Furthermore, a predetermined cutoff point should be established for classifying patients into subgroups with low and high biomarker

concentrations. In addition, acceptable quality archival tissue should be available from a sufficient number of patients from an appropriate prospective trial. This is necessary to achieve adequate statistical power and for the patients included in the study to be representative of those participating in the trial. Finally, the results from archival samples should be validated with samples from other similar trials (69). An example of prospective-retrospective validation was the identification of mutant K-RAS as a companion biomarker for predicting resistance to anti-EGFR antibodies in patients with advanced CRC.

In contrast to the all-comers approach, the enrichment design type of trial includes only patients with biomarker-positive disease. Enrichment design trials may be appropriate when the biology of the candidate marker is well established, an analytically validated assay is available for its measurement, and existing pre-clinical/early clinical data suggest predictive potential (63). This type of design can potentially result in faster codevelopment of a drug and its companion biomarker than the all-comers approach. Trials that have used the enrichment design strategy include those that evaluated trastuzumab in HER2-positive breast cancer patients, anti-BRAF agents in BRAF-positive melanoma patients, and crizotinib to treat ALK-positive NSCLC patients.

Although the enrichment design can help identify patients who will potentially derive a clinical benefit, i.e., those patients positive for the relevant biomarker, the enrichment design is unable to provide predictive information in the biomarker-negative population. The question may therefore remain whether potentially drug-responsive patients could be missed with this type of trial (65). For example, although preclinical studies indicated that *ERBB2* gene amplification/overexpression was necessary for response to trastuzumab, unplanned retrospective analyses of clinical trials suggested that some *ERBB2*-negative patients might benefit from this monoclonal antibody (70, 71). This preliminary finding obviously requires prospective validation before trastuzumab could be recommended for treatment of *HER2*-negative patients.

Analytical Validation of Biomarker Assays

In addition to clinical validation, analytical validation of the companion biomarker assay with respect to reproducibility (intra- and interassay), accuracy, analytical specificity, and analytical sensitivity is necessary before clinical use (72). Analytical validation should be completed before the definitive clinical trial is started to ensure that the assay is sufficiently stable in performance for use throughout the trial (59). Additional important requirements for a clinically used biomarker

are a standardized assay and detailed guidelines for its measurement. These guidelines should include recommendations on preanalytical (sample handling and storage), analytical (positive and negative controls and QC samples), and postanalytical (reporting and interpretation of result) criteria. Such guidelines are currently available for ER and HER2 in breast cancer (12, 31) but need to be developed for other companion biomarkers, such as mutation testing for *KRAS* and *EGFR* in CRC and NSCLC, respectively. For laboratories performing these assays for clinical use, it is essential to perform regular internal QC measurements, have established assay acceptance and rejection criteria, participate in external quality assurance programs, and be accredited by an appropriate organization, e.g., CLIA in the US. The external quality assurance should include clinical interpretation of results as well as the assessment of interlaboratory variation. Finally, it may be desirable to perform technical and clinical audits on an ongoing basis to see if the test is performing as expected (73).

Conclusions

Progress in the development of predictive companion biomarkers in oncology has greatly accelerated in recent years. Most if not all of the available predictive companion biomarkers are, however, single analytes such as specific mutated genes or specific proteins. Looking to the future, it is likely that single-analyte tests will be replaced by multianalyte tests such as gene expression profiles, panels of mutated genes, and exome and whole-genome sequencing. Clinical implementation of these technologies will present major logistical challenges that include the routine performance of technically demanding assays in a timely manner; implementation of internal QC and external quality assessment programs; relatively high initial setup costs; clinical validation, regulation, and reimbursement; and data reporting and storage (74). Overcoming these challenges should eventually facilitate the availability of personalized treatment for many patients with cancer.

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