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Personalized medicine and pharmacogenetic biomarkers: progress in molecular oncology testing

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Abstract

In the field of oncology, clinical molecular diagnostics and biomarker discoveries are constantly advancing as the intricate molecular mechanisms that transform a normal cell into an aberrant state in concert with the dysregulation of alternative complementary pathways are increasingly understood. Progress in biomarker technology, coupled with the companion clinical diagnostic laboratory tests, continue to advance this field, where individualized and customized treatment appropriate for each individual patient define the standard of care. Here, we discuss the current commonly used predictive pharmacogenetic biomarkers in clinical oncology molecular testing: *BRAF*V600E for vemurafenib in melanoma; *EML4–ALK* for crizotinib and *EGFR* for erlotinib and gefitinib in non-small-cell lung cancer; *KRAS* against the use of cetuximab and panitumumab in colorectal cancer; *ERBB2* (*HER2/neu*) for trastuzumab in breast cancer; *BCR–ABL* for

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tyrosine kinase inhibitors in chronic myeloid leukemia; and *PML/RAR α* for all-*trans*-retinoic acid and arsenic trioxide treatment for acute promyelocytic leukemia.

Keywords

biomarker; cancer; clinical laboratory; clinical utility; molecular diagnostics; oncology; personalized medicine; pharmacogenetic; predictive medicine; testing

With the rapid evolution of genetic and genomic technologies revolutionizing our approach to prognosis, screening and targeting of therapies, the age of personalized and predictive medicine has not only defined how clinical practice is evolving today, but also portends to how it will be practiced in the future. Personalized medicine has its underpinning in the clinical molecular testing of biomarkers, which can be prognostic, predictive, pharmacodynamic or diagnostic [1–3]. Prognostic biomarkers have an association with clinical outcomes, such as overall survival or recurrence-free survival, independent of treatment [4–6]. Predictive biomarkers assess the likely benefit of a specific treatment to a specific patient, and thus are used to make clinical decisions [1,4,6]. Pharmacodynamic biomarkers measure the effect of a drug on the disease, whereas diagnostic biomarkers are used to establish the particular disease that is present in the patient sample [7,8]. Even though the progress in biomarker technology coupled with the companion clinical diagnostic laboratory tests will continue to advance medicine, where individualized and customized treatment appropriate for each individual patient will continue to define the standard of care, the clinical application of molecular diagnostics in predicting outcomes that may be clinically actionable has seen disproportionate acceptance and uptake across different medical fields [9]. The level of evidence for qualifying the clinical utility of any biomarker needs to be rigorous, and guidelines will undoubtedly evolve as the field advances [10].

In oncology, clinical molecular diagnostics and biomarker discoveries are constantly advancing as the intricate molecular mechanisms that transform a normal cell to an aberrant state in concert with the dysregulation of alternative complementary pathways are increasingly understood. Exploiting this knowledge of biomarkers led to the implementation of monoclonal antibodies and small-molecule tyrosine kinase inhibitors that target EGFR in colorectal cancers and non-small-cell lung carcinoma (NSCLC) [11]. Another case of the utility of predictive biomarkers comes in the anticipated use of poly-ADP (ribose) polymerase inhibitors in *BRCA1/2*-deficient tumors [12,13]. Even though the prostate-specific antigen test for screening in prostate cancer has limitations and is still controversial, the US FDA has approved the use of the prostate-specific antigen test along with a digital rectal exam to help detect prostate cancer and for monitoring recurrence in men aged 50 years and older. Clinicians have used cancer antigen 125 for ovarian cancer and carcinoembryonic antigen for colon cancer and other types of cancers for decades. The importance and necessity of these biomarkers are highlighted by the enormous healthcare expenditure on cancer drugs, and the estimated savings from patient selection and stratification based on the results of these biomarker diagnostic tests on predictive biomarkers with demonstrated clinical utility [14–17]. With predictive testing and patient stratification, not only is there a benefit of reducing unnecessary treatment, there is the additional benefit of avoiding toxic effects of the therapeutic regimen, thus decreasing morbidity, as in the case of trastuzumab and cardiotoxicity in breast cancer treatment [18].

The success stories of clinically useful pharmacogenetic predictive biomarkers in oncology thus far have come mostly from retrospective analyses of clinical trial data and impromptu genetic analyses, as exemplified by *KRAS* status and poor response to cetuximab and panitumumab [19,20]. A systematic prospective approach with current technologies

available is defining how biomarker discoveries are made in tandem with drug development [21]. A variety of high-throughput approaches, including the use of massively parallel next-generation sequencing, single nucleotide polymorphism analysis and transcript profiling by microarray have been employed to discover new predictive biomarkers [22]. Even though these approaches may identify genes and proteins that correspond to disease progression or response to therapeutics, the information may be difficult to integrate with the mechanisms and pathways involved in tumor phenotype or drug action [17,23]. Thus, developing platforms that allow functional biomarkers to be rationalized in the context of mechanism and pathway for tumor killing by the drug are of utmost importance to support clinical drug development [24]. Recently, by applying a next-generation sequencing assay, the identification of novel *ALK* and *RET* gene fusions from colorectal cancer and NSCLC biopsies may eventually result in a clinically actionable predictive biomarker with further prospective clinical trials using RET kinase inhibitors [25].

Traditionally, cancer diagnosis has been classified according to anatomic origin, microscopic morphology and protein-based tests such as immunohistochemistry. Other useful means of diagnosis and monitoring include cell surface markers for leukemia and lymphoma, specific cytokine production and other nonspecific markers, such as Ig clonality in lymphoid tumors. Medical oncologists select the most appropriate therapy based on these characteristics and the extent of spread and staging of the tumor. In recent years, the clinical molecular testing of predictive pharmacogenetic biomarkers of high clinical utility has ushered in the era of personalized medicine in clinical oncology. In this review, we discuss the current commonly used predictive biomarkers in clinical molecular oncology testing (Table 1): *BRAF*V600E for vemurafenib in melanoma; *EML4-ALK* for crizotinib and *EGFR* for erlotinib and gefitinib in NSCLC; *KRAS* against the use of cetuximab and panitumumab in colorectal cancer; *ERBB2* (*HER2*/neu) for trastuzumab in breast cancer; *BCR-ABL* for tyrosine kinase inhibitors in chronic myeloid leukemia (CML); and *PML/RAR α* for all-*trans*-retinoic acid and arsenic trioxide treatment for acute promyelocytic leukemia (APL).

Clinical molecular diagnostics & cancer genetics

BRAF V600E for vemurafenib in melanoma

Melanoma is the leading cause of death from skin disease with prognosis ranging from good if detected early to poor if the cancer has spread beyond the skin and nearby lymph nodes. An understanding of the molecular pathogenesis of melanoma has provided important insights that recently led to the development of targeted therapies for specific subsets of patients with *BRAF*V600E mutation with metastatic melanoma. Activating mutations in *BRAF* are present in approximately 40–60% of advanced melanomas [26,27]. In 80–90% of cases, this activating mutation consists of the substitution of glutamic acid for valine at amino acid 600 (V600E mutation) in exon 15. Advanced melanomas with a mutation in *BRAF* appear to have some clinical differences that are associated with a more aggressive clinical course [27]. For this biomarker, Roche has developed an FDA-approved companion biomarker real-time PCR (RT-PCR) assay on the Roche cobas® 4800. This assay has been shown to be able to detect the mutation when the mutation constitutes only 10% of a mixture with wild-type *BRAF* gene (i.e., a ratio of 90:10 of wild-type:mutated *BRAF*). Hairy-cell leukemia is an uncommon hematological malignancy (2% of all leukemia) with good prognosis, characterized by accumulation of abnormal B lymphocytes. Whole exome sequencing has identified five mis-sense somatic clonal mutations, including a heterozygous mutation in *BRAF* that results in the V600E variant [28,29]. Tiacchi *et al.* and Boyd *et al.* have also demonstrated sensitive, reliable, high-resolution melting analysis and allele-specific PCR qualitative assays to confirm the V600E mutation in hairy-cell leukemia [30,31].

Vemurafenib is a specific inhibitor of activated BRAF, and has been shown to significantly increase survival in melanoma patients whose tumor contains a V600E mutation in the *BRAF* gene [32]. Vemurafenib produces rapid tumor regression in the vast majority of patients with V600E-mutant melanoma, including those with extensive tumor burden and significant disease-related symptoms. Overall survival was significantly increased in patients assigned to vemurafenib compared with dacarbazine (estimated 6-month survival rates: 84 vs 64%; hazard ratio for death: 0.37; 95% CI: 0.26–0.55). Progression-free survival was significantly longer in those initially treated with vemurafenib (median: 5.3 vs 1.6 months; hazard ratio: 0.26; 95% CI: 0.20–0.33). The objective response rate was significantly higher with vemurafenib (48 vs 4%). Continued treatment appears necessary to maintain efficacy. Side effects are typically modest and do not generally restrict or limit treatment. Another specific BRAF inhibitor, GSK2118436, has also shown significant activity in patients with metastatic melanoma and is undergoing Phase III study [32,33]. *BRAF* testing and inhibition is also potentially relevant to other cancers in which *BRAF* mutations are common, such as papillary cancer of the thyroid.

***EML4*–*ALK* for crizotinib in NSCLC**

Another major development in molecular diagnostic and clinical oncology is the discovery of the *EML4*–*ALK* fusion oncogene, which arises from an inversion on the short arm of chromosome 2, inv(2)(p21p23) that joins exons 1–13 of *EML4* to exons 20–29 of *ALK* [34]. The resulting chimeric protein, *EML4*–*ALK*, contains an N-terminus derived from *EML4* and a C-terminus containing the entire intracellular tyrosine kinase domain of *ALK*. This fusion oncogene rearrangement defines a distinct clinicopathologic subset of NSCLC, with overall incidence of approximately 5% [35–37]. However, in never or light smokers, the frequency of *ALK* positivity was approximately 22%, and among never or light smokers who did not have an *EGFR* mutation, the frequency was 33% [38]. Compared with small-cell carcinoma, NSCLCs are relatively insensitive to chemotherapy. When possible, NSCLCs are surgically resected, although neoadjuvant and adjuvant chemotherapy are increasingly used. NSCLCs mostly tend to be squamous cell carcinoma, large cell carcinoma and adenocarcinoma, with the *ALK* mutation composing a small fraction of all NSCLC cases.

It only took 4 short years from the publication of the discovery of *ALK*-rearranged NSCLC to the conditional approval by the FDA of the *EML4*–*ALK* inhibitor, crizotinib, in August 2011. This may not have happened without the rapid development and validation of the companion diagnostic test Vysis *ALK* Break Apart FISH Probe Kit (CE-marked from Abbott Molecular; approved in September 2010). To identify *ALK* rearrangements, FISH is performed on formalin-fixed, paraffin-embedded tumors and defined as positive if >15% of tumor cells have split signals. The break-apart probes include two differently colored (red and green) probes that flank the highly conserved translocation breakpoint within *ALK*. In wild-type cells, the overlying red and green probes result in a yellow (fused) signal while in *ALK*-rearranged cells, these probes are separated and individual red and green signals are observed. Other methods to identify *ALK* activation include immunohistochemistry, which is not yet the gold standard [39]. The *EML4*–*ALK* fusion transcript can also be demonstrated with RT-PCR [40]. The efficacy of crizotinib is quite impressive; a recent report observed an overall response rate of 57% and rate of stable disease of 33% [41]. Historically, the response rate in NSCLC in the second-line setting is approximately 10% [42].

***EGFR* for erlotinib & gefitinib in NSCLC**

The EGF receptor (EGFR) is a transmembrane protein with cytoplasmic kinase activity that transduces important growth factor signals from the extracellular milieu to the cell. Early nonmetastatic NSCLCs are usually not very sensitive to chemotherapy or radiation, so

surgery is the preferred treatment of choice with adjuvant chemotherapy. For advanced metastatic NSCLC, a wide variety of chemotherapies are used. Studies have suggested that for advanced NSCLC patients with *EGFR*-mutant tumors, initial therapy with a tyrosine kinase inhibitor instead of chemotherapy may be the best choice of treatment [43]. In the USA, the currently available *EGFR* tyrosine kinase inhibitor, erlotinib, is FDA-approved as monotherapy for NSCLC as a second-line, and in the maintenance setting irrespective of *EGFR* status based on modest but statistically significant improvement in overall and progression-free survival, as demonstrated in the SATURN maintenance study (ClinicalTrials.gov identifier: NCT01194050 [101]) and NCIC BR.21 trial (ClinicalTrials.gov identifier: NCT00036647 [102]) in relapsed setting [44,45]. Therefore, mutation testing has become the standard of care to identify these patients.

DNA mutational analysis is the preferred method to assess *EGFR* status. Over the years, a multitude of techniques have been developed with varying sensitivities to detect known and *de novo* mutations, with differing instrumentations, reagents, assay runtimes and costs [46]. The peptide nucleic acid-locked nucleic acid PCR clamp is one method capable of detecting *EGFR* mutations in a background of wild-type *EGFR* [47]. This method employs fluorescent primers with preferred amplification of the mutant sequence, which is then detected by locked nucleic acids to increase specificity. The sensitivity and specificity of the peptide nucleic acid-locked nucleic acid PCR clamp method are 97 and 100%, respectively [47]. Many commercial *EGFR* mutation detection kits are available, such as from Genzyme and QIAGEN. The Roche cobas® *EGFR* Mutation Test is CE-marked, and identifies 41 mutations across exons 18, 19, 20 and 21. Currently, tumor samples are required, whether for primary lung lesion or metastasis, and are normally formalin-fixed and paraffin-embedded. One future direction is the testing of surrogate samples such as bronchial alveolar lavage, sputum, pleural fluid or serum.

***KRAS* against cetuximab or panitumumab in colorectal cancer**

In contrast to the above biomarkers that select patients who can benefit from applications of molecular-targeted treatments, the *KRAS* mutation in colorectal cancer selects against patients who will not benefit from anti-*EGFR* receptor therapy, namely cetuximab or panitumumab. Even though colorectal cancer is one of the most common causes of cancer in both sexes, with increased and effective screening strategies prognosis is good with early detection. Mutations in the *KRAS* oncogene are overexpressed in colorectal cancer but are common in many other types of cancers, including pancreatic, lung, and ovarian cancer (~20%) [48]. It is a key element in the MAPK, JAK–STAT and PI3K cell-signaling pathways, acting as a key signal transducer for a number of cellular receptors. Mutations in the gene lead to abnormal cellular growth, proliferation and differentiation as a result of the activation of cell signaling. Most methods utilized to detect mutations, including nucleic acid sequencing, allele-specific PCR methods, single-strand conformational polymorphism analysis, melt-curve analysis and probe hybridization, employ PCR to amplify exons 2 and 3 of the gene, and distinguish wild-type from mutant sequences in key codons 12 and 13 [49]. No specific methodology is recommended, as all methods in current clinical use appear to have adequate clinical sensitivity for predicting a lack of response to cetuximab and panitumumab [50].

***ERBB2 (HER2/neu)* for trastuzumab in breast cancer**

Breast cancer is responsible for approximately 14% of cancer deaths in women worldwide. The prognosis and survival rate vary greatly depending on the sex and geographical location of the patient, as well as cancer type, staging and treatment. Mutations in *BRCA1* and *BRCA2* account for 5–10% of breast cancers in Caucasian women in the USA [51–53]. The 185delAG and 5382insC mutations in *BRCA1*, and 6174delT mutation in *BRCA2*, are most

commonly found in the Ashkenazi Jewish population [54]. Mutations in several other genes, such as *TP53*, *PTEN*, *CHEK2*, *MLH1* and *MSH2* are also associated with hereditary forms of breast cancers [55,56].

The hormone receptor status of the tumor, whether estrogen receptor (ER) or progesterone receptor, can predict the outcome to suppression therapy with tamoxifen or raloxifene [57,58]. Tamoxifen competes with estrogen for binding to the ER and has been used as first-line therapy for decades. If the tumor is hormone receptor-negative, then hEGF receptor 2 (HER2/neu) status will determine the efficacy of trastuzumab and lapatinib. There is evidence of crosstalk between ER and HER2/neu signaling pathways during breast cancer development, leading to the expression of multiple receptors [59]. Aromatase inhibitors, such as third-generation letrozole, have been shown to be more effective than tamoxifen at blocking tumor progression, independent of HER2/neu [60]. In breast cancers coexpressing HER2/neu and hormone receptor, aromatase inhibitors in combination with lapatinib have been shown to significantly improve disease outcome [60]. A serine protease inhibitor targeting the urokinase plasminogen activator system is currently in Phase II trial in patients with metastatic, *HER2*-negative breast cancer [61]. Before the FDA revoked its conditional approval of bevacizumab in 2011, the monoclonal antibody against VEGF-A was used in conjunction with chemotherapy for *HER2*-negative metastatic breast cancer [62].

Mechanisms of distinguishing breast cancer subtypes include histopathology and molecular pathology. In the last 15 years, microarrays have allowed for the study of the expression of multiple genes and the use of expression patterns as an indicator of breast cancer progression [63,64]. An emerging area of research is the correlation of biomarkers with the behavior of breast cancer subtypes. In the more aggressive triple-negative breast cancers, the presence of biomarker PKC α or the lack of nuclear biomarker ER β , is associated with more aggressive breast cancer behavior, endocrine resistance and poorer prognosis [65]. Another recent discovery suggests that 14–3–3 theta/tau and tBID can predict neoadjuvant chemotherapy resistance in ER-positive breast cancers [66]. Upregulation of heat shock protein 90 (HSP90) mRNA expression appears to predict the aggressive behavior of *HER2*-negative breast cancer [67]. BRCA1-inter-ribosomal entry sequence overexpression is associated with mechanisms directed at avoiding apoptosis, and triggers aggressive tumor formation, especially in *HER2*-positive or triple-negative/basal-like breast cancers [68].

***BCR–ABL* for tyrosine kinase inhibitors in CML**

CML is a clonal bone marrow stem cell disorder characterized by the proliferation of myeloid cells in the bone marrow. The understanding of the molecular pathogenesis of CML and the development of therapy to target the causative molecular defect have led to dramatic improvements in patient survival and quality of life. Patients with CML have the chromosomal abnormality t(9;22)(q34;q11.2), which results in the *BCR–ABL* fusion gene. The enhanced tyrosine kinase activity of *BCR–ABL* is responsible for activation of several signal transduction pathways. This results in the leukemic phenotype of CML cells, which encompasses deregulated proliferation, reduced adherence to the bone marrow stroma and defective apoptotic response to mutagenic stimuli [69]. In 90–95% of cases, the translocation is recognized by routine karyotyping. In the remaining cases, the chromosomal rearrangement is complex or cytogenetically cryptic, and the translocation can only be detected by FISH or RT-PCR [70].

In the absence of therapy, patients with CML eventually progress from chronic phase into a transformed phase, characterized by deteriorating hematologic parameters and worsening performance status. Conventional chemotherapy improves median survival by approximately 4 years, but does little to delay the onset of accelerated phase or blast phase [71]. The understanding of the abnormal signaling in CML cells led to the design and

synthesis of small molecules that target the tyrosine kinase activity of BCR–ABL, of which imatinib was the first to be successfully used. Imatinib competes with ATP for binding to the BCR–ABL kinase domain, thus preventing phosphorylation of tyrosine residues. Interruption of this oncogenic signal is very effective for control of the disease, particularly when used in the chronic phase. However, the emergence of subclones of leukemic progenitor cells with point mutations that prevent the binding of the inhibitor to the kinase domain of BCR–ABL can lead to drug resistance. The second-generation compounds, nilotinib and dasatinib, can circumvent this form of drug failure in the case of most kinase domain mutations associated with imatinib resistance [69,72].

The most important prognostic indicator is the response to treatment at the hematologic, cytogenetic and molecular level [73,74]. Currently the complete cytogenetic response rate to imatinib is 70–90%, with a 5-year progression-free survival and overall survival between 80–95% [69]. Despite the efficacy of imatinib, it is not curative, and transcripts of *BCR–ABL* remain detectable by quantitative RT-PCR in most patients [75]. In some hematological cancers (e.g., acute lymphoblastic leukemia, CML and APL) as opposed to solid tumors, minimal residual disease (MRD) testing determines effectiveness of treatment, compares efficacy of different treatments, and monitors patient remission status and recurrence. Therefore, patients with CML need to be continually monitored in order to detect the level of *BCR–ABL* transcripts in the blood or bone marrow, as well as to detect evidence of cytogenetic remission or evolution [70,75,76]. Measurement of low-level disease or MRD using molecular tests is becoming the gold standard of measuring response to therapy, owing to its higher sensitivity compared with other routine techniques. Equipment used by different laboratories vary (Applied Biosystems® 7500 and 7900, Roche's LightCycler®, Corbett RotorGene™ [QIAGEN], Cepheid products, Stratagene products and Biorad's CFX™ series) with the choice more often dictated by cost and workload [77].

***PML/RARα* for all-*trans*-retinoic acid and arsenic trioxide treatment in APL**

APL constitutes 5–8% of acute myeloid leukemia (AML) cases, with an abnormal accumulation of promyelocytes in the blood and bone marrow [69,78]. Prompt diagnosis is essential because of the high frequency of life-threatening disseminated intravascular coagulation. The t(15;17)(q22;q12) results in fusion of the promyelocytic gene (*PML*) on chromosome 15 with the retinoic acid receptor (*RARα*) gene on chromosome 17. The *PML–RARα* fusion protein mediates a block in myeloid differentiation. The blasts are highly sensitive to anthracycline-based chemotherapy, and differentiate in response to all-*trans*-retinoic acid and arsenic trioxide treatment [78]. All-*trans*-retinoic acid targets the *RARα* component of the fusion protein, whereas arsenic trioxide targets *PML*, causing maturation and apoptosis.

Three breakpoint regions are described on the *PML* gene at band q22 of chromosome 15 [79]. Cytogenetics, FISH, monoclonal anti-PML antibodies or RT-PCR is necessary for genetic confirmation of the aberrant *PML–RARα* fusion [80]; RT-PCR is the only technique that can identify the *PML–RARα* isoform useful for the monitoring of MRD [81,82]. Quantitative RT-PCR technology improves the predictive value of MRD monitoring. It is used to assess response to treatment and evaluate prognosis of disease, and therefore guides therapy in order to reduce the rate of relapse and to increase the rate of cure in high-risk patients [83]. Sequential RT-PCR monitoring provides the strongest predictor of relapse-free survival in APL, and provides a valid strategy to reduce rates of clinical relapse when coupled with preemptive therapy [81]. In adult patients who achieve complete remission, the prognosis is better than for any other category of AML. Once considered the most malignant human leukemia associated with the worst prognosis, APL has been transformed in the past few decades into the most frequently curable, with advances in diagnostic molecular testing,

sensitive MRD monitoring by PCR, definition of relapse-risk categories and adoption of risk-adapted strategies [69,84].

Summary

*BRAF*V600E for vemurafenib in melanoma and hairy-cell leukemia:

- Activating mutations in *BRAF* are present in approximately 40–60% of advanced melanomas. In 80–90% of cases, this activating mutation consists of the substitution of glutamic acid for valine at amino acid 600 in exon 15;
- Vemurafenib is a specific inhibitor of activated *BRAF* and has been shown to significantly increase survival in patients whose tumor contains a V600E mutation in the *BRAF* gene. The use of vemurafenib should be limited to patients whose tumor contains this mutation;
- *BRAF* testing and inhibition is also potentially relevant to other cancers in which *BRAF* mutations are common, such as papillary cancer of the thyroid.

EML4-ALK for crizotinib in NSCLC:

- *EML4-ALK* fusion oncogene arises from an inversion on the short arm of chromosome 2, inv(2)(p21p23), that joins exons 1–13 of *EML4* to exons 20–29 of *ALK*. The resulting chimeric protein, EML4–ALK, contains an N-terminus derived from EML4 and a C-terminus containing the entire intracellular tyrosine kinase domain of ALK;
- This fusion oncogene rearrangement defines a distinct clinicopathologic subset of NSCLC with an overall incidence of approximately 5%;
- Conditional approval by the FDA of the EML4–ALK inhibitor, crizotinib, 4 years after the discovery of *ALK*-rearranged NSCLC, may not have happened without the rapid development and validation of the companion diagnostic test.

EGFR for erlotinib and gefitinib in NSCLC:

- For advanced NSCLC patients with *EGFR*-mutant tumors, initial therapy with a tyrosine kinase inhibitor instead of chemotherapy may be the best choice of treatment.

KRAS against cetuximab or panitumumab in colorectal cancer:

- The *KRAS* mutation in colorectal cancer selects against patients who will not benefit from anti-EGFR receptor therapy, namely cetuximab or panitumumab.

ERBB2 (*HER2/neu*) for trastuzumab in breast cancer:

- The hormone receptor status of the tumor, whether ER or progesterone receptor, can predict the outcome of hormone suppression therapy with tamoxifen or raloxifene;
- In hormone receptor-negative tumors, *HER2/neu* status will probably determine the efficacy of trastuzumab and lapatinib.

BCR-ABL for tyrosine kinase inhibitors in CML:

- Patients with CML have the chromosomal abnormality t(9;22) (q34;q11.2), which results in the *BCR-ABL* fusion gene;
- Imatinib competes with ATP for binding to the BCR–ABL kinase domain, thus preventing phosphorylation of tyrosine residues. Interruption of this oncogenic

signal is very effective for control of the disease, particularly when used in chronic phase;

- Despite the efficacy of imatinib, it is not curative, and transcripts of *BCR-ABL* remain detectable by quantitative RT-PCR in most patients. Therefore, patients with CML need to be monitored continually to detect the level of BCR-ABL transcripts in the blood or bone marrow, as well as to detect evidence of cytogenetic remission or evolution;
- Emergence of subclones of leukemic progenitor cells with point mutations that prevent the binding of the inhibitor to the kinase domain of BCR-ABL can lead to drug resistance. The second-generation compounds nilotinib and dasatinib can circumvent this form of drug failure.

PML/RAR α for all-*trans*-retinoic acid and arsenic trioxide treatment in APL:

- APL constitutes 5–8% of AML cases;
- Prompt diagnosis is essential because of the high frequency of life-threatening disseminated intravascular coagulation;
- The t(15;17)(q22;q12) results in fusion of the promyelocytic gene on chromosome 15 with the retinoic acid receptor gene on chromosome 17;
- The blasts are highly sensitive to anthracycline-based chemotherapy and differentiate in response to all-*trans*-retinoic acid and arsenic trioxide treatment.

Expert commentary

With advances and implementation of clinical molecular diagnostics, personalized medicine in oncology has taken great strides, with predictive biomarkers guiding both therapy and monitoring of disease progression or remission. The progress in biomarker technology, coupled with companion clinical diagnostic laboratory tests, will continue to advance medicine where individualized and customized treatment appropriate for each individual patient will continue to define the standard of care. Not only are they relatively less toxic with less side effects than conventional chemotherapy, some targeted therapies are also more efficacious in tumor types where conventional chemotherapy previously provided little or no benefit. Many of the targeted therapies are orally administered, and are therefore more convenient for the patient. Unfortunately, the administration of many targeted agents results primarily in partial response or stable disease, with few complete remissions, and most targeted agents are not curative. Some of the drawbacks of such clinical molecular diagnostics include potential loss or diminished value of diagnostic or prognostic biomarkers in the setting of previously instituted therapies. Furthermore, many molecular diagnostic modalities have specimen requirements that preclude the use of size-limited or pauci-cellular specimens for multiple testing, such as needle core biopsies of lung tissue for *EGFR* and *ALK* fusion testing in NSCLC. However, careful consideration of preanalytic variables and emerging technologies for clinical molecular testing will likely abrogate such issues. The elucidation of molecular biomarkers, as well as their use in design and implementation of targeted therapies, is shifting paradigms in cancer chemotherapy, from tissue- or disease-based therapeutic regimens to molecular target-based protocols. Certainly, the availability of ever-increasing molecular data sets will hasten these advancements.

Five-year view

Discovery of new biomarkers will employ high-throughput methodologies through prospective hypothesis-driven testing. With the ‘thousand-dollar’ whole-genome sequencing within reach in the next few years, it will not be the cost of genotyping or sequencing that

will deter the progress of biomarker discovery and utilization. Even though these approaches may identify genes and proteins that correspond to disease progression or response to therapeutics, it may be difficult to integrate this information with the mechanisms and pathways involved in tumor phenotype or drug action. Drug development, clinical validation and eventual implementation of these biomarkers can be supported by the rationalization of biomarkers in the context of mechanism and pathway for tumor killing and drug response.

As more targets for cellular inhibition are discovered, including abnormal targets that drive malignant cell proliferation or prolong survival, the efficacy of individualized therapy will continue to improve. Normal nonmutated targets will also be uncovered, and if blocked or stimulated, will stop malignant cells from proliferation or differentiation and apoptosis. These discoveries will facilitate and boost targeted drug therapy development, such as FMS-like receptor tyrosine kinase-3 inhibitor for AML and KRAS inhibitors. Personalized therapy will also improve with the optimization of complete pathway blockade, such as adding lapatinib and pertuzumab to trastuzumab in the *HER2*/neu pathway. Another future direction is the improvement of efficacy and decreased toxicity via use of antibody–drug conjugates, such as brentuximab for Hodgkin’s lymphoma and trastuzumab–DM1 for breast cancer.

Individualized tumor pharmacogenetic analysis will continue to improve. There will be more knowledge regarding the optimal dose and administration schedule of targeted agents. There will also be more clinical guidelines pertaining to combining targeted agents with conventional therapy, or using multiple targeted agents together, concomitantly or sequentially. For these advances to transpire, scientists will need to continue to pursue the molecular and genetic abnormalities of the full spectrum of tumor types, and thus cancer patients of all types are invaluable in all phases of clinical trials.

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Key issues

- Personalized medicine in clinical oncology has its underpinning in the clinical molecular testing of biomarkers, which can be diagnostic, prognostic, pharmacodynamic and/or predictive.
- The importance and necessity of these predictive biomarkers are highlighted by the enormous healthcare expenditure on chemotherapy, the estimated savings, and the avoidance of toxic effects and morbidity from patient selection and stratification based on clinical molecular testing.
- Current commonly used predictive biomarkers of high clinical utility in clinical oncology molecular testing include: *BRAF*V600E for vemurafenib in melanoma; *MLL4-ALK* for crizotinib and *EGFR* for erlotinib and gefitinib in non-small-cell lung cancer; *KRAS* against the use of cetuximab and panitumumab in colorectal cancer; *ERBB2 (HER2/neu)* for trastuzumab in breast cancer; *BCR-ABL* for tyrosine kinase inhibitors in chronic myeloid leukemia; and *PML/RAR α* for all-*trans*-retinoic acid and arsenic trioxide treatment for acute promyelocytic leukemia.
- The elucidation of molecular biomarkers and their use in design and implementation of targeted therapies is shifting paradigms in cancer chemotherapy, from tissue- or disease-based therapeutic regimens to molecular target-based protocols.
- Individualized tumor pharmacogenetic analysis will continue to improve and will translate into optimal dosing and scheduling of targeted agents. There will also be more guidance as to combining targeted agents with conventional therapy or using multiple targeted agents together, concomitantly or sequentially.

Table 1

Clinical utility and targeted therapeutics for molecular biomarkers in oncology.

Molecular biomarker	Prevalence	Clinical utility	Select targeted therapeutics
<i>BRAF</i> mutation	40–60% metastatic melanoma; 90%: V600E mutation	Therapeutic target; prognostic	Vemurafenib GSK2118436/LGX818 RO5212054 RAF265 XL281
<i>EML4-ALK</i> fusion gene	5% NSCLC total; 22% of NSCLC in non- or light- smokers	Therapeutic target; prognostic	Crizotinib CH5424802 AP26113
<i>EGFR</i> mutation	10% NSCLC (US; 35% East Asians); more common in females and those who have never smoked vs those who have	Therapeutic target; prognostic	Erlotinib Gefitinib Afatinib CO-1686 Cetuximab
<i>KRAS</i> mutation	15–25% lung adenocarcinoma; 40% colorectal cancer	Negative predictor of benefit to anti- EGFR therapy (antibody therapy for colorectal cancer, and small molecule inhibitor for lung cancer)	None
<i>ERBB2 (HER2/neu)</i> amplification	20% breast cancer; 7–27% gastric cancer	Therapeutic target; prognostic	Trastuzumab Lapatinib Ertumaxomab MM-111
<i>BCR-ABL</i> fusion gene	Detectable in 98% of chronic myelogenous leukemia and 5– 20% of acute lymphoblastic leukemia	Diagnostic; therapeutic target; prognostic; minimal residual disease marker	Imatinib Dasatinib Nilotinib Radotinib INNO-406 DCC-2036
<i>PML-RARα</i> fusion gene	>95% of acute promyelocytic leukemia	Diagnostic; therapeutic target; prognostic; minimal residual disease marker	All- <i>trans</i> -retinoic acid

NSCLC: Non-small-cell lung cancer.