Applied molecular profiling: evidence-based decision-making for anticancer therapy

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Applied molecular profiling is a method for helping clinicians select the most appropriate therapy for a patient with cancer by determining the level of gene and/or protein expression within the cancer and comparing that expression pattern with the expression profiles of cancers with known outcomes. This approach facilitates the development and selection of tumor-specific therapies based on the identification of biomarkers within a tumor. Molecular characterization techniques such as immunohistochemistry, microarray analysis, and fluorescence in situ hybridization have facilitated identification and validation of a number of important solid tumor biomarkers, including HER2/neu, *EGFR*, *EML4/ALK*, and KIT, and can also be used to identify biomarkers (eg, *BCR-ABL*, CD20, CD30) in various hematologic malignancies. It is of note that molecular profiling can be used to identify targets in tumors for which a therapeutic agent may already be available, thus avoiding the administration of an unproven investigational agent. As the field of molecular profiling continues to evolve and next-generation techniques such as exome sequencing – sequencing 1% of the genome – and whole gene sequencing gain currency, biomarker identification and analysis will become less expensive and more efficient, and possibly allow for a pathway-oriented approach to treatment selection. Wider acceptance and use of molecular profiling should therefore help practicing physicians and oncology researchers keep pace with advances in the understanding of oncogenic expression in various malignancies and encourage the use of molecular profiling in earlier stages of cancer rather than as an option of last resort.

Applied molecular profiling combines molecular medicine and bioinformatics to select the most appropriate therapy for a patient with cancer. The concept of molecular characterization for cancer identification and treatment is not new, but one that has been contemplated and pursued since before the completion of the human genome sequence.[1] Rather than classifying a cancer according to the morphological appearance of the cells and surrounding tissue, molecular characterization allows for the classification of cancerous tissue by determining the level of gene and/or protein expression within the cancer and using a predetermined algorithm to compare that expression pattern with the expression profiles of cancers with known outcomes. The algorithm stratifies the cancer into an outcome class derived from similar expression profiles, or yields a survival probability.[1]

On the basis of those initial stratifications, molecular profiling allows for the development and selection of biomarker-specific therapies that could have a significant impact on cancer care. With molecular profiling, a select patient population with expression of a specific biomarker can be treated with a regimen that targets the biomarker, with potentially less toxicity than that observed with traditional chemotherapy. This targeted approach may yield prolonged, durable responses. Even in the absence of a significant survival difference, when the targeted therapy is an oral drug it will likely appeal to patients who would rather receive oral therapy than cytotoxic chemotherapy.

A sampling of types of molecular characterization analyses is provided in Table 1. The field of molecular profiling has developed to the point where companies are offering analysis platforms that are supported by input from advisors with tumor-specific expertise as well as the examination of hundreds of thousands of abstracts and manuscripts from the clinical literature that link

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TABLE 1 Molecular characterization technologies for identifying and analyzing tumor-specific biomarkers

Technology platform	Function
Immunohistochemistry (IHC) analysis	Determines the expression level of proteins in cells of a tissue section through the binding of antibodies to antigens in the tissue.
Fluorescence in situ hybridization (FISH)	Detects gene deletions, amplifications, translocations, and fusions.
Polymerase chain reaction (PCR)	Amplifies a single copy or a few copies of a DNA sample across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
Reverse transcriptase polymerase chain reaction (RT-PCR)	A variant of PCR by which a strand of RNA is reverse transcribed into its DNA complement, complementary DNA (cDNA), using the enzyme reverse transcriptase; the resulting cDNA is amplified using PCR.
Restriction fragment length polymorphism (RFLP)	A DNA profiling technique by which differences in homologous DNA sequences are detected by the presence of fragments of different lengths.
Sequencing	Identifies mutations in a targeted gene by determining the DNA sequence.

biomarkers to specific drug associations. As the field continues to evolve and the literature is updated with new information on biomarkers and drug associations, increasing numbers of patients are expected to benefit from tailored therapeutic approaches.

Biomarkers identified and validated through molecular profiling

HER2/neu

The promise of molecular profiling in solid tumors was first realized with the development of trastuzumab, which was approved based on findings in a phase 3 clinical trial in which patients with breast cancer were prospectively screened for the presence of the *HER2/neu* gene. This trial involved a selected population of 469 patients in whom *HER2/neu* was overexpressed and the findings demonstrated durable objective responses, improved survival compared with standard chemotherapy without trastuzumab, a longer time to disease progression, and acceptable tolerability in these patients.[2] By contrast, studying an unselected population likely would have required thousands of patients to generate data that were sufficiently robust for the drug to be approved (see Figure 1). It has been estimated that 23,586 randomized patients – 50 times as many as in the targeted trastuzumab trial – would have been required for an untargeted trial to demonstrate the same effect, assuming that trastuzumab was completely ineffective in patients who tested negative on a predictive responsiveness assay. If assay-negative patients were also found to benefit from trastuzumab, an untargeted trial would require 1,256 patients, a sample size that would still be 2.67 times greater than the targeted trial.[3] At an estimated average per-patient cost of more than $26,000 for a phase 3 trial,[4] it would have been considerably more expensive to conduct such a trial in an unselected patient population.

EGFR in NSCLC

Information linking biomarkers to specific therapies is not always obtained during the pre-approval stage. In the case of erlotinib, knowledge of its specificity for activating epidermal growth factor receptor (EGFR) tyrosine kinase mutations was realized retrospectively (ie, after the drug had been approved), years after the conclusion of the pivotal BR.21 trial, in which erlotinib was found to prolong survival in patients with non–small-cell lung cancer (NSCLC) after first- or second-line chemotherapy.[5] Similarly, a postapproval investigation in patients with NSCLC demonstrated that *EGFR* mutations may also

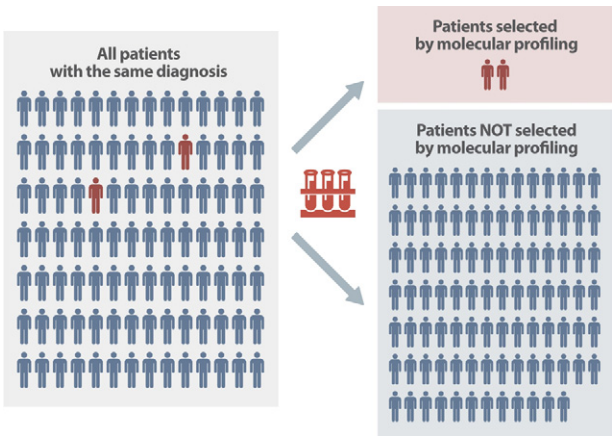

FIGURE 1 By using molecular profiling, these patients are stratified for a trial in which only a small number of patients who have the target that is to be studied (red) are enrolled in the trial rather than the remaining patients who lack the target (blue) and would not be selected for enrollment in this particular molecular profiling-directed study.

predict sensitivity to gefitinib,[6] which has a similar mechanism of action to that of erlotinib. Those findings have been supported by more recent data. In a 2009 pan-Asian study of *EGFR*-mutation-positive NSCLC patients who were nonsmokers or former light smokers, gefitinib was associated with significantly longer progression-free survival (PFS) compared with carboplatin–paclitaxel combined.[7] In a 2010 Japanese study of patients with advanced NSCLC who were selected on the basis of *EGFR* mutations, first-line gefitinib significantly improved PFS, with acceptable toxicity, compared with carboplatin–paclitaxel combined.[8] Other recent studies have demonstrated the sensitivity of activating *EGFR* mutations involving exon 19 deletions or exon 21 point mutations to erlotinib and gefitinib. These mutations have emerged as strong predictors of response to these agents.[9,10]

EML4/ALK translocation in lung cancer

The discovery of a fusion gene comprised of portions of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*) gene in NSCLC cells was reported in 2007.[11] The discovery was pivotal to the approval of the ALK inhibitor crizotinib, which induced shrinkage or stabilization of metastatic NSCLC tumors in 90% of 82 patients who carried the *EML4/ALK* fusion gene, with at least 30% shrinkage in 57% of treated patients.[12,14] A retrospective analysis of this cohort showed that crizotinib is associated with improved survival compared with that observed in crizotinib-naïve controls; the authors noted that *ALK* rearrangement "is not a favorable prognostic factor in advanced NSCLC."[15] Again, the use of molecular profiling was a critical factor in yielding these findings. As already noted, without molecular profiling, a conventional approach to screening patients would have required thousands of patients to generate pivotal data.

KIT in gastrointestinal stromal tumors

KIT, which is also known as c-KIT, CD117, or mast/stem cell growth factor receptor (SCFR), is a tyrosine kinase receptor that is mutated and activated in gastrointestinal stromal tumor (GIST) cells.[16,17] Imatinib is a tyrosine kinase inhibitor (TKI) that blocks KIT, thereby impeding cell proliferation and inducing apoptosis of GIST cells.[18] It is indicated for treatment of KIT-positive unresectable and/or metastatic GIST, as well as for adjuvant treatment following complete gross resection of KIT-positive GIST.[18] Sequencing of *KIT* has become especially important in light of development of resistance to imatinib because it may drive the development of therapies for patients with GIST that is resistant or refractory to imatinib.

Hematologic applications of molecular profiling

Imatinib is one of the first targeted therapies that essentially changed the way we think about anticancer therapy, based on its inhibition of *BCR-ABL*, the abnormal tyrosine kinase created by the Philadelphia chromosome translocation in chronic myeloid leukemia (CML).[18] That translocation is found in more than 90% of patients with CML,[19] making *BCR-ABL* an important biomarker in hematologic malignancies. Screening for *BCR-ABL* can thus help identify appropriate candidates for imatinib therapy, which is indicated for the treatment of newly diagnosed Philadelphia chromosome-positive CML (Ph+ CML), and for Ph+ CML in blast crisis, accelerated phase, or chronic phase after interferon therapy.[18]

Rituximab is a monoclonal antibody that binds to the protein CD20, which is found on the surface of B cells. This agent is used to treat diseases that are characterized by overexpression, overactivity, or dysfunction of B cells, including hematological diseases such as leukemias and lymphomas. Rituximab is indicated for the treatment of CD20-positive B-cell non-Hodgkin lymphoma (NHL), both as a single agent and in combination with chemotherapy; chronic lymphocytic leukemia (CLL), in combination with fludarabine and cyclophosphamide; rheumatoid arthritis, in combination with methotrexate; and Wegener's granulomatosis and microscopic polyangiitis, in combination with glucocorticoids.[20] Its approval for NHL was based on a phase 2 trial in 37 patients with relapsed B-cell lymphoma that expressed the CD20 antigen. In that trial, results with rituximab compared favorably with standard chemotherapy, though rituximab had a superior safety profile.[21]

Brentuximab is a monoclonal antibody that targets the cell membrane protein CD30, which is generally expressed by Reed-Sternberg cells, the defining characteristic of Hodgkin lymphoma.[22,23] Brentuximab has been approved for the treatment of Hodgkin lymphoma after failure of autologous stem-cell transplant or after failure of at least 2 prior multiagent chemotherapy regimens in noncandidates for autologous stem-cell transplant, as well as for the treatment of systemic anaplastic large-cell lymphoma after failure of at least 1 prior multiagent chemotherapy regimen.[24] Its approval was based on a phase 2 trial in which 34% of 102 patients with relapsed or refractory Hodgkin lymphoma achieved complete remission and another 40% had partial remission; tumor volume was reduced in 94% of patients.[25]

Molecular profiling of hematologic malignancies typically involves flow sorting of leukemia or lymphoma cells in a flow cytometer with antibodies. Although the process

differs from immunohistochemistry (IHC) and other molecular characterization techniques used to assay solid tumors, information about proteins that are frequently expressed in hematologic malignancies can provide a good indication of the likelihood of response to agents such as rituximab and brentuximab.

Practical applications of biomarker analysis and molecular profiling

Target Now pilot effort

Molecular profiling can be used to answer an important question that arises when caring for patients who have been referred for phase 1 clinical trials: if one studied the patients' tumors carefully enough, would there be targets in their tumors for which a therapeutic agent might already be available? Presumably, treating the patient with that therapeutic agent might be better than administering the phase 1 agent, because one would have a better understanding of the available agent's dose, schedule of administration, and side effect profile.[26]

That question was first addressed in a study known as the Target Now pilot effort, in which investigators performed IHC assays for up to 13 targets, such as HER2/neu, KIT, estrogen receptor (ER), as well as a 2-color oligonucleotide microarray (OMA) with 17,085 unique probes, on tumors from 112 patients referred for phase 1 study evaluation. The patients had exhausted conventional chemotherapy options and were undergoing procedures for cancer-related complications such as ascites and obstruction. Paraffin-embedded tumor samples were submitted for IHC analysis, which identified at least 1 potential target (eg, ER) in 74% of patients. In addition, at least 1 potential target (eg, thymidylate synthase) was identified in 99% of patients whose frozen tumor samples were submitted for OMA analysis. The findings suggested that even patients with a history of extensive prior treatment who are referred for phase 1 studies have tumors that can harbor treatment-actionable targets, such as ER. In addition, the pilot effort underscored the need for a prospective trial of the IHC–microarray approach to evaluate its utility for patients with advanced refractory cancer.[26]

Bisgrove trial

Further validation of the molecular profiling approach was provided by a pilot study known as the Bisgrove trial. The investigators identified Food and Drug Administration-approved agents through molecular profiling of a patient tumor and compared the patient's PFS on the selected agent with the patient's PFS on the previous treatment on which progression had occurred. Tissue samples from 86 patients with refractory metastatic solid tumors (ie, refractory to at least 2 prior therapies) were submitted for molecular characterization that included IHC, fluorescence in situ hybridization (FISH), and OMA. The molecular profiling approach was deemed beneficial for any patient with a PFS ratio (PFS on molecular profiling-selected therapy/time to progression [TTP] on prior therapy) of ≥ 1.3. The 20 patients who did not undergo molecular profiling experienced declining performance status or withdrew consent for additional therapy. A molecular target was detected in 98% (84/86) of patients whose tumors underwent molecular characterization, and 66 of those patients were treated using molecular profiling. Twenty-seven percent (18/66) of patients had a PFS ratio ≥ 1.3 (95% CI, 17%-38%; $P = .007$); that is, PFS was longer on a molecular profiling-suggested regimen than on the regimen on which the patient had recently experienced progression.[27]

According to the Bisgrove investigators, the study demonstrated the feasibility of identifying molecular targets in patients' tumors from multiple centers. The outcomes were significant, durable, and had an impact on overall survival in a very sick patient population.[27] In addition, the molecular characterization technologies that were evaluated in the study were found to be "sufficiently robust to allow selection of additional treatment for this patient population in a fashion superior to that of an experienced clinician's best judgment."[28] The trial demonstrated the practicability of obtaining "high-quality, fresh research tumor biopsies" from patients with advanced disease who were enrolled at multiple sites, and is considered innovative for its establishment of "a novel algorithm for the use of unique molecular profiles to determine an individual patient's treatment."[28] The Bisgrove trial was also notable for demonstrating the feasibility of using the PFS ratio, which essentially compares a patient's TTP while on pretrial therapy with PFS observed during the trial.[28]

Molecular profiling in previously treated metastatic pancreatic cancer

The utility of molecular profiling in biomarker identification and analysis was recently demonstrated in an ongoing phase 2 study involving 35 patients with metastatic pancreatic cancer whose biopsied tumor samples underwent IHC, comparative genomic hybridization, and OMA. Topoisomerase 1 or 2 and thymidylate synthase were the most common targets identified. Commonly recommended agents or regimens against these targets included FOLFIRI, FOLFOX, irinotecan, and doxorubicin. In most patients, molecular profiling identified at least 2 targets for therapy and a noncross-resistant regimen could be implemented, suggesting that the tumors

from this patient population are "target-rich." In addition to demonstrating the feasibility of molecular profiling in second- and third-line pancreatic cancer, the findings suggest that obtaining tissue through percutaneous core biopsy is adequate for analysis. Patients in the study are currently being followed for 1-year survival.[29]

Other commercially available assays and services

Outside of individual assays for specific advanced cancers (eg, *EGFR* for lung cancer, HER2/neu for breast cancer), there are several products and services that are currently accessible to providers and their patients that take a broader survey of tumors using molecular profiling techniques. Target Now is the only assay that has been tested in a prospective study and published.[27] The current version of the assay includes qPCR (quantitative polymerase chain reaction), IHC, FISH, and select gene sequencing. FoundationOne offers next-generation sequencing of 182 cancer-related genes. PRÉCIS Precision Medicine assays include tumor, response, and resistance biomarkers for NSCLC and colon cancer. Another service for whole genome sequencing is offered by Illumina called Individual Genome Sequencing.

Future directions in molecular profiling

In the last few years, molecular characterization technologies have advanced to the point where increasing numbers of clinicians are using molecular profiling to identify tumor-specific biomarkers and to select appropriate therapies for their patients. Such advances will yield more sophisticated techniques to identify and measure therapeutic targets, thereby improving patient management. Next-generation sequencing techniques, including exome sequencing (ie, sequencing 1% of the genome) and whole gene sequencing (WGS), are expected to attract increasing attention. Compared with traditional single-gene sequencing, exome sequencing provides enhanced throughput and reduced cost per gene sequenced, which is a tremendous advantage when one needs to sequence multiple genes. Although WGS offers the additional advantage of detection of structural rearrangements, its high cost (currently 6 times greater than that of exome sequencing) and resource requirements (such as machine time and lab space for processing and analyzing massive amounts of data) will not make it feasible for routine practice in the near future.[30]

Nevertheless, the price of molecular characterization modalities is expected to decline as data analysis becomes more efficient, which could result in faster turnaround times. Advances in molecular characterization technologies will yield information on multiple biomarkers in a matter of hours or days, rather than waiting 1 to 2 weeks for results for 3 or 4 genes. This will be the next major change in oncology practice and molecular profiling.

As molecular profiling continues to evolve, the field may allow for the adoption and use of pathway-oriented therapy, as opposed to selecting a single therapeutic target for a disease. A recent next-generation sequencing study in metastatic triple negative breast cancer (mTNBC) points the way to the pathway approach. In the study, tissue samples from 14 patients who were previously treated with chemotherapy underwent WGS and transcriptome sequencing to identify mutations to guide therapeutic targeting within available phase 1 or 2 clinical trials. The sequencing revealed numerous known and novel mutations in mTNBC (Figure 2). For example, all patients' cancers had alterations that would activate the mitogen-activated protein kinase (MAPK) pathway, but through different mechanisms in different patients (eg, *BRAF* amplification or overexpression, *NF1* homozygous deletion, consistent *IQGAP3* overexpression). All patients' tumors also harbored mutations that would activate the phosphoinositide 3 (PI3) kinase/AKT pathway (eg, *PTEN* homozygous deletion or downregulation, consistent *INPP4B* downregulation, *FBXW7* homozygous deletion, *ERAS* overexpression). According to the investigators, this is the first report of unique somatic genomic events that significantly alter the *ERBB4* locus, leading to its loss in 5 of 7 patients' tumors. One chemotherapy-refractory mTNBC patient, with a high-level *BRAF* amplification or overexpression and downregulation of *PTEN* and *INPP4B*, had a major response to a combination regimen consisting of an MEK inhibitor and an AKT (protein kinase B) inhibitor in a phase 1 study.[31]

The study, which involved comprehensive genomic and transcriptomic sequencing of mTNBCs, shed light on the importance of coactivation of the MAPK and PI3K/AKT pathways (albeit through different mutational mechanisms). In addition, the approach used in this study supports the possible use of combination therapy (ie, MEK inhibitors plus AKT inhibitors) to co-inhibit these pathways in mTNBC.[29] The study's use of molecular profiling thus points the way toward the more intelligent design of trials to identify mTNBC patients with PI3K/AKT pathway alterations, possibly leading to the approval of agents or regimens that inhibit both MEK and AKT. Indeed, an ongoing phase I study is investigating the effectiveness of this combination approach.

It may be several years before new molecular characterization techniques such as WGS are considered practical for routine use, but the field of molecular profiling is already helping clinicians select evidence-based, tumor-specific therapies for patients who oth-

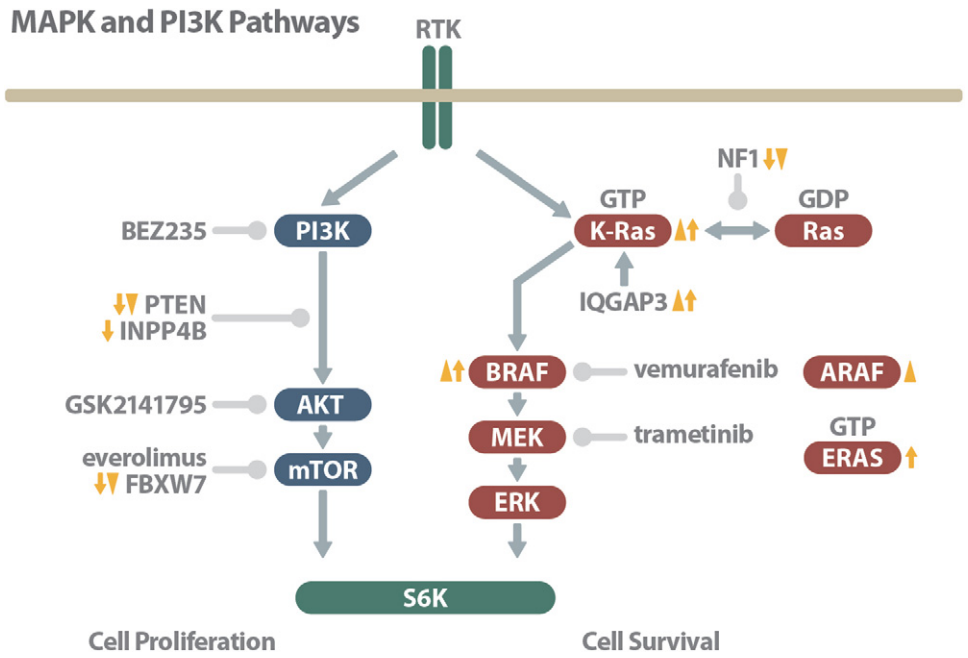

FIGURE 2 This figure depicts the signaling protein alterations that are representative in the MAPK (red) and PI3K (blue) pathways in mTNBC from molecular profiling.[31] The receptor tyrosine kinase (RTK) in the cancer is activated and signaling downstream is activated to the final common component (S6K, green) that stimulates cell proliferation and survival.
Key: upward orange triangle, amplification of gene; upward orange arrow, overexpression of mRNA; downward orange triangle, deletion of gene; downward orange arrow, underexpression of mRNA; gray line with solid circle, inhibitory signal; gray arrow, stimulatory signal.
Drug names and main mechanisms of action: BEZ235, PI3K inhibitor; GSK2141795, AKT inhibitor; vemurafenib, BRAF inhibitor; trametinib, MEK inhibitor.

erwise would have exhausted their treatment options. Enhanced understanding of the roles of various oncogenes in the growth and proliferation of specific malignancies has made tumor-based genomic sequencing and analysis more widely accepted. As more is learned about cancer biomarkers and the techniques to measure and analyze their expression, molecular profiling may eventually be used in earlier stages of cancer rather than as a last-resort option when all other alternatives have been exhausted.

References

1. Cross D, Burmeister JK. The promise of molecular profiling for cancer identification and treatment. *Clin Med Res.* 2004;2:147-150.
2. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001;344:783-792.
3. Simon R, Maitournam A. Evaluating the efficiency of targeted designs for randomized trials. *Clin Cancer Res.* 2004;10:6759-6763.
4. Phase 3 clinical trial costs exceed $26,000 per patient [news release]. Research Triangle Park, NC: Cutting Edge Information; October 12, 2006. http://www.lifesciencesworld.com/news/view/11080. Accessed June 26, 2012.
5. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med.* 2005;353:123-132.
6. Paez JG, Janne PA, Lee JC, et al. *EGFR* mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science.* 2004;304:1497-1500.
7. Mok TS, Wu Y-L, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med.* 2009;361:947-957.
8. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR . *N Engl J Med.* 2010;362:2380-2388.
9. Jackman DM, Miller VA, Cioffredi L-A, et al. Impact of epidermal growth factor receptor and *KRAS* mutations on clinical outcomes in previously untreated non-small cell lung cancer patients: results of an online tumor registry of clinical trials. *Clin Cancer Res.* 2009;15:5267-5273.
10. Rosell R, Taron M , Reguart N, et al. Epidermal growth factor receptor activation: how exon 19 and 21 mutations changed our understanding of the pathway. *Clin Cancer Res.* 2006;12:7222-7231.
11. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer. *Nature.* 2007;448:561-566.
12. Bang Y, Kwak EL, Shaw AT, et al. Clinical activity of the oral ALK inhibitor PF-02341066 in ALK-positive patients with non-small cell lung cancer [ASCO abstract 3]. *J Clin Oncol.* 2010;28(*suppl*):S18.
13. Southall J. ALK inhibitor crizotinib has high response rate in patients with ALK-positive NSCLC. *HemOnc Today.* 2010 June 5. http://www.healio.com/hematology-oncology/lung-cancer/news/online/%7B7F093ECD-D7CD-420A-86E2-F47AC2497224%7D/ALK-inhibitor-crizotinib-has-high-response-rate-in-patients-with-ALK-positive-NSCLC. Accessed June 19, 2012.

14. Winslow R, Loftus P. Advances come in war on cancer. *Wall Street Journal*. 2010 June 7. http://online.wsj.com/article/SB10001424052748704002104575291103764336126.html?mod=WSJ_WSJ_US_News_3. Accessed June 19, 2012.
15. Shaw AT, Yeap BY, Solomon BJ, et al. Effect of crizotinib on overall survival in patients with advanced non-small-cell lung cancer harboring ALK gene rearrangement: a retrospective analysis. *Lancet Oncol*. 2011; 12:1004-1012.
16. Rubin BP, Singer S, Tsao C, et al. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res*. 2001;61: 8118-8121.
17. Allander SV, Nupponen NN, Ringner M, et al. Gastrointestinal stromal tumors with *KIT* mutations exhibit remarkably homogeneous gene expression profile. *Cancer Res* 2001;61:8624-8628.
18. Gleevec (imatinib mesylate) [package insert]. East Hanover, NJ: Novartis Pharmaceuticals Corporation; 2012.
19. Kurzrock R, Kantarjian H, Druker BJ, Talpaz M. Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. *Ann Intern Med*. 2003;138:819-830.
20. Rituxan (rituximab) [package insert]. South San Francisco, CA: Genentech Inc; 2012.
21. Maloney DG, Grillo-Lopez A, White CA, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood*. 1997; 90:2188-2195.
22. Hsu PL, Hsu SM. Autocrine growth regulation of CD30 ligand in CD30-expressing Reed-Sternberg cells: distinction between Hodgkin's disease and anaplastic large cell lymphoma. *Lab Invest*. 2000;80:1111-1119.
23. Su C-C, Chiu H-H, Chang C-C, et al. CD30 is involved in inhibition of T-cell proliferation by Hodgkin's Reed-Sternberg cells. *Cancer Res*. 2004;64:2148-2152.
24. Adcetris (brentuximab vedotin) [package insert]. Bothell, WA: Seattle Genetics Inc; 2011.
25. Positive data from pivotal trial of brentuximab vedotin (SGN-35) in relapsed or refractory Hodgkin lymphoma at ASH annual meeting [news release]. Bothell, WA: Seattle Genetics; Dec ember 6, 2010. http://investor.seagen.com/phoenix.zhtml?c=124860&p=irol-newsArticle&ID=1504306&highlight=. Accessed June 19, 2012.
26. von Hoff DD, Penny R, Shack S, et al. Frequency of potential therapeutic targets identified by immunohistochemistry (IHC) and DNA microarray (DMA) in tumors from patients who have progressed on multiple therapeutic agents [ASCO abstract 3071]. *J Clin Oncol*. 2006;24(suppl):S18.
27. von Hoff D, Stephenson JJ Jr, Rosen P, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *J Clin Oncol*. 2010;28:4877-4883.
28. Doroshow JH. Selecting systemic cancer therapy one patient at a time: is there a role for molecular profiling of individual patients with advanced solid tumors? *J Clin Oncol*. 2010;28: 4869-4871.
29. Ramanathan RK, Barrett MB, Weiss GJ, et al. Phase II study of therapy selected by molecular profiling in patients with previously treated metastatic pancreatic cancer: a study of the Stand Up to Cancer (SU2C) Consortium [AACR abstract LB-221]. *Cancer Research*. 2012;72(suppl 1)
30. Biesecker LG, Shianna KV, Mullikin JC. Exome sequencing: the expert view. *Genome Biology*. 2011;12:128.
31. O'Shaughnessy J, Craig DW, Kiefer J, et al. Next generation sequencing reveals co-activating events in the MAPK and PI3K/AKT pathways in metastatic triple negative breast cancers. *Cancer Res*. 2011;71(suppl 24):S104-S105