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Precision Oncology Medicine: The Clinical Relevance of Patient Specific Biomarkers Used to Optimize Cancer Treatment

Keith T. Schmidt, PharmD¹, Cindy H. Chau, PharmD, PhD², Douglas K. Price, PhD², and William D. Figg, PharmD^{1,2}

¹Clinical Pharmacology Program, Office of the Clinical Director, National Cancer Institute, NIH, Bethesda, MD, USA

²Molecular Pharmacology Section, Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD, USA

Abstract

Precision medicine in oncology is the result of an increasing awareness of patient specific clinical features coupled with the development of genomic-based diagnostics and targeted therapeutics. Companion diagnostics designed for specific drug-target pairs were the first to widely utilize clinically applicable tumor biomarkers (e.g. HER2, EGFR), directing treatment for patients whose tumors exhibit a mutation susceptible to a FDA approved targeted therapy (e.g. trastuzumab, erlotinib). Clinically relevant germline mutations in drug metabolizing enzymes and transporters (e.g. TPMT, DPYD) have been shown to impact drug response, providing rationale for individualized dosing to optimize treatment. The use of multigene expression-based assays to analyze an array of prognostic biomarkers have been shown to help direct treatment decisions, especially in breast cancer (e.g. Oncotype DX). More recently, the use of Next-Generation Sequencing to detect many potential “actionable” cancer molecular alterations is further shifting the one gene-one drug paradigm towards a more comprehensive, multi-gene approach. Currently, many clinical trials (e.g. NCI-MATCH, NCI-MPACT) are assessing novel diagnostic tools with a combination of different targeted therapeutics, while also examining tumor biomarkers that were previously unexplored in a variety of cancer histologies. Results from ongoing trials like the NCI-MATCH will help determine the clinical utility and future development of the precision-medicine approach.

Keywords

Precision Medicine; Oncology; Molecular Targeted Therapy; Next-Generation Sequencing

Introduction

Precision medicine in cancer care relies on the use of genomic technologies at the point-of-care to inform clinical treatment decisions. This allows for more accurate and efficient prediction of individualized therapies that is most suited for specific patients. Advancement

in the field is the result of recent development of biological databases, increased affordability and reliability of powerful methods to characterize patient tumors (such as genomics, proteomics, metabolomics, improved cellular assays and platforms), and computational tools for analyzing large omics data sets. This revolution has given rise to cancer landscape studies identifying key oncogenic drivers, inter- and intratumoral genetic heterogeneities and therapies to specifically target these alterations that confer clinical benefit.^{1, 2} Ultimately, the goal is to build the evidence base in cancer genomics needed to guide clinical practice.

The concept of targeted therapy focuses on finding relevant unique molecular abnormalities associated with specific cancers. These cancer biomarkers, which include both germ-line and somatic mutations, may influence disease outcome and/or response to therapy and can be classified as prognostic (associated with disease outcome) or predictive (associated with drug response). Selection of a particular anticancer therapy is based on the presence of the actionable target and interfering with its function in driving cancer cell growth or progression. Information on key genomic changes, including mutations, somatic copy number alterations, and polymorphisms affecting drug metabolism, has already helped shape the development and use of some of the newest targeted cancer treatments, underscoring the importance of cancer genomics in advancing personalized medicine. The purpose of this review is to highlight the clinically relevant biomarkers and molecular profiling platforms in precision oncology medicine. The first section will focus on prognostic biomarkers, discussing drug-target pairs and their companion diagnostic tests. The second section will focus on predictive biomarkers of drug sensitivity/resistance, metabolism, and toxicity. The final section will highlight current efforts to improve the advancing field of precision oncology medicine, including new technology platforms and trial designs for implementing precision medicine.

Clinically relevant drug-target biomarkers

The Food and Drug Administration (FDA) have approved several targeted therapies that incorporate specific mutations essential for drug efficacy along with an approved companion diagnostic test for tumor molecular profiling. Common methodologies include in situ hybridization (ISH), immunohistochemistry (IHC), real-time polymerase chain reaction (RT-PCR) amplification, and DNA sequencing; the latter two are used to detect the presence or absence of a specific genetic mutation. All current FDA approved companion diagnostics are summarized in Table 1. The next section will discuss targeted therapeutics approved by the FDA along with their companion diagnostic tests to illustrate examples of actionable mutations and their matching drugs (drug-target pairs).

HER2 Overexpression/Amplification

The human epidermal growth factor receptor (*HER2*) gene is overexpressed in 15–20% of breast cancers and in other cancer types such as gastric, colon, head and neck. *HER2* heterodimerizes with other transmembrane tyrosine kinase receptors (EGFR, *HER3*, *HER4*) without ligand binding and activation of these pathways promotes tumorigenesis in addition to malignant phenotypic characteristics.^{3, 4} *HER2*-positive tumors are also associated with a

more aggressive tumor, poor prognosis, and shorter survival.⁵ Determination of HER2 status relies on using IHC for measuring protein overexpression or ISH for gene amplification (Table 1).^{6, 7}

Four drugs are approved to target HER2 (trastuzumab, pertuzumab, ado-trastuzumab emtansine, and lapatinib) along with their companion diagnostics.⁸ Trastuzumab, the first HER2 targeting monoclonal antibody, has clinical activity in women with HER2 positive breast cancer as monotherapy⁹ and in combination with chemotherapy.^{9–15} The FDA-approved drug label for pertuzumab requires that patients be tested for HER2 protein overexpression or *HER2* gene amplification, as determined by an accurate and validated FDA-approved assay. A pivotal trial conducted by Slamon *et al.* provided rationale for developing a combination regimen that increased clinical benefit with the addition of trastuzumab to traditional chemotherapy. The trial also highlighted the importance of chemotherapy selection, as combination therapy of trastuzumab with anthracyclines and cyclophosphamide resulted in severe cardiotoxicity.¹⁶ Trastuzumab with docetaxel or vinorelbine provided optimal treatment regimens with synergistic interaction.¹⁷ A phase III trial evaluated trastuzumab in combination with docetaxel or vinorelbine in metastatic HER2 positive breast cancer. The study did not show a difference in overall survival (OS) between the two regimens, but the trastuzumab with vinorelbine arm had significantly fewer adverse events.¹⁸

Pertuzumab is a distinct agent from trastuzumab that occupies a different extracellular subdomain of HER2 to achieve inhibition of downstream signaling. With pertuzumab binding to subdomain II and trastuzumab binding to subdomain IV, a dual inhibitory mechanism occurs without competitive interaction.¹⁹ Pertuzumab (P) received accelerated approval (in breast cancer patients with evidence of HER2 overexpression/amplification) in combination with docetaxel and trastuzumab after clinically significant improvements in progression free survival (PFS) was observed over trastuzumab (T) and docetaxel (D) alone (HR 0.68; 95% CI 0.56 to 0.84; $P < 0.001$).^{19, 20} The five-year follow-up survival analysis showed a continued benefit of P added to T+D that persisted over time, supporting the association between pathologic complete response rate and improvements in long-term outcomes.²¹

Two additional HER2 targeting agents, Ado-trastuzumab emtansine (T-DM1) and lapatinib, are viable treatment options for refractory HER2-positive metastatic breast cancer. TDM1, an antibody drug conjugate (ADC), retains the functionality of trastuzumab by binding to HER2 on tumor cells. Upon internalization, the DM1 moiety is released and binds to tubulin, thereby disrupting microtubule assembly/disassembly dynamics and inhibiting cell division and the proliferation of cancer cells that overexpress HER2.²² Lapatinib is a dual tyrosine kinase inhibitor (TKI) of HER2 and Epidermal Growth Factor Receptor (EGFR).^{23, 24} Following the phase III TH3RESA trial, TDM-1 demonstrating an improved PFS (HR 0.528, 95% CI [0.422–0.661] $p < 0.001$) and should be considered as a new standard for patients with HER2-positive advanced breast cancer who have previously received trastuzumab and lapatinib.²⁵ However, in the first line treatment setting, results of the phase III MARIANNE trial found that HER2-positive metastatic breast cancer patients treated with T-DM1 plus pertuzumab had similar PFS compared with those treated with

trastuzumab plus a taxane-based chemotherapy.²⁶ Similarly, lapatinib failed to show an improved response as a part of first line treatment,²⁷ but did demonstrate an increased time to progression (TTP) as a second line therapy in combination with capecitabine versus capecitabine alone (8.4 months vs. 4.4 months respectively, HR 0.49, 95% CI [0.34 to 0.71] $p<0.001$).²⁸ A comparison of T-DM1 to lapatinib and capecitabine in the second line treatment setting showed T-DM1 had less toxicity and a significant OS improvement (30.9 months vs. 25.1 months, HR 0.68, 95% CI [0.55 to 0.85] $p<0.001$).²⁹ In current practice, T-DM1 is the preferred second-line therapy for HER2-positive metastatic breast cancer.³⁰

Current clinical practice guidelines recommend HER2 testing on every primary invasive breast cancer and on metastatic sites (if Stage IV and specimen becomes available), especially in patients who previously tested HER2 negative and present with disease recurrence. The decision to recommend HER2-targeted treatment should be delayed if HER2 status cannot be determined and additional testing to establish tumor HER2 status is necessary to guide therapeutic decisions.³¹

ALK

Somatic rearrangements of the anaplastic lymphoma kinase (*ALK*) create common oncogenic activation pathways in Non-Small Cell Lung Cancer (NSCLC). Like HER2, ALK is a cell surface protein that regulates cell signaling pathways. A number of genes have been noted to translocate and fuse with ALK, leading to a variety of different ALK variants.^{32–34} The most predominant ALK fusion protein is formed in combination with echinoderm microtubule-associated protein like-4 (EML-4), which occurs in 4–8% of NSCLC.^{35, 36} The ALK fusion protein is associated with several downstream targets, including mitogen associated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), janus kinase (JAK), RAS-related protein (RAP-1), and signal transducer and activator of transcription (STAT). ALK activation leads to cell growth and differentiation, while limiting apoptosis.³⁷ The current FDA approved platforms for ALK rearrangement detection utilize FISH and, more recently, IHC. Historically, FISH has been the more reliable method of detection, but higher cost and longer processing times has led to increased interest in the development of IHC detection. The recently approved IHC platform demonstrated an improved positive predictive value that is comparable to FISH, allowing for its use as a diagnostic test.³⁸

Crizotinib was the first FDA approved agent for ALK-positive lung cancer based on two clinical trials in the first line treatment setting. The PROFILE 1007 study demonstrated improvement in PFS for crizotinib and is superior to standard chemotherapy (7.7 months vs 3.0 months, HR: 4.9, 95% CI 0.37 to 0.64, $P<0.001$) in patients with previously treated, advanced NSCLC with ALK rearrangement.³⁹ The PROFILE 1014 study found crizotinib was superior to standard first-line pemetrexed-plus-platinum chemotherapy (PFS 10.9 months vs. 7.0 months, HR 0.82, 95% CI 0.54 to 1.26, $p<0.001$) in patients with previously untreated advanced ALK-positive NSCLC.⁴⁰ Mutations that confer resistance to crizotinib provided the rationale for the development of second-generation ALK inhibitors. Ceritinib targets the L1196M gatekeeper mutation and alectinib targets the L1196M, R1174L, and R1275Q mutations.⁴¹ Accelerated approvals were granted for ceritinib (2014) and alectinib (2015) for patients with ALK-positive NSCLC with disease progression on or who are

intolerant to crizotinib. A phase I study that demonstrated ceritinib was highly active with responses observed in patients with various resistance mutations in ALK and in patients without detectable mutations.⁴² Two phase II studies showed that patients who have become resistant to crizotinib responded well to alectinib, with an additional advantage of being effective against brain metastases.⁴³ A phase III trial in treatment naïve ALK-positive NSCLC patients comparing alectinib and crizotinib head-to-head is currently ongoing (ClinicalTrials.gov Identifier: NCT02075840). Molecular profiling of tumors that become resistant to initial treatment with an ALK inhibitor will help in the selection of second- and third-line treatments as a number of novel agents targeting specific resistance mechanisms are in clinical trials.

EGFR

Epidermal growth factor receptor (EGFR) is expressed on the cell surface and initial studies with the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib demonstrated biologic and clinical activity in only a relatively limited subset of lung cancers.⁴⁴ Subsequent studies demonstrated that the highest response rates were observed in patients with somatic mutations within the EGFR-TK domain, particularly exon 19 deletion (Del19), exon 21 L858R, and exon 18 G719X,⁴⁵ while exon 20 T790M mutation is associated with acquired resistance to TKI therapy.⁴⁶

Activating *EGFR* mutations are commonly observed in patients with adenocarcinomas, no prior history of smoking, as well as in females and those of Asian descent; use of the EGFR-TKIs gefitinib, erlotinib, and afatinib is limited to patients who have known activating *EGFR* mutations. Prior to the recognition that *EGFR* mutations sensitize tumors to EGFR-TKIs, gefitinib originally received accelerated approval in 2003 for the treatment of patients with advanced NSCLC after progression on platinum doublet chemotherapy and docetaxel. It was voluntarily withdrawn from the market after subsequent confirmatory trials failed to verify clinical benefit. In 2015, gefitinib was FDA approved for first-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 L858R substitution mutations as detected by the companion test *therascreen* EGFR RGQ PCR Kit. Data from the IFUM (IRESSA Follow-Up Measure) trial showed an overall response rate of about 50%, with a median duration of response of 6 months.⁴⁷ These results were further supported by the IPASS (IRESSA Pan-Asia Study) trial, which assessed gefitinib versus carboplatin/paclitaxel for first-line treatment in the same population. Patients who were positive for an activating *EGFR* mutation demonstrated significantly longer PFS of 10.8 months in the gefitinib group versus 5.4 months for carboplatin/paclitaxel.^{48–50} The OPTIMAL study compared erlotinib to carboplatin and gemcitabine in Stage IIIB or IV NSCLC patients with confirmed Del19 or L858R mutation, reporting a superior benefit in median PFS (13.1 months vs. 4.6 months, HR 0.16, 95% CI 0.10 to 0.26, $p < 0.0001$).⁵¹ In 2013, erlotinib was approved for first-line treatment of NSCLC whose tumors have EGFR exon 19 deletions or exon 21 L858R mutations as detected by the companion diagnostic cobas EGFR Mutation Test.

Subsequent EGFR TKI development has refined kinase inhibition to select for the T790M mutation. The *EGFR* mutation T790M is found in approximately half of patients with

acquired resistance EGFR-TKIs. Second generation EGFR TKIs (e.g. afatinib) are designed to target mutant EGFR better than wild type EGFR. Afatinib binds to EGFR, HER2 and HER4, irreversibly inhibiting tyrosine kinase autophosphorylation; it also inhibits transphosphorylation of HER3. Afatinib has also shown activity against the T790M mutation.⁵² It was FDA approved for first line treatment in patients with NSCLC with confirmed Del19 or L858R mutation as detected by the diagnostic companion test theascreen *EGFR* RGQ PCR Kit.^{53, 54} The third generation EGFR TKI that specifically targets T790M, osimertinib, was recently approved in November 2015 for metastatic EGFR T790M mutation-positive NSCLC in patients who have progressed during or after EGFR TKI therapy.⁵⁵ The FDA also approved the first companion diagnostic test (cobas EGFR Mutation Test v2) for detecting the T790M resistance mutation.

In contrast to the clear link between *EGFR* mutation status and EGFR-TKI response, the presence of *EGFR* mutations was not predictive of response with the EGFR monoclonal antibody cetuximab; however EGFR expression on IHC may be predictive of response.^{56, 57} As such, cetuximab is not yet FDA-approved for NSCLC. Mutations in *EGFR* and *ALK* are mutually exclusive in patients with NSCLC; the presence of one mutation in lieu of another can influence response to targeted therapy. Current guidelines recommend testing all patients with metastatic NSCLC for the presence of activating *EGFR* mutations, and to use an EGFR-TKI as first-line therapy in this specific patient population.

BRAF

RAF kinases are part of the mitogen-activated protein (MAP) kinase pathway (Ras-Raf-MEK-ERK) involved in cell proliferation and survival with an overwhelming frequency of being aberrantly activated in cancer. BRAF mutations and other BRAF anomalies (amplifications, fusions) have been detected in various tumor types. BRAF is mutated in about 15% of all cancers with BRAF mutation frequency of 40%–60% for melanoma and 100% for hairy cell leukemia.^{58, 59} In BRAF-mutated cancers, the V600E mutation represents approximately 70% to 90% of all mutations in BRAF and the V600K mutation is found at a frequency of 7% to 19%.^{58, 60}

Targeted inhibition of BRAF with vemurafenib⁶¹ or dabrafenib⁶² (TKIs targeting the BRAF V600E mutation) or direct inhibition of MEK with trametinib⁶³ (TKI targeting BRAF V600E or V600K mutation) confers high response rates and statistically significant survival benefits over traditional chemotherapy, leading to their FDA approvals as monotherapies for the treatment of metastatic melanoma containing a V600 BRAF mutation. Monotherapy with vemurafenib, dabrafenib, or trametinib eventually leads to acquired resistance and more aggressive recurrent disease. Many patients treated with BRAF inhibitors are prone to treatment resistance 6 to 8 months following treatment, with reactivation of the RAF/MEK/ERK pathway often resulting in secondary cutaneous squamous-cell carcinoma (CSCC).^{64–68} Combination treatment of a BRAF and a MEK inhibitor, dabrafenib plus trametinib, can further increase the rate and durability of treatment responses and lengthen the survival benefit conferred by single-agent treatment as demonstrated by the COMBI-DT phase III study (dabrafenib and trametinib versus dabrafenib alone, 9.4 vs. 5.8 months respectively, HR = 0.39, 95% CI 0.25 to 0.62,

$P < 0.001$).⁶⁹ In addition, combination therapy reduced the rate of secondary CSCC by 12% (19% vs. 7%).⁶⁹ The COMBI-V trial, another phase III trial that compared the same combination therapy versus vemurafenib, yielded similar results.⁷⁰ The FDA granted accelerated approval to trametinib and dabrafenib for use in combination in the treatment of patients with unresectable or metastatic melanoma with a BRAF V600E or V600K mutation as detected by the FDA-approved companion diagnostic tests.

PD-L1 Expression

Distinct from the aforementioned targeted therapies, recently approved cancer immunotherapy drugs (e.g. nivolumab and pembrolizumab) stimulate or restore the ability of the immune system to fight cancer.⁷¹ A recent review by *Lee et al.* highlighted the lack of clinically relevant predictive biomarkers to select patients who would be appropriate for immunotherapy.⁷¹ Currently, the only clinically validated predictive marker is the expression of Programmed Death Ligand-1 (PD-L1). The KEYNOTE-001 trial showed that PD-L1 expression of greater than 50% as determined by IHC staining was correlated with an improved overall response rate (ORR) when patients with platinum therapy refractory metastatic NSCLC were treated with pembrolizumab, a Programmed Death Receptor-1 (PD-1) inhibitor (PD-L1 staining 50% versus PD-L1 staining of 1–49%, 45.2% vs. 16.5% respectively).⁷² The FDA granted accelerated approval of pembrolizumab for use in the treatment of patients with metastatic NSCLC whose tumors express PD-L1 as determined by an FDA-approved test following disease progression on or after platinum-containing chemotherapy.

Drug Response & Toxicity Biomarkers

Precision oncology medicine is centered on the concept of predicting which patients are more likely to respond to specific cancer therapies and to determine optimum individualized therapies. In addition to patient prognosis and tumor response, tumor biomarkers are also associated with a drug's metabolism, response, and toxicity. Clinically relevant germline mutations that have been shown to impact drug response include thiopurine-S-methyl transferase (TPMT), uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1), and cytochrome P450 2D6 (CYP2D6). These examples will be briefly discussed in the next section along with mention of actionable prescribing decisions from either the FDA-approved drug labels or the Clinical Pharmacogenomics Implementation Consortium (CPIC) published genotype-based drug guidelines to assist in optimizing drug therapy.⁷³ Examples of drugs indicated for the treatment of cancer with pharmacogenomic biomarkers of toxicity included in the FDA-approved drug label are summarized in Table 2.

TPMT is ubiquitously expressed throughout the human body and catalyzes the S-methylation of thiopurines, such as 6-mercaptopurine (6-MP) and thioguanine, into inactive compounds. Variant alleles of *TPMT* (primarily *2, *3A, *3C) result in reduced enzyme activity, exposing patients to higher drug concentrations (approximately 10-fold) and subsequently increasing their risk of severe myelosuppression.^{74–76} Toxicity from this gene variation occurs in 1 of every 300 Caucasian patients and presents a high positive predictive value of 67–100%.⁷⁷ Therefore, *TPMT* genotyping or phenotyping can identify patients who

are homozygous deficient or who have low/intermediate TPMT activity, which predispose them to drug toxicity. The CPIC published initial dosing guidelines for TPMT in 2011 (updated in 2013).^{78, 79}

Dihydropyrimidine dehydrogenase (DPD), encoded by *DPYD*, is another polymorphic enzyme that is a predictor of myelosuppression. DPD is the rate limiting enzyme in the metabolism of 5-fluorouracil (5-FU) and capecitabine. Only a small fraction of patients who experience grade 3 to 4 toxicity have documented DPD mutations.⁸⁰ The most predominant *DPD* mutations noted in the literature, *DPYD**2A, *13, and rs67376798, account for 20–50% of patients with treatment-related toxicity. These mutations have a positive predictive value of only 62%.⁷⁷ CPIC published fluoropyrimidine dosing recommendations for the three DPD alleles⁸⁰ whereas both FDA-approved drug labels only mention the potential for severe toxicity in patients with DPD deficiency with no accompanying genetic testing or dosing recommendations.

UGT1A1 is a polymorphic phase II enzyme responsible for conjugating activated irinotecan, SN-38, to a glucuronide inactive metabolite, SN-38G. Mutations in *UGT1A1* can result in significant reductions in glucuronidation, resulting in increased exposure of SN-38 and an increased risk of toxicity.^{81–84} The *28 and *6 alleles are the clinically relevant variants that are associated with irinotecan-related diarrhea and neutropenia. Studies of irinotecan pharmacogenetics have mainly focused on the association of *UGT1A1**28 allele to irinotecan-related toxicity. The clinical utility of *UGT1A1**28 genotyping for pre-emptive dose reductions remains to be determined since studies to date on whether *28 affects treatment efficacy have been contradictory and since most episodes of severe toxicity are managed by dose reduction in subsequent cycles.⁸⁵ Despite differences in patient population and regimens, the general consensus from studies on *UGT1A1* genotype-directed dosing of irinotecan is that patients with the *28/*28 genotype are at the highest risk of irinotecan-related toxicity and require a dose reduction of up to 40%. Since 2005, the FDA has recommended a reduction of the initial irinotecan dose (by at least one level) for individuals who are *UGT1A1**28 homozygous variant because they are at increased risk for neutropenia.

CYP2D6 is necessary for tamoxifen activation and metabolism to the more potent 4-hydroxy-tamoxifen and endoxifen metabolites.^{86, 87} Mutations in *CYP2D6* can alter enzyme activity affecting the extent of drug metabolism with individuals classified as poor metabolizers, intermediate metabolizers, extensive metabolizers or ultrarapid metabolizers.⁸⁸ This results in altered concentrations of endoxifen in the serum and consequently affecting patient response rates. The data on CYP2D6–tamoxifen association studies to determine the clinical utility of *CYP2D6* genotype-guided tamoxifen therapy remain inconclusive. Decreased CYP2D6 enzymatic activity has also been shown to impact on increased breast cancer recurrence rates in poor and intermediate metabolizers compared to extensive metabolizers.⁸⁹ Several studies have investigated the correlation between tamoxifen and CYP2D6 genotype and did not demonstrate a clinical association between CYP2D6 and tamoxifen outcomes;^{90, 91} however, these studies have been plagued by criticisms in study design and lack of uniformity in study results.⁹² Other meta-analyses studies have demonstrated that while a clear gene-exposure effect was able to partially explain the

interindividual variability in plasma concentrations of endoxifen, a clear exposure-response effect remained controversial.^{93, 94} Therefore, individualized treatment of tamoxifen based on genotyping has not yet met consensus and the FDA-approved drug label for tamoxifen does not discuss genetic testing for *CYP2D6*.

Technological Advances for Precision Medicine

Oncotype DX and other multigene-based assays

Improvements in molecular profiling techniques have given rise to the identification of gene signatures used to define cancer subtypes to help guide treatment decisions, making the transition from a single gene assay to a multigene panel inevitable. The development of multigene expression-based assays (e.g., Oncotype DX, MammaPrint, Mammostrat, and Prosigna) has resulted in a paradigm shift in the management and treatment of breast cancer, particularly in the setting of early stage breast cancer. Oncotype DX, a 21-gene expression (including HER2 amplification) RT-PCR assay, is used to estimate a woman's risk of recurrence of early-stage, hormone-receptor-positive breast cancer. The assay generates a score ranking a patient's 10-year risk of recurrence, ranging from low (<18), intermediate (18–30), and high (>30) risk category patients, to also determine whether the addition of chemotherapy is beneficial after breast cancer surgery.⁹⁵

Several studies have evaluated the utility of the Oncotype DX test.^{96–98} A recent meta-analysis seeking to assess the impact of Oncotype DX on clinical decision making and net chemotherapy change found that personalized medicine via Oncotype DX in breast cancer appears to aid physician decisions and improve treatment response.⁹⁹ The Oncotype DX test is the only one of the four genomic tests with results that has been clinically validated. While studies on the MammaPrint, Mammostrat, and Prosigna tests are promising, these three tests aren't widely used to help make treatment decisions. At the time of writing, the Oncotype DX test is the only genomic test for early-stage breast cancer that is included in the National Comprehensive Cancer Center Network (NCCN) and the American Society of Clinical Oncology (ASCO) treatment guidelines. The Oncotype DX test for ductal carcinoma in situ (DCIS) recurrence is relatively new and not yet included in the ASCO or NCCN DCIS treatment guidelines.

Other genetic assays are in development for other cancer subtypes, most notably colon cancer and prostate cancer. In colon cancer, the 12-gene Oncotype DX colon cancer assay stratifies stage II and III patients based on a similar risk score. For stage II patients, the score determines the necessity for adjuvant chemotherapy following resection, while in stage III patients the score dictates the addition of oxaliplatin to 5-fluorouracil therapy.¹⁰⁰ Several studies, most notably the QUASAR, CALGB 9581, and NSABP C-07 studies^{101–103} provide evidence to support the use of this test in patients. A similar approach is currently in development for prostate cancer, deriving predictive relationships between several specific biomarkers. These include PSA (prostate specific antigen), AR-V7 (an androgen receptor splice variant), and gene methylation patterns. Diagnostics (e.g. Prolairis, ConfirmMDx, and Oncotype DX) are in development to determine patients eligible for active surveillance and the risk of adverse events following prostatectomy.¹⁰⁴

Next generation sequencing

The rapid development of molecularly targeted cancer therapeutics has expanded the utility of multigene sequencing panels for detecting tumor-specific mutations. The development of next generation sequencing (NGS) and associated target sequence enrichment technologies are robust platforms that can detect these “actionable” cancer molecular alterations in a large number of genes in a single multiplexed assay.^{105–107} As a result of these large-scale technologies, precision medicine has shifted from a one gene-one drug paradigm to a multigene-many drugs model.¹⁰⁸

The development of NGS-based companion diagnostics will become more relevant in the near future. For example, analytically validated assays are essential such is the case for the assay development and analytical validation of a custom NGS-based mutation-detection assay that can be used for screening patients for enrollment into the NCI-Molecular Profiling-Based Assignment of Cancer Therapy (NCI-MPACT), and to assess the utility of applying sequencing data to the selection of treatment in cancer patients.¹⁰⁹ In addition, FDA has recently created and launched the precision FDA web platform, a community research and development portal that allows for testing, piloting, and validating existing and new bioinformatics approaches to NGS processing.

Despite continuous advances in high-throughput genomic sequencing technologies, challenges in successful implementation of precision medicine also exist. For example, access to tumor tissue for profiling is especially complicated, subject to sampling bias and can be limiting for certain types of cancers. A potential technology that may address the difficulty in obtaining biopsies is the use of liquid biopsy techniques, which involve the use of circulating tumor cells or circulating tumor DNA to identify genomic alterations and track patient’s genomic landscape over time. Moreover, *Donnenberg et al.* recently described the challenge of using cancer genomics to describe processes underlying therapy resistance and to target cancer stem cells, primarily by inhibiting the bidirectional properties of the epithelial-to-mesenchymal transition (EMT).¹¹⁰ Another challenge is in determining the clinical feasibility of applying high-throughput sequencing data to query a panel of “actionable” cancer gene mutations and to incorporate this approach into clinical decision making to specify the use of many targeted agents.^{1, 111, 112} This has led to a new generation of genomic-based clinical trials to define the validity and utility of cancer genomic data to identify clinically relevant actionable mutations and select appropriate therapeutics strategies based on the patients’ tumor molecular profiles.

A framework for genomically-guided personalized therapy was recently proposed.¹¹³ Four major criterion for use of this methodology have been outlined for incorporation into routine decision making. First, there must be confidence in next generation sequencing to accurately call genetic alterations and determine the patient’s tumor genomic profile. Second, the clinical implications of the patient’s genomic profile must be determined, primarily focusing on current prognosis and identification of potential predictive biomarkers. Third, relevant FDA-approved drugs must be identified in addition to relevant clinical trials that outline the potential of the indicated treatment. Finally, an assessment of scientific evidence of each of the indicated agents in the context of the patient’s specific genomic alterations should yield an appropriate clinical decision.¹¹³

Future Perspectives: Strategies to Implement Precision Medicine

Optimal trial design for genomics-based clinical studies remains critical. "Basket" (or bucket) trials are genotype-focused evaluating a single drug on a specific mutation or mutations across various cancer types.^{114–116} Within such a histology-agnostic trial, patients with the different types of cancer can be grouped into separate study arms (or baskets), allowing separate analysis of patient responses with each type of cancer as well as to assess the impact of the drug on the entire group of the patients as a whole. A basket trial design is especially advantageous when the mutation or cancer type is rare as it provides an important opportunity to test therapies for rare cancers (possessing the eligible molecular abnormality), which are severely underrepresented in clinical trials. "Umbrella" trials are designed to test the impact of different drugs targeting different mutations either in a single cancer subtype or in a variety of tumor subtypes.¹¹⁶ After analysis of the molecular profile of each patient's tumor, a molecularly-guided algorithm is formulated to determine an individualized treatment plan. "Hybrid" trials represent a mix of "umbrella" and "basket" trial components, incorporating either multiple "umbrella" subtrials (same histology, different molecular aberrations), or multiple "basket" subtrials (same molecular aberrations, different histologies) into one protocol.¹¹⁷ Examples of all three types of trials are shown in Table 3.

The NCI has recently revealed a bold new trial designed to examine the utility of genomically informed personalized therapy. The NCI-MATCH (Molecular Analysis for Therapy Choice) trial plans to screen 3,000 patients and enroll 1,000 adults with advanced solid tumors and lymphomas that are refractory to therapy or for which there is no standard therapy. Structured as a multi-arm phase II trial, the study will analyze 4000 different variants across 143 genes. Using the results of this test, the patient will be assigned treatment with one of 20 drugs with either FDA approved or investigational-based actionable mutations. Built into the protocol is the ability to re-biopsy and transfer the patient to another arm of the study based on new genomic alterations present. Each arm acts as a single arm open-label trial within the confines of one large trial and will not be accompanied by a control arm. The primary endpoint for each arm is overall response rate, with investigators seeking a minimum of at least 5 of 31 patients (16%) achieving at least a partial response to treatment.^{118, 119} It is likely that many lessons will be learned from NCI-MATCH trial and others like it. Hopefully, results from these trials will contribute evidence toward clinical validation and clinical utility of using molecular information to guide precision medicine-based approach to therapy such that a consensus on the level of evidence that is needed to use a molecular abnormality to choose a treatment would be reached.

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Table 1**Drug-Targets and their FDA Approved Companion Diagnostic Tests**

Drug	Target	Indication	Diagnostic Tests
Trastuzumab	HER2/Neu Amplification [£]	Breast Cancer	Bond Oracle Her2 IHC System
			INFORM HER2 DUAL ISH DNA Probe Cocktail
			INSITE HER-2/NEU KIT
			SPOT-LIGHT HER2 CISH Kit
			PATHWAY ANTI-HER-2/NEU (4B5) Rabbit Monoclonal Primary Antibody
		Localized, Lymph Node Negative Breast Cancer	INFORM HER-2/NEU
Trastuzumab/Pertuzumab/Ado-Trastuzumab Emtansine		Breast Cancer & Gastric Cancer	HER2 CISH PharmDx Kit
			PATHVYSION HER-2 DNA Probe Kit
Crizotinib	ALK rearrangement	NSCLC	HER2 FISH PharmDx Kit
			HERCEPTEST
Crizotinib	ALK rearrangement	NSCLC	VENTANA ALK (D5F3) CDx Assay
			VYSIS ALK Break Apart FISH Probe Kit
Afatinib	EGFR - Exon 19 deletion or L858R	NSCLC	therascreen EGFR RGQ PCR Kit
Erlotinib			cobas EGFR Mutation Test
Gefitinib			therascreen EGFR RGQ PCR Kit
Osimertinib	EGFR – T790M	NSCLC	cobas EGFR Mutation Test v2 [€]
Cetuximab/Panitumumab	EGFR Expression	CRC	DAKO EGFR PharmDx Kit
	KRAS - Codon 12/13		cobas KRAS Mutation Test
			therascreen KRAS RGQ PCR Kit
Dabrafenib/Trametinib	BRAF V600E	Melanoma	THxID BRAF Kit [¥]
			cobas 4800 BRAF V600 Mutation Test
Pembrolizumab	PD-L1 Expression	NSCLC	PD-L1 IHC 22C3 pharmDx
Imatinib Mesylate	c-Kit	GIST	DAKO C-KIT PharmDx
	KIT D816V	ASM	KIT D816V Mutation Detection by PCR
	PDGFRB	MDS/MPD	PDGFRB FISH
Olaparib	Germline BRCA1/BRCA2	Ovarian cancer	BRACAnalysis CDx
Venetoclax	17p deletion	CLL	VYSIS CLL FISH PROBE KIT

[£]Diagnostic tests for the detection of HER2/Neu Amplification vary in specificity for particular tumor histologies and/or drug treatment. Refer to the specific diagnostic test package insert for more complete information about the most appropriate use of a specific diagnostic test.

[€]The cobas EGFR Mutation Test v2, used to detect the T790M mutation indicated for osimertinib, can also detect Exon 19 deletions and the L858R mutation indicated for erlotinib.

[¥]Also used for the detection of the BRAF V600K mutation indicated for the use of trametinib alone or in combination with dabrafenib.

HER2: Human Epidermal Growth Factor Receptor; IHC: Immunohistochemistry; FISH: Fluorescent *In Situ* Hybridization; ALK: Anaplastic Lymphoma Kinase; NSCLC: Non-Small Cell Lung Cancer; EGFR: Epidermal Growth Factor Receptor; PCR: Polymerase Chain Reaction; CRC: Colorectal Cancer; PD-L1: Programmed Death-Ligand 1; GIST: Gastro-Intestinal Stromal Tumor; ASM: Aggressive Systemic Mastocytosis;

PDGFRB: Platelet-Derived Growth Factor Receptor Beta; MDS: Myelodysplastic Syndrome; MPD: Myeloproliferative Disorder; CLL: Chronic Lymphocytic Leukemia.

(Adapted from the U.S. Food and Drug Administration's "List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools)", <http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm>, date accessed 4/27/2016.)

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Table 2**Examples of Biomarkers of Toxicity Included in Oncology Drug Labeling**

Drug	Toxicity Biomarker	Associated Adverse Event	Clinical Recommendation
Mercaptopurine Thioguanine	TPMT (*2, *3A, *3C)	Myelosuppression	Patients are at risk for severe toxicity and generally require substantial dose reduction. Testing for TPMT gene polymorphism should be considered in patients who experience severe bone marrow toxicities. Homozygous deficient patients may require up to a 90% dose reduction.
Cisplatin		Ototoxicity	Genetic factors may contribute to cisplatin-induced ototoxicity (association has not been consistent across populations and study designs)
Fluorouracil Capecitabine	DPD (partial or complete deficiency)	Increased drug exposure [£]	Withhold or permanently discontinue drug with evidence of acute early-onset or unusually severe toxicity, which may indicate near complete or total absence of DPD activity. No dose has been proven safe in patients with absent DPD activity
Irinotecan	UGT1A1*28/*28	Neutropenia	Patients are at increased risk for toxicity following initiation of irinotecan treatment. A reduction in starting dose by at least one level of irinotecan should be considered for patients. The precise dose reduction is not known, subsequent dose reductions should be considered based on individual patient tolerance to treatment.
Nilotinib Pazopanib		Hyperbilirubinemia	Competitive inhibitor of UGT1A1. Association with hyperbilirubinemia and potential increase in concentration of drugs that are UGT1A1 substrates.
Dabrafenib	G6PD deficiency	Hemolytic anemia	Monitor patients with G6PD deficiency for signs of hemolytic anemia.
Lapatinib	HLA-DQA1*02:01 HLA-DRB1*07:01	Hepatotoxicity	Monitor liver function in all patients regardless of genotype.

[£]No specific toxicities are mentioned in the package inserts. Potential adverse events include mucositis, diarrhea, neutropenia, and neurotoxicity.

TPMT: Thiopurine-S-Methyl Transferase; DPD: Dihydropyrimidine Dehydrogenase; UGT1A1: Uridine-Diphosphate Glucuronosyltransferase 1A1; G6PD: Glucose-6-Phosphate Dehydrogenase

(Adapted from the U.S. Food and Drug Administration's "Table of Pharmacogenomic Biomarkers in Drug Labeling", <http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>, date accessed 4/27/2016. Clinical Recommendations are adapted from the U.S. Food and Drug Administration's most recent drug labeling for mercaptopurine, thioguanine, cisplatin, fluorouracil, capecitabine, irinotecan, nilotinib, pazopanib, lapatinib, and dabrafenib.

Table 3

A – Selected Clinical Trials that feature a Basket Trial Design						
Trial ^(REF) & Identifier(s)	Study Design	Malignancy Type	Predictive Biomarkers	Drug Therapy	Platform Utilized	Primary Endpoints
SIGNATURE [£] NCT01833169 NCT01981187 NCT02002689 NCT01885195 NCT02187783 NCT02160041 NCT02186821 NCT01831726	Non-Randomized Phase II	Advanced Solid Tumors Multiple Myeloma B-Cell Non-Hodgkin Lymphoma	--- PI3K BRAF V600 PTCHI, SMO RAS, RAF, MEK CDK4, CDK6 FGFR ALK, ROS1 FGFR, PDGFR, VEGF, cKIT, FLT3, CSFR1, Trk, RET	--- BKM120 LGX818 LDE225 MEK162 LEE011 BGJ398 Ceritinib Dovitinib	Indicated tumor biomarkers are identified via a CLIA-certified laboratory diagnostic test prior to trial enrollment	Rate of Clinical Benefit
CUSTOM ¹²⁰ NCT01306045	Non-randomized Phase II	NSCLC, SCLC, Thymic Carcinoma	EGFR KRAS, BRAF, HRAS, BRAF PIK3CA, AKT, PTEN KIT, PDGFRA HER2	Erlotinib Selumetinib MK2206 Sunitinib Lapatinib	Pyro-sequencing Sequenom Mass-Array NGS FISH	ORR, OS
SHIVA ¹²¹ NCT01771458	Randomized Phase II	Refractory Metastatic Solid Tumor	KIT, ABL1/2, RET PI3KCA, AKT1/2/3, mTOR, RAPTOR, RICTOR, PTEN, STK11, INPP4B BRAF PDGFRA/B, FLT3 EGFR HER2 SRC, EPHA2, LCK, YES1 ER, PR AR	Imatinib Everolimus --- --- Vemurafenib Sorafenib Erlotinib Lapatinib + Trastuzumab Dasatinib Tamoxifen or Letrozole Abiraterone	IHC NGS Gene copy number alterations	PFS
CREATE NCT01524926	Non-Randomized Phase II	Anaplastic Large Cell Lymphoma Inflammatory Myofibroblastic Tumor Papillary Renal Cell Carcinoma Type I Alveolar Soft Part Sarcoma Clear Cell Sarcoma Alveolar Rhabdomyosarcoma	ALK, MET	Crizotinib	Diagnostic testing is not required for this trial	ORR
My Pathway NCT02091141	Non-Randomized Phase II	Advanced Solid Tumor	EGFR HER2 BRAF SMO, PTCHI	Erlotinib, Pertuzumab, Trastuzumab Vemurafenib Vismodegib	CLIA-certified laboratory diagnostic test	ORR

A – Selected Clinical Trials that feature a Basket Trial Design						
Trial ^(REF) & Identifier(s)	Study Design	Malignancy Type	Predictive Biomarkers	Drug Therapy	Platform Utilized	Primary Endpoints
VE-BASKET ¹²² NCT01524978	Non-Randomized Phase II	BRAF V600E-mutated advanced solid tumors	BRAF	Vemurafenib (in combination with cetuximab in patients with colorectal cancer)	Targeted Genotyping via Companion Diagnostic	ORR
GSK BRAF-V600E NCT02034110	Non-Randomized Phase II	Various BRAF V600E positive tumors	BRAF	Dabrafenib, Trametinib	Local CLIA-certified laboratory diagnostic test and Centralized Companion Diagnostic Test	ORR

B – Selected Clinical Trials that feature an Umbrella Trial Design						
Trial ^(REF) & Identifier(s)	Study Design	Malignancy Type	Predictive Biomarkers	Drug Therapy	Platform Utilized	Primary Endpoints
ALCHEMIST NCT02194738, NCT02201992 NCT02193282	--- Screening, Randomized Phase III	Stage IB-III/IA NSCLC Adjuvant Setting	--- --- ALK fusion EGFR Exon 19 deletion or L858R	--- --- Crizotinib Erlotinib	--- --- FISH Direct Sequencing	OS
BATTLE-2 ¹²³ NCT01248247	Randomized Phase II	Refractory NSCLC	Expression Analysis - pAKT, PTEN, HIF-1 α , LKB1 Mutation Analysis - PI3KCA, BRAF, AKT1, HRAS, NRAS, MAP2K1, MET, CTNNB1, STK11	Erlotinib Erlotinib + MK-2206 Selumetinib + MK-2206 Sorafenib	IHC, NGS Mutation analysis (Sequenom), mRNA pathway activation (Affymetrix), Protein profiling	8 week DCR
ISPY-2 ¹²⁴ NCT01042379	Randomized Phase II	Non-metastatic Breast Cancer Neoadjuvant Setting	Baseline Assessment – ER, PR, HER2, and Mammagraprint status Exploratory Biomarkers - Hsp90, HER2, HER3, IGF1R, PI3K, AKT, MAPK, MEK, cMET, mTOR	PT-AC AMG386 $\pm$ Trastuzumab Ganitumab + Metformin MK-2206 $\pm$ Trastuzumab T-DM1 + Pertuzumab Pertuzumab + Trastuzumab Ganetespib ABT-888 Neritinib PLX3397 Pembrolizumab + Paclitaxel	IHC FISH Mammagraprint	pCR
LUNG-MAP NCT02154490	Randomized Phase II/III	Squamous Cell Lung Carcinoma (Stage IV and Recurrent)	PIK3CA CDK4, CDK6, CCND1/2/3 FGFR1/2/3 HGF/cMET	Taselisib, Palbociclib, AZD4547 Rilotumumab, Erlotinib Nivolumab, Ipilimumab, Docetaxel, Durvalumab	Tissue submitted to Foundation Medicine for broad platform CLIA biomarker profiling	PFS

B – Selected Clinical Trials that feature an Umbrella Trial Design					
Trial ^(REF) & Identifier(s)	Study Design	Malignancy Type	Predictive Biomarkers	Drug Therapy	Platform Utilized
NLM1 ²⁵ NCT02664935	Non-Randomized Phase II	NSCLC (Adenocarcinoma or Squamous Cell Carcinoma)	FGFR2/3 mTORC1/2, LKB1 CDK4, CDK6, p16, KRAS, CCND1 ALK, MET, ROS1 NRAS, NF1 PI3K, PIK3CA, AKT, PTEN EGFR (T790M) PD-L1 (no actionable mutation)	AZD4547 AZD2014 Palbociclib Crizotinib Selumetinib + Docetaxel AZD5363 AZD9291 MED14736	NGS panel of 28 genes (adaptable for new biomarkers)
SAFIR-02 -# NCT02299999 NCT02117167	Randomized Phase II	Metastatic Breast Cancer Metastatic NSCLC	mTOR FGFR AKT HER2, EGFR MEK VEGF, EGFR PD-L1 (no actionable mutation) AR PARP	AZD2014 AZD4547 AZD5363 AZD8931 Selumetinib Vandetanib MED14736 Bicalutamide Olaparib Standard Chemotherapeutic Agents <i>Erlotinib, Pemetrexed</i>	CGH array NGS
					PFS

C – Selected Trials that feature a Hybrid Trial Design					
Trial & Identifier	Study Design	Malignancy Type	Predictive Biomarkers	Drug Therapy	Platform Utilized
NCL-MATCH -# NCT02465060	Non-randomized Phase II	Advanced Solid Tumors, Lymphomas	EGFR/HER2 activating mutations EGFR T790M HER2 amplification ALK, ROS1 BRAF V600 BRAF fusions, NF1, GNAQ, GNA11 NF2 loss cKIT PIK3CA PTEN SMO or PTCH1 DDR2	Afatinib AZD9291 Ado-trastuzumab entansine Crizotinib Dabrafenib + Trametinib Trametinib Defactinib Sunitinib Taselsib GSK2636771 Vismodegib Dasatinib	NGS (4000 variants across 143 genes)
NCL-MPACT NCT01827384	Randomized Phase II	Advanced Solid Tumors	DNA Repair --- PI3K RAS, RAF, MEK	Temozolamide + ABT-888 Carboplatin + MK-1775 Everolimus Tremetinib	NGS-based mutation-detection assay
TAPUR NCT02693535	Non-Randomized Phase II	Advanced Solid Tumors, Multiple Myeloma, B-Cell Non-Hodgkin Lymphoma	VEGFR Bcr-abl, SRC, LYN, LCK ALK, ROS1, MET CDKN2A/p16, CDK4, CDK6 CSF1R, PDGFR, VEGFR	Axitinib Bosutinib Crizotinib Palbociclib Sunitinib	Genomic or IHC test integrated with TAPUR platform - €
					ORR or PFS

C – Selected Trials that feature a Hybrid Trial Design					
Trial & Identifier	Study Design	Malignancy Type	Predictive Biomarkers	Drug Therapy	Platform Utilized
			mTOR, TSC EGFR BRAF V600E HER2 PTCH1 KRAS, NRAS, BRAF Bcr-abl, SRC, KIT, PDGFR, EphA2, FYN, LCK, YES1	Temsirolimus Erlotinib Vemurafenib + Cobimetinib Trastuzumab + Pertuzumab Vismodegib Cetuximab Dasatinib	

£ SIGNATURE is separated into several registered clinical trials, each designed to assess one targeted therapeutic.

¥ For the Biomarkers and Drug Therapy – Bold Text Indicates for both Breast Cancer and NSCLC, Italicized Text Indicates for NSCLC Only, Normal Text Indicates for Breast Cancer Only.

It is anticipated that more targeted therapeutics will be included in this trial.

€ CLIA approved, CAP-accredited, or NIH Genetic Test Registry diagnostic tests are obtained outside of the protocol at the discretion of the patient’s clinical oncologist. The patient’s clinical oncologist selects appropriate therapy (on or off protocol) with the option to consult a Molecular Tumor Board. (More information provided at <http://www.clinicaltrials.gov> or <http://www.tapur.org>)

PI3K: Phosphoinositide 3-Kinase; PTCH1: Protein patched homolog 1; SMO: Smoothed, frizzled class receptor; CDK: Cyclin Dependent Kinase; MEK: MAP/ERK Kinase; FGFR: Fibroblast Growth Factor Receptor; ALK: Anaplastic Lymphoma Kinase; PDGFR: Platelet-Derived Growth Factor Receptor; VEGF: Vascular Endothelial Growth Factor; FLT3: Fetal Liver Tyrosine Kinase 3; CSFR1: Colony Stimulating Factor 1 Receptor; Trk: Tyrosine Kinase; CLIA: Clinical Laboratory Improvement Amendments; NSCLC: Non-Small Cell Lung Cancer; SCLC: Small Cell Lung Cancer; EGFR: Epidermal Growth Factor Receptor; PIK3CA: Phosphoinositide 3-Kinase Catalytic Subunit Alpha; ER: Estrogen Receptor; PR: Progesterone Receptor; AR: Androgen Receptor; PTEN: Phosphatase and Tensin Homolog; PDGFRA: Platelet-Derived Growth Factor Receptor Alpha; HER2: Human Epidermal Growth Factor Receptor; NGS: Next-Generation Sequencing; FISH: Fluorescent *In Situ* Hybridization; ORR: Overall Response Rate; OS: Overall Survival; mTOR: Mammalian Target of Rapamycin; RAPTOR: Regulatory-associated Protein of mTOR; RICTOR: RPTOR Independent Companion of mTOR; STK11: Serine/Threonine Kinase 11; INPP4B: Inositol Polyphosphate-4-Phosphatase, Type II B; IHC: Immunohistochemistry; PFS: Progression Free Survival; HIF-1α: Hypoxia-Inducible Factor 1α; LKB1: Liver Kinase B1; DCR: Disease Control Rate; Hsp90: Heat-Shock Protein 90; IGF1R: Insulin-like Growth Factor Receptor; MAPK: Mitogen-activated Protein Kinase; HGF: Hepatocyte Growth Factor; PT-AC: Paclitaxel and Trastuzumab followed by Doxorubicin and Cyclophosphamide; pCR: rate of pathological Complete Response; mTORC: Mammalian Target of Rapamycin Complex; NF1: Neurofibromatosis Type 1; PARP: Poly (ADP-ribose) Polymerase; CGH: Comparative Genomic Hybridization; PD-L1: Programmed Death Ligand 1; GNAQ: Guanine Nucleotide-Binding Protein, Q Polypeptide; GNA11: Guanine Nucleotide Binding Protein, Alpha 11; DDR2: Discoidin Domain Receptor 2; VEGFR: Vascular Endothelial Growth Factor Receptor; TSC: Tuberous Sclerosis Complex.