PART I
Principles of Targeted Therapies
CHAPTER 1

Toward Personalized Therapy for Cancer

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Introduction

Personalized cancer care is grounded in the principle that the patient’s genotype and molecular characterization of a tumor and its microenvironment can identify the most effective cancer management for each patient while reducing toxicity. By tailoring therapy to a specific tumor, the approach of personalized cancer therapy is expected to save critical treatment time and healthcare costs by avoiding the selection of less beneficial therapies. Thus, the objective of personalized cancer therapy is to harvest information about the tumor—its DNA, RNA, proteins, and metabolism—within the context of the tumor microenvironment and the patient’s genotype to inform treatment decisions. However, much work remains to be done before this concept can be translated from the research environment to the clinical setting.

Several complementary components are necessary to achieve personalized medicine throughout the continuum of cancer care (Figure 1.1). The first phase of personalized cancer care includes risk assessment, in order to identify patients at higher cancer risk, appropriately modifying screening strategies and frequency, and offering preventive strategies. Once a cancer diagnosis is made, the care of the patient enters the second phase of personalized care—molecular characterization of the tumor to assess patient prognosis. Accordingly, patients at a high risk of recurrence can receive more intensive therapy, while patients at low risk may receive less toxic systemic therapy or may avoid additional therapy altogether.

The third phase in personalized care involves in-depth molecular characterization of the tumor to identify potential therapeutic targets and to test for established and putative predictive markers, that is, markers predictive of response to specific therapies. Markers predictive of adverse events can be used to select regimens with the least toxicity. Early response to therapy may be monitored with pharmacodynamic markers of response.

Furthermore, as efficacy of treatment for recurrent disease improves, a growing role for biomarkers is likely to develop in monitoring early recurrence and providing a personalized program for survivorship. Although currently standardized follow-up schedules based on cancer histology and stage exist for most cancer types, more precise determination of expected prognosis (i.e., likelihood of recurrence) based on molecular subtype would personalize cancer follow-up, including the frequency of follow-up visits and the need for specialist follow-up. As many cancer treatments have long-term unintended effects, personalized survivorship programs can offer more intensive screening for patients at higher risk of developing these side effects.

Personalized Targeted Therapy

Principles of Molecular Therapeutics

Even in therapy-sensitive cancers such as breast cancer, only a subgroup of cancer patients achieve a pathologic complete response with currently available standard chemotherapy, underscoring the need to develop novel targeted therapies. Therefore, an important component of personalized therapy is the delivery of individualized “targeted” therapy, directed toward molecular aberrations in specific tumors. The principle of molecular therapy is to target molecular differences between cancer cells and normal cells. To implement molecular therapeutics, targets must first be identified using genomic and proteomic techniques. Notably, numerous differences exist between cancer cells and normal cells, differentiating between cancer “drivers” that play a key role in cancer progression and survival and “passengers” that are present but not critical for cancer maintenance. Furthermore, “hitchhiking” mutants conferring survival advantage to the cancer cell are much more common than driver mutations, leading to challenges in developing effective therapeutic targets. Thus, the molecular therapeutic approach allows a tailor-made strategy to target specific tumor characteristics, while using a drug that does not target normal cells.

Predictors of Response for Patient Selection

In addition to the need for compelling therapeutic targets, drugs that inhibit the identified targets, ideally through selective inhibition, are necessary to minimize off-target toxicity. Biomarkers to detect the presence of the target within the tumor are employed to select patients who will benefit from the targeted therapy. Often, the presence of the target is pursued as a potential predictive marker; however, expression of the target itself may not be sufficient to
### Figure 1.1 The cancer care continuum for personalized medicine.

#### Pharmacodynamic Markers of Response

Early in drug development, pharmacodynamic markers of biological effect must be discovered to determine whether the putative target is inhibited by the novel therapeutic agent and to measure the extent of target inhibition and downstream signaling inhibition. Biological inhibition of the target can be assessed in surrogate tissue samples, such as skin biopsies, hair follicles, peripheral blood mononuclear cells, or platelets. However, ultimately there is value added in determining the effect of the drug on tumor cells by obtaining pre-treatment and on-treatment biopsies.

Another important goal for molecular therapeutics is the development of early biomarkers of response. The traditional approach to assessing response in clinical trials has been to treat patients for two to three cycles and then evaluate treatment response with repeat imaging. However, with the implementation of targeted therapies, the discovery of pharmacodynamic markers of response that can assess response earlier would spare patients from unnecessary toxicity, save the healthcare system the cost of administering ineffective therapy, and facilitate the transfer to alternate therapeutic regimens without further disease progression. Through assessment of biomarkers pre-treatment and on-treatment with repeat biopsies, pharmacodynamic markers of response within the tumor can be examined after only one cycle of therapy or even earlier; likewise, the biopsy assessment would permit correlation with radiographic response or clinical benefit on standard response assessment. In addition, an on-treatment biopsy can provide further information about adaptive responses to the current treatment. This insight can assist in planning future studies of rational combinatorial therapy. An area yet to be explored is the use of individual adaptive responses to personalize combination therapies chosen.

Although obtaining pre- and on-treatment biopsies to assess pharmacodynamic markers of response is theoretically appealing, this process presents several challenges. One barrier to early...
assessment of treatment response is that measurement of target inhibition itself may be difficult. Pathway activation is often determined through assessment of phosphorylation of downstream mediators, and phospho-specific residues are known to be relatively unstable. The acquisition of a biopsy may also change the readout of the pathway and cell proliferation. Cold ischemia time and intratumoral heterogeneity of the specimen can alter the measurable targets within the sample. There are no widely accepted approaches for quantitative assessment of downstream signaling, though IHC, reverse-phase protein array (RPMA), enzyme-linked immunosorbent assay (ELISA), and bead-based multiplex proteomics are all currently utilized, they have limitations. To minimize variability in assessment of treatment response, researchers and clinicians must collaborate to optimize and standardize specimen collection and assay selection for each desired target within a tissue type. Another valid concern is the significant cost added to clinical trials by pre- and on-treatment biopsies. Further, these biopsies introduce additional problems, such as biopsy quality and potential increased morbidity. Despite the increasing number of early trials incorporating biopsies for pharmacodynamic assessment, only a small fraction of phase I trials that included biomarkers made use of the biomarker results for dose selection. Some have proposed that if the drug does not show preliminary evidence of antitumor efficacy in an early trial, the biomarkers will be uninformative. However, even if antitumor efficacy is not observed, pharmacodynamic assessment may serve other important roles, such as determining whether there was lack of or insufficient target inhibition. These results could uncover the need to modify treatment dose or schedule. Furthermore, if there were inhibition of target but with inadequate treatment response, the information gathered from the biopsy could suggest that the target may not be the primary driver in that tumor type or that there may be alternate resistance mechanisms within the tumor.

Early Successes in Personalized Therapy

Despite the challenges to biomarker selection, targeted therapy development, and treatment response assessment, the field of personalized cancer therapy has generated early successes incorporating biomarkers and targeted therapies to transform cancer treatment.

Prognostic Stratification and Prediction of Chemotherapy Benefit in Hormone Receptor-Positive Breast Cancer

Several RNA-based prognosticators have recently been developed. Two of these commercially available multi-marker assays for breast cancer prognostication are notable as they are widely utilized. In two independent analyses of phase III clinical trials, one in node-negative and one in node-positive breast cancer with tamoxifen-alone control arms, the Oncotype Dx (Genomic Health) RT-PCR-based 21-gene recurrence score was shown to identify a group of patients with low recurrence scores, who do not appear to benefit from chemotherapy and a second group, with high scores, who do benefit from chemotherapy. The role of chemotherapy in breast cancer patients with hormone receptor-positive, node-negative, intermediate recurrence score tumors and hormone receptor-positive, node-positive, low and intermediate recurrence score tumors is being assessed prospectively in the TAILORx and RxPONDER studies, respectively. In a non-randomized clinical setting, the MammaPrint 70-gene signature was shown to be prognostic in node-negative and 1–3 node-positive patients and to predict chemotherapy benefit in the high-risk group. The MammaPrint is being prospectively validated in the large adjuvant MINDACT (Microarray In Node-negative Disease May Avoid Chemotherapy) clinical trial. For both Oncotype and MammaPrint assays, the discordance rates between the assay prediction and clinical-pathologic risk categories are approximately 30%. Clinical utility studies demonstrate that assay use results in a change in treatment decision in 25–30% of cases, most commonly from chemo-endocrine therapy to endocrine therapy alone. The widespread use of these tools in clinical practice suggests that clinicians not only are seeking prognostic tools to assist in counseling patients and treatment planning but also are willing to modify their clinical practice to incorporate new technological adjuncts.

HER2-Targeted Therapy in Breast Cancer

Twenty percent of breast cancers display HER2 amplification, which is associated with a poorer prognosis compared to those without HER2 overexpression. However, treatment of these breast cancers in the adjuvant setting with trastuzumab, a monoclonal antibody targeting the extracellular domain of the protein encoded for by HER2, has been shown to improve survival in both early stage and metastatic breast cancers with HER2 amplification. This initial success was rapidly followed by development of additional anti-HER2 therapies such as lapatinib, pertuzumab, and T-DM1. Even still, many HER2-positive tumors do not respond to HER2-targeted therapy, suggesting that additional biomarkers are needed to predict intrinsic resistance and the emergence of acquired resistance.

BRAF Inhibitors in BRAF Mutant Melanoma

B-Raf is a member of the Raf kinase family of serine-threonine kinases that activates the MAP/ERK signaling pathway. Mutations in BRAF have been detected in 40–60% of melanomas. Less than a decade after the development of the RAF inhibitor vemurafenib, a phase III randomized trial confirmed that the BRAF V600E mutation was a response-specific predictive biomarker for treatment of melanoma with vemurafenib. Patients with therapy-naive metastatic melanoma harboring the BRAF V600E mutation had significantly longer progression free and overall survival when treated with vemurafenib compared to standard chemotherapy. The rapid clinical development of B-Raf inhibitors exemplifies how molecular identification of a driver aberration can be rapidly translated into a clinically effective therapy. However, in spite of the impressive response rates (48% for vemurafenib compared with 5% for dacarbazine), responses were short-lived, demonstrating the need for combinatorial therapy with other drugs or immunotherapy to obtain durable responses.

Strategies for Comprehensive Molecular Characterization

With the advent of high-throughput technologies, interest in the utilization of multmarker technologies to assist in tumor molecular classification and selection of optimal personalized therapy has intensified. A brief summary of strategies commonly utilized for comprehensive molecular characterization is provided below and in Table 1.1.
### Table 1.1 Strategies for comprehensive molecular characterization.

<table>
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<tr>
<th>Technology</th>
<th>Detection target</th>
<th>Tissue requirement</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>DNA Hot spot mutation testing</td>
<td>Single nucleotide variations</td>
<td>Blood, fresh/frozen tissue or FFPE</td>
<td>Minimal DNA required</td>
<td>Limited to hot spot mutations assayed</td>
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<tr>
<td>DNA Targeted gene sequencing</td>
<td>Mutations in candidate genes</td>
<td>Fresh/frozen tissue or FFPE</td>
<td>Cost effective</td>
<td>Higher throughput</td>
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<tr>
<td>DNA Whole exome and genome sequencing</td>
<td>Mutations</td>
<td>Fresh or high-quality frozen tissue</td>
<td>Complete sequencing of open reading frames</td>
<td>Larger amount of tissue required</td>
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<tr>
<td>DNA DNA methylation screening</td>
<td>Methylation</td>
<td>Blood, fresh or high-quality frozen tissue</td>
<td>Valuable target discovery</td>
<td>Must differentiate germline SNPs from somatic mutations</td>
</tr>
<tr>
<td>RNA Quantitative PCR</td>
<td>Relative gene expression</td>
<td>Blood, fresh or high-quality frozen tissue</td>
<td>Monitor treatment effect on pathway expression</td>
<td>Quantitates relative to housekeeping gene</td>
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<tr>
<td>RNA Microarray-based gene expression profiling</td>
<td>Relative mRNA or miRNA expression</td>
<td>Blood, fresh or high-quality frozen tissue</td>
<td>Monitor treatment effect on pathway expression</td>
<td>Reproducibility of results due to sample preparation and type of platform</td>
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<tr>
<td>RNA RNA sequencing</td>
<td>Absolute RNA abundance, splicing variants, mutations, fusions</td>
<td>Fresh/frozen tissue</td>
<td>Monitor treatment effect on pathways</td>
<td>“Reads” are proxies for mRNA abundance</td>
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<tr>
<td>RNA Ribosome footprinting</td>
<td>Quantitate expression</td>
<td>Fresh/frozen tissue</td>
<td>Information on protein abundance regulation</td>
<td>Does not quantify proteins but only translation efficiency</td>
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<tr>
<td>Protein Stable isotopic labeling with amino acids in cell culture (SILAC)</td>
<td>Relative protein concentration</td>
<td>Fresh/frozen tissue or FFPE</td>
<td>High throughput</td>
<td>Isotopic labeling may not be feasible</td>
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<tr>
<td>Protein High-resolution tandem mass spectrometry RPPA</td>
<td>Absolute protein quantification</td>
<td>Blood, fresh/frozen tissue or FFPE</td>
<td>Cost effective</td>
<td>Dependent on calibration or reference standards</td>
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<tr>
<td>Protein Immunohistochemistry</td>
<td>Relative protein expression and activation</td>
<td>Fresh/frozen tissue or FFPE</td>
<td>Cost effective</td>
<td>Proteins must have high-quality antibodies available</td>
</tr>
<tr>
<td>Protein Metabolomics</td>
<td>Metabolite expression and pathway activation</td>
<td>Blood, urine, or fresh/frozen tissue</td>
<td>High throughput</td>
<td>Proteins must have high-quality antibodies available</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>Metabolite expression and pathway activation</td>
<td>Blood, fresh/frozen tissue</td>
<td>High throughput</td>
<td>Low throughput</td>
</tr>
</tbody>
</table>

Sample harvest conditions can alter results
Genomic Profiling

Much of the effort in biomarker discovery for personalized cancer therapy has been directed at genomic markers, in part because of targeted therapies entering the market with DNA-based predictive markers, such as BRAF V600E as a predictor of response to BRAF inhibitors, and also because of the recent advances allowing multiple genomic testing to be performed in a rapid, reproducible, and relatively cost-effective manner.

Recently, several high-throughput genotyping methods have moved into the Clinical Laboratory Improvement Amendment (CLIA) environment including the MassARRAY System (Sequenom), SNPShot technology (Applied Biosystems), and ion semiconductor sequencing (Ion Torrent Technology). Multiplex hot spot mutation testing, also referred to as high-throughput SNP genotyping, has many advantages: requiring minimal DNA, accommodating formalin-fixed paraffin-embedded (FFPE) tissue, processing multiple samples simultaneously, detecting mutations present in a small proportion (5%) of cells, and being relatively cost-effective. However, this technique is limited to evaluating only the hot spot mutations being assayed. Hot spot genotyping does not have the capability to provide full coverage of all tumor suppressor genes, to detect new mutations in known cancer-related genes, or to discover novel cancer-related genes.

In addition to high-throughput SNP genotyping, targeted sequencing has recently become available in the CLIA environment. Target enrichment allows for selective capture of genomic regions of interest (usually exomes) and subsequent sequencing of cancer-relevant genes, including actionable targets, and common mutations. This technique has several benefits: complete sequencing of genes, including tumor suppressor genes; directing analytical resources to the most relevant genes in a select panel (e.g., 200–400), and accommodating FFPE tissue. The drawbacks of targeted exome sequencing are the larger amounts of tissue required, the need to differentiate germline SNPs from somatic mutations, and the limitations of novel gene discovery resulting from the limited gene panel. Sequencing alone will also not capture other critical alterations such as epigenetic changes.

As the cost for whole exome sequencing (WES) and whole genome sequencing (WGS) has decreased, the utility of these approaches in personalized cancer therapy is now being explored. The advantage of these techniques is the comprehensive genomic analysis of the tumor, yielding mutational, gene copy number, and rearrangement data. This complete examination can detect changes resulting in oncogene activation or tumor suppressor gene inactivation, perhaps uncovering alterations in the exon or genome that are essential for the maintenance of the malignant phenotype. In addition, the genomic data harvested from WES and WGS can aid in the development of novel targeted therapies, and assist in the selection of currently available treatments likely to be most effective. However, the minimum quantity of DNA required is significantly greater than other genomic techniques, and WES/WGS analysis of FFPE samples is only being optimized now. Even still, WES and WGS are prone to high rates of false positive and false negative calls, especially in samples with low tumor cellularity, necessitating validation with additional technologies. Possible solutions to these concerns are creating a standardized algorithm for calling single nucleotide variants (SNVs) and stringent standards to assess the reliability of calls in the CLIA environment. Despite the development of tools to assist in calling SNVs, hurdles to incorporating WES and WGS in personalized cancer therapy involve predicting the functional impact of every mutation and prioritizing each mutation as a driver or passenger. In addition, the large amount of data generated from WES/WGS creates considerable challenges to the capacity and security of information storage, as well as to the timely turnaround of bioinformatic analysis.

Next-generation targeted sequencing and WES/WGS approaches also have the advantage of providing information on DNA copy number. Other high-throughput technologies being pursued to assess copy number alterations include comparative genomic hybridization, single nucleotide polymorphism arrays, digital karyotyping, and molecular inversion probes.\(^7\)\(^8\)

Epigenetic Profiling

Genomic technologies can detect genetic alterations that yield response-predictive biomarkers; however, the frequency of mutations in some cancers is quite low. An alternative to mutational analysis is epigenetic or DNA methylation screening. Epigenetic profiling of immortalized cancer cell lines can uncover associations between methylated genes and therapeutic sensitivity. Inactivation of DNA mismatch repair genes can be assessed using epigenetic tools that can then provide prognostic stratification for clinical application, such as CpG island methylator phenotype (CIMP) in colorectal cancer.\(^9\)\(^10\) In addition, methylation screening can detect activation of oncogenic signaling through the silencing of pathway signaling regulators. To detect mechanisms of resistance, methylation screening of a tumor pre- and post-treatment can identify epigenetic changes following chemotheraphy that may alter the anti-tumor efficacy of other agents, such as the use of the methylating agent temozolomide based on the methylation status of the MGMT promoter in glioblastoma.\(^9\)\(^11\)

Transcriptional Profiling

To produce an individualized signature of a patient’s tumor, gene expression profiling utilizes mRNA, microRNA, and non-coding RNA. This unique transcriptome can then be used for classification of unique molecular subtypes, prognostic assessment, and to predict therapeutic responsiveness of tumors. In addition, transcriptional profiling of cancers before and after neoadjuvant systemic therapy can provide crucial information about the effect of treatment on the regulation of pathways and biological processes, potentially revealing new targets for therapy.\(^12\)

Other technologies such as exon junction arrays and genome tiling arrays use probes to the expected splice sites for each gene, thus allowing detection of splicing isoforms. As interest in massive parallel sequencing of RNA (RNA-seq) has intensified, RNA-based technologies are continuing to evolve rapidly. Compared with traditional transcriptional profiling with microarray technology, RNA-seq has the ability to detect other abnormalities in the cancer transcriptome in addition to changes in RNA expression, including alternative splicing, novel transcripts, and gene fusion.\(^13\)\(^14\) Furthermore, RT-PCR-based multiplex assays, such as Oncotype Dx, that assess expression of selected RNA panels are likely to have sustained utility.

Proteomic Profiling

IHC is a well-validated tool to assess therapeutic biomarkers, such as the estrogen receptor in breast cancer. However, IHC has limitations as a low-throughput technology requiring larger amounts of sample and considerable clinical manpower and expense to process and interpret each biomarker of interest. An advantage of IHC is its visualization of the protein of interest within the tumor, providing information about intratumoral location and tissue morphology.
The development of a multiplex method for IHC could transport this worthwhile and validated tool into the realm of personalized oncology. Other assays, such as ELISA, and newer-generation assays, such as bead-based multiplexed proteomic assays, can allow for assessment of a panel of proteins but still present challenges regarding not only linear range and challenges in absolute quantitation but also scalability to a large sample set. Mass-spectrometry-based proteomics remains a powerful discovery tool. RPPA is a protein array design that allows the measurement of protein expression levels in a large number of biological samples simultaneously in a quantitative manner. Briefly, lysates from cell lines, tissue lysates, or biological fluids can be spotted onto reverse-phase protein microarrays and probed with a panel of high-quality, monospecific antibodies. RPPA is a relatively cost-effective, high-throughput method to identify cancer subtypes, resistance biomarkers, and functional pathways. One drawback of RPPA is that it is limited to proteins for which high-quality antibodies are available. Given that most biomarkers and drug targets are proteins, proteomics has an advantage over transcriptional profiling for monitoring therapeutic response, discovering novel targets, and exposing mechanisms of pathway resistance in a personalized manner. The proteomic signature can also guide treatment selection by stratifying tumors into molecular categories and allowing the clinical team to incorporate the most efficacious therapy into the treatment plan. Further, RPPA is mainly utilized through comparison of a sample with other samples in a set. Approaches to normalize expression of a sample compared to controls are needed to transition this approach from a discovery tool to a point-of-care assay. Large-scale validation of proteomic signatures as well as proteomic profiles must be achieved before proteomic profiles can enter widespread clinical use.

Metabolomics

Metabolomics utilizes mass spectrometry, nuclear magnetic resonance, and gas and liquid chromatography to reveal small molecule metabolites and metabolic pathway alterations essential for the maintenance of the malignant phenotype. Metabolic screening can also aid in the early detection of cancers, especially those for which screening is difficult, by assessing biomarkers not only in tumor tissues but also in patients’ body fluids. Alterations in mitochondrial metabolism, a characteristic of invasive cancer, can differentiate malignancy from normal tissues. *In vivo* metabolic screening for staging and monitoring cancer is achieved through PET imaging technology that capitalizes on the increased metabolic activity of malignant cells to expose residual disease and metastases. Alterations in imaging may also occur with different tumor subtypes. For example, mutations in the isocitrate dehydrogenase (IDH) genes are frequently found in gliomas. This results in the production of an oncometabolite, 2-hydroxyglutarate (2-HG), which can be detected noninvasively in gliomas with IDH mutations using magnetic resonance spectroscopy. Greater emphasis on big data, and sharing of clinically annotated high-throughput data, is likely to improve predictive algorithms.

### A Personalized Approach to Investigational Therapy Selection

Increasingly, genomic characterization directs patients to specific clinical trials targeting the aberrant gene product or downstream signaling pathways. Routine comprehensive testing of patients with advanced disease could facilitate faster delivery of effective therapies to patients, while enriching for patients with matched aberrations in targeted therapy trials and accelerating accrual in those trials. Comprehensive testing is of the greatest value to patients who are interested in participating in clinical trials, are potentially eligible for therapeutic trials, and are able to access a menu of actively accruing targeted therapy trials. Unfortunately, most patients have limited access to pathway-matched investigational targeted therapies due to lack of relevant trials or lack of availability in early clinical trials.

Standardized molecular testing in the CJI environment can facilitate a variety of phase II clinical trial designs (Figure 1.2). In one commonly used approach, a biomarker can be used for patient selection for treatment with an agent targeting that alteration or the downstream pathway activated by that alteration (Figure 1.2a), or for randomization between standard of care or the targeted therapy (Figure 1.2b). Alternately, the biomarker can be used for prospective stratification, but all patients may be treated in a single arm study with the targeted therapy (Figure 1.2e) or randomized to targeted therapy or standard therapy (Figure 1.2d). In umbrella trials, patients are allocated to one of several treatments based on their biomarker profile (Figure 1.2e). In adaptive trials, patients are initially randomly allocated but subsequently allocated by biomarkers linked to therapeutic approaches discovered in the initial part of the treatment (Figure 1.2f) (e.g., ISPY 2, BATTLE trials). One common strategy is to match patients to trials based on a chosen molecular aberration, such as enrolling patients with PIK3CA-mutant breast cancer in trials that have the presence of a PIK3CA mutation as an eligibility criterion (Figure 1.2a). However, biomarker assessment can also enrich trials without biomarker eligibility criteria through the enrollment of patients with pathway aberrations, for example, matching patients with PIK3CA mutations in trials with agents targeting the PI3K pathway. Ultimately, this technique may enhance the clinical benefit achieved for enrolled patients, as supported by a study in which matching treatment delivery to patient tumor genotype improved response rate even in early clinical trials. However, it should be noted that the response and clinical benefit rates observed in this study may not be representative of the general population due to small sample size and lack of randomization.

There is also increasing interest in treating individual patients through off-label use of drugs targeting an aberration approved for another indication or compassionate use of agents in clinical trials that do not fit the patient characteristics. Cancer centers need to determine ways to facilitate treatment of these patients on “N-of-1” studies (Figure 1.2g). N-of-1 trials use clincopathologic logic molecular characteristics to select an individualized therapy plan. The most significant challenge is in quantifying benefit of an N-of-1 effort; the test is of the process and not the individual biomarker/drug pairing. Clinical benefit has been suggested in “N-of-1” settings by measuring time-to-progression on the trial drug compared to time-to-progression on the most recent treatment.
Figure 1.2 Biomarker-driven clinical trials. (a, b) Biomarker-selected single arm trials may deliver targeted therapy to patients selected for a specific genomic alteration or other biomarker or may randomize patients with a biomarker to investigational therapy or standard of care therapy. (c, d) Patients may be stratified based on the biomarker status, and patients with or without the biomarker may receive the investigational therapy or they may be randomized between standard of care therapy and the investigational arm. (e) In umbrella trials, multiple biomarkers may be simultaneously assessed, and patients allocated to treatment based on biomarker status. (f) In adaptive trials, initially patients can be randomly allocated between treatment arms, but subsequently the allocation is adaptive, based on disease control or other short-term endpoints in each biomarker subtype for each therapy. (g) In N-of-1 trials, patients are given a therapeutic regimen assessed to match their genomic/molecular profile most closely.
Collaborations with industry are needed to access novel investigational drugs that may be of benefit in these trials. Biomarker-selected trials for rare alterations are especially challenging. Collaboration across institutions is necessary to enroll across a variety of institutions, usually leveraging local testing for enrollment. The efficiency of such trials is increased with the increasing utilization of "basket trials," trials that test the efficacy of agents either in a histology-independent manner, or by accruing a variety of tumor types, with planned analysis in disease-specific cohorts.

With increasing multiplex testing, patients are frequently found to have more than one actionable alteration. Novel strategies are needed to rapidly test novel combination therapies, either targeting more than one alteration at a time, or targeting one actionable gene and additional survival pathway shown to be associated with intrinsic or acquired resistance. The design of clinical trials inherently poses significant challenges not only to biomarker discovery but also to validating the benefit of targeted therapies. Phase I trials often contain heavily pretreated patients, adding additional complexity to the challenges of tumor heterogeneity and molecular evolution. Phase II trials can have small sample sizes with inadequate power for biomarker validation. In addition, to validate a biomarker, patients with and without the biomarker must be treated with a drug; however, this methodology also raises ethical concerns about treating patients in non-marker matched trials if strong rationale exists for the biomarker's predictive benefit. However, if genomic markers were used at the onset during the development of a drug and therapies were proven effective, studies in populations with and without markers still would have been of importance to determine the predictive value of a marker. Another challenge is that even in phase III trials of targeted therapies may yield few objective responders as many novel treatments are cytotoxic but not cytoreductive. One commonly utilized strategy for discovery in phase I, II, and III trials is to select patients for additional analysis through an unusual responder protocol; this method focuses efforts on patients who fail to respond as predicted or who achieve notably better results on a treatment regimen.

Challenges to Personalized Cancer Therapy

Although personalized therapy holds much promise, biomarker-based treatment is utilized in only a few cancer types at this time. Furthermore, no data has demonstrated that comprehensive molecular profiling provides added value for the patient or decreases healthcare costs. Before personalized therapy can be implemented, several challenges must be overcome; these obstacles are discussed below.

Tumor Heterogeneity

From variation among the proportion of cancer cells having specific mutations to variation among the types of mutations, tumors have considerable heterogeneity. Presently available multiplex technologies can detect mutations that exist in as few as 5% of a tumor's cells, in-depth sequencing may be able to detect even rarer tumors. It is currently not known whether a minimum proportion of tumor cells must contain a somatic mutation to observe an effect on tumor biology, response to targeted therapies, or resistance to alternate pathway inhibitors. The relative tumor cellularity also influences the "percentage mutant" through the relative proportion of normal DNA in the total DNA analyzed. In select cases with low tumor cellularity, microdissection of tumor cells may be necessary prior to genomic screening, resulting in markedly increased cost. Addressing this issue is critical to determining the genomic sequencing coverage-depth necessary to make clinical decisions.

Molecular Evolution

Biomarker assessment of archival tissue, typically the primary tumor specimen, often serves as the basis for patient treatment decisions. However, tumors evolve as the disease progresses, acquiring additional mutations that provide a growth or survival advantage and selecting for a population of subclones. In a study of pancreatic cancer metastases and matched primary tumors, sequencing revealed that the clonal populations that resulted in distant metastases were also represented in the primary tumor; however, these clones had genetically evolved from the parental non-metastatic clone. Furthermore, it is not yet known whether "founder mutations" that exist in parental clones or "progressor mutations" that arise following clonal evolution are better therapeutic targets. It is also unclear if the concordance of biomarkers between primary and recurrent tumors differ by cancer tissue of origin. In breast cancer, discordance in the standard of care markers—estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2 (HER2)—between the primary tumor and metastases has been observed and is associated with poorer outcomes. Similarly, there is discordance in immunohistochemical markers of phosphatidylinositol 3-kinase (PI3K) pathway activation, as well as PIK3CA mutational status between primary and recurrent breast cancers. Interestingly, the discordance observed is not only attributable to metastases acquiring additional aberrations but also from loss of aberrations that had been detected in the primary tumor. In contrast to PIK3CA mutation status in breast cancer, a high concordance in X-Ras status between primary tumors and matched liver metastases has been reported to inform the selection of samples for biomarker assessment, additional studies are needed to establish the concordance of key biomarkers among different cancer tissues of origin and different metastatic sites.

Cancer cells adapt and acquire resistance through several mechanisms upon prolonged treatment with targeted therapy. One method is through loss of the target, as observed in a study of breast cancer patients treated with neoadjuvant trastuzumab-based chemotherapy, on post-treatment biopsy, a third of the samples from patients who did not have a complete pathologic response now displayed loss of the HER2 amplification that had been present in their pretreatment biopsies. Another means by which cancers develop resistance is the acquisition of additional genomic aberrations. In lung cancer, a second mutation in EGFR (T790M) and MET amplification have been described as two mechanisms of drug resistance to the EGFR inhibitors erlotinib and gefitinib. Subpopulations of cells with MET amplification were identified even prior to drug exposure, suggesting that drug treatment effectively selects for these subpopulations. To reveal mechanisms of acquired drug resistance, sequential tumor biopsies and systemic genetic and histological analyses were performed in 37 patients with drug-resistant non-small cell lung cancers (NSCLC) harboring EGFR mutations. Across sequential biopsies, every tumor retained the activating EGFR mutations; some developed known mechanisms of resistance, including the EGFR T790M mutation and MET amplifications. Others displayed novel genetic changes, including EGFR amplification, PIK3CA mutations, and markers of epithelial-to-mesenchymal transition. Some tumors transformed into small cell lung cancers (SCLC) that were sensitive to standard SCLC treatments. Serial biopsies in three patients revealed that
Biomedical Informatics and Decision Support
An additional challenge to personalized cancer therapy is the bioinformatics and medical informatics capacity and medical decision support that must be leveraged to provide reliable results in a timely fashion and to supply the clinical team with accessible tools for designing treatment strategies. The rapidly growing field of bioinformatics must evolve with technology to develop standardized algorithms for calling SNVs, predicting functional impact of mutations, and prioritizing mutations to differentiate drivers from passenger mutations. Institutions must also build a medical informatics infrastructure to facilitate therapy selection and monitoring. Systems need to be built to analyze and inform the expected outcome for a patient treated with standard regimens based on their molecular profile. These teams must also monitor patient toxicity and the response to treatment of the primary tumor and any metastases, while analyzing that data in the context of the patient’s biomarker status. Medical decision support must provide clinicians with user-friendly software to identify and prioritize actionable targets revealed in biomarker assessment, while incorporating patient health information, prior treatment response, and relevant data from published literature. The personnel and technological needs to design and support these valuable clinical tools add substantial cost.

Resource Allocation
The implementation of personalized cancer medicine is a costly endeavor. In light of the current economic decline, policymakers and insurance providers are scrutinizing healthcare spending and rising costs. Although better validated and thus more reliable, CLIA testing for biomarker assessment is expensive. To contain costs and maintain accuracy, CLIA laboratories that perform large volumes of high-throughput testing, analysis, and validation must be designated as testing centers for the comprehensive tumor assessment that is critical to personalized cancer therapy. Academic cancer centers with governmental and philanthropic funding to support and advance biomarker testing must assume a leadership role in the personalized cancer therapy movement. These centers must maintain current grants and seek out additional funding sources to maintain ongoing research into targeted therapies and the clinical trials essential for their evaluation. Lastly, buy-in from insurance providers is crucial to the success of personalized cancer therapy, as partial or complete failure to reimburse for comprehensive testing and cancer treatment—including biomarker analysis, routine pre-, on-, and post-treatment biopsies, and novel targeted therapies—will hinder advancement of the field and restrict the personalization of treatment to the affluent minority.

Collaboration and Regulation
To execute cancer treatment in a personalized manner, academia and industry must collaborate on biomarker discovery and validation to develop novel targeted therapies and expedite their evaluation in clinical trials. Likewise, clinicians and researchers must connect in order to translate laboratory breakthroughs into predictive biomarkers, innovative treatment design. Increased studies on biomarkers of toxicity can also lead to reduction of patient toxicity. Furthermore, knowledge-sharing and collaborative training of clinical and research personnel will improve communication and effectiveness in the multidisciplinary team approach to cancer treatment. Clinical research committees and institutional review boards must recruit, maintain, and develop members with expertise in biomarkers, genomics, and proteomics, to ensure the safety of patients while genetic aberrations were lost after cessation of EGFR inhibitor treatment, yet these cancers then became sensitive to a second round of EGFR inhibitor treatment.

These studies emphasize the importance of ex-characterizing tumors at the time of relapse to identify mechanisms of resistance and to design more efficacious combinatorial therapies. Assessing cancers throughout the disease course introduces additional challenges: the cost associated with image-guided biopsies, concerns about biopsy quality, the morbidity of biopsies, and the understanding that all distant sites of metastasis may not develop identical mechanisms of resistance. To evaluate the molecular evolution of a patient’s cancer in a safer and more cost-effective manner, less invasive approaches—including biomarker analysis of circulating tumor cells or circulating free DNA and functional imaging—must be optimized for clinical application.

Strategies for Biomarker Analysis
The traditional approach to biomarker analysis is the point-of-care assessment of relevant biomarkers prior to treatment with a specific therapy, such as BRAF mutational screening to select treatments for metastatic melanoma. There are now increasing numbers of phase I and II trials accruing patients with specific somatic mutations, thereby necessitating pretreatment testing. Although the approach limits the quantity of biomarkers assessed, this strategy introduces delays in treatment initiation for patients. Even in highly efficient molecular diagnostic labs, the retrieval of archival tissue blocks often requires many days to weeks, especially if the samples are in an extramural location. After several weeks of waiting, many patients learn their tumors lack the necessary biomarker for trial eligibility and thus will have experienced an unnecessary treatment delay. This delay can be even more sizeable for biomarkers requiring WES or WGS, as the testing and analysis takes several weeks, well beyond a clinically acceptable window for biomarker turnaround time for treatment planning.

One strategy to prevent these delays in biomarker analysis is comprehensive multi-marker testing performed at the time of presentation, allowing for earlier determination of treatment options. However, this approach relies on the examination of archival blocks and thus may not encompass changes due to molecular evolution. Alternatively, point-of-care biopsies can be obtained, eliminating the time lost in the retrieval of archival tissue and potentially accounting for molecular evolution. This approach introduces additional costs and morbidity to obtain the biopsy and does not eliminate the delays in biomarker assessment.

Biopsy and Tumor Specimen Quality
Biopsy quality is another challenge to implementation of personalized oncology. Even with dedicated radiologists and pathologists, one study of lung cancer patients found that 16.7% of research biopsies are inadequate for biomarker assessment.16 This percentage may be even higher in other tumor types, in patients who have received several previous treatment regimens, and in the application to technologies requiring large DNA quantities and high tumor cellularity. Furthermore, as more patients receive effective neoadjuvant therapy, even surgical excisions may provide samples with minimal tumor cellularity. Surgical samples also deteriorate with prolonged specimen storage, resulting in lower-quality data even from robust DNA-based assays. These quality and quantity concerns underscore the need to develop biomarker analysis of minimally invasive samples, including circulating tumor cells, circulating free DNA and bone marrow micrometastases.
also not introducing delays to the approval of research protocols and clinical trials.

Summary

Personalized cancer therapy stems from the premise that comprehensive molecular characterization of the patient’s tumor will result in the most effective treatment design for each patient while minimizing individual toxicity. Utilizing current and developing assays, each tumor can be assessed for preventative, prognostic, and predictive biomarkers, while also uncovering novel actionable targets. Early successes, such as BRAF mutation screening of melanomas to select for treatment with BRAF inhibitors, have provided encouragement to a field facing significant challenges. Tumor heterogeneity and molecular evolution add considerable complexity to biomarker assessment and treatment design, while funding, personnel, and regulatory issues are a cause for concern at the institutional level. Although much must be accomplished before this model enters widespread use in the clinic, we must not be discouraged. Personalized cancer therapy long-term will not only be in the best interest of our patients but also financially advantageous. Our goal must be to treat each and every patient with the most effective treatment, the first time, to attain prolonged responses and ultimately to achieve cures.

References