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Implementation of Biomarker-Driven Cancer Therapy: Existing Tools and Remaining Gaps

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Abstract

There has been growing interest in biomarker-driven personalized cancer therapy, also known as precision medicine. Recently, dozens of molecular tests, including next generation sequencing, have been developed to detect biomarkers that have the potential to predict response of cancers to particular targeted therapies. However, detection of cancer-related biomarkers is only the first step in the battle. Deciding what therapy options to pursue can also be daunting, especially when tumors harbor more than one potentially actionable aberration. Further, different mutations/variants in a single gene may have different functional consequences, and response to targeted agents may be context dependent. However, early clinical trials with new molecular entities are increasingly conducted in a biomarker-selected fashion, and even when trials are not biomarker-selected, much effort is placed on enrolling patients onto clinical trials where they have the highest probability of response. We review available molecular tests and therapy discerning tools, including tools available for assessing functional consequences of molecular alterations and tools for finding applicable clinical trials, which exist to help bridge the gap between detection of cancer-related biomarker to the initiation of biomarker-matched targeted therapies.

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Introduction

The development of trastuzumab for human epidermal growth factor 2 (HER2)-positive breast cancer dramatically altered the outcome of patients with HER2-positive disease, providing a prime example for the success of biomarker-driven, personalized cancer therapy. Objective response rates for first line trastuzumab treatment in patients with and without HER2 gene amplification by fluorescence *in situ* hybridization (FISH) analysis were 34% and 7% respectively (Vogel *et al.*, 2002), illustrating the efficacy of targeted therapies in tumors harboring molecular aberrations predicted to mediate therapy sensitivity. Currently, over a dozen targeted therapies have been approved with companion diagnostic tests, assays that predict tumor response to therapy, aimed at assisting in the decision of which targeted therapy strategies should be utilized for specific patients. These include assessment of *BRAF* V600 mutations for administration of anti-RAF and anti-MEK therapies in melanoma and *BCR-ABL* gene fusions for the use of imatinib in treating chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL).

The implementation of personalized cancer therapy is based on the ability to detect a molecular biomarker that predicts prognosis, sensitivity or resistance to specific single agent or combination therapies, or specific therapy-associated toxicities. Recently, much of the ongoing effort has been directed at identifying predictive markers of benefit. The goal of this clinical approach is to deliver treatment regimens targeting the underlying molecular alterations for each tumor, putatively increasing the likelihood of response to the selected therapy. For example, original clinical responses of chemotherapy resistant non-small cell lung cancer (NSCLC) patients to anti-epidermal growth factor receptor (EGFR) small molecule inhibitors in patients selected based on expression of the EGFR in tumors showed a modest but statistically significant response rates of 10–20% (Shepherd *et al.*, 2005). This actually led to the clinical approval of these agents as single-agent therapy for chemotherapy-refractory metastatic NSCLC. However, subsequent studies of patients responding to therapy (the first successful unusual responder effort) uncovered activating EGFR kinase domain mutations within a subset of NSCLC tumors strongly correlated with clinical responses to EGFR tyrosine kinase inhibitors (TKIs) (Lynch *et al.*, 2004; Paez *et al.*, 2004; Pao *et al.*, 2004). Indeed, studies have now shown that prescreening NSCLC patients for activating *EGFR* mutations for anti-EGFR TKI therapy leads to a substantial increase in response rates (>50%) and overall survival (Inoue *et al.*, 2009; McDermott & Settleman, 2009; Sequist *et al.*, 2008; van Zandwijk *et al.*, 2007).

Several hundred targeted therapies are in clinical or late preclinical development, resulting in an urgent and growing need for companion diagnostic tests. The development of next generation sequencing (NGS) technology has facilitated multiplexed testing of genomic aberrations in one tumor sample, which could dramatically enhance test turnaround time and also allow for tissue sparing approaches for assessment of multiple alterations, thus, increasing therapeutic options while potentially reducing cost of testing. In this paper, we will link available molecular tests and biomarker-driven targeted therapies to tools that are available for helping match patients to targeted therapies and/or clinical trials.

What Are Biomarkers And How Are They Detected?

A biomarker is a measurable and quantifiable biological molecule, which can be a protein, DNA, RNA, or biological compound, which acts as an indicator for a specific biological state or condition. In the context of personalized cancer therapy, biomarkers are used to determine patient prognosis and predict which patients will have the highest likelihood of responding to selected therapies or have adverse side effects with particular therapies. Biomarker tests are currently being used to predict the likelihood of benefit which integrates both sensitivity and resistance to targeted therapies. For example, tumors with *BRAF* V600 mutations are highly sensitive to vemurafenib therapy (Chapman *et al.*, 2011; Flaherty *et al.*, 2010; Rubinstein *et al.*, 2010; Sosman *et al.*, 2012). Conversely, *BRAF* wild-type status in melanoma suggests possible resistance to vemurafenib therapy (Bollag *et al.*, 2010). In fact, BRAF inhibitors may actually enhance tumor growth in *BRAF* wild-type tumors (Hatzivassiliou *et al.*, 2010). Therefore, detection of the lack and presence of activating *BRAF* mutations are clearly critical to direct the use of vemurafenib therapy.

In the United States, molecular diagnostic tests to be used for patient care, as with all patient care-related tests, have to be performed using assays and laboratories that are highly accurate, reproducible and satisfy Clinical Laboratory Improvement Amendments (CLIA) regulations. Multiple CLIA approved assays exist for cancer diagnostics that can test for protein or nucleic acid (DNA and RNA) biomarkers. Table 1 lists examples of CLIA-approved commercially available biomarker assays and their associated therapies and tumor types that are available for personalized cancer therapy. Below, we review some of the common technologies used to detect protein expression, single nucleotide alterations, gene copy number changes, and chromosomal rearrangements.

Immunohistochemistry (IHC)

IHC is a technique used to detect protein or phospho-protein expression within a histological tissue specimen. This technique utilizes molecule-specific antibodies coupled to colorimetric dyes for visualization of labeled molecules under a microscope. IHC is commonly used for cancer diagnosis, such as detection of tumor-type relevant markers to establish tumor primary diagnosis or for tumor subtyping. However, IHC is also used as a companion diagnostic assay for treatment with certain targeted therapies (Table 1), as is the case with the use of estrogen receptor (ER), progesterone receptor (PR) and HER2 IHC assays before administering ER-targeted or HER2-targeted therapy.

In Situ Hybridization (ISH)

ISH is a technique that hybridizes a complementary DNA or RNA probe to a specific DNA or RNA sequence within a specimen, which in the clinical setting is typically an FFPE tissue slide. The most common hybridization probes used for this technique are either chromogenic (CISH) or fluorescent (FISH). The complementary hybridization probes can work to determine the number of copies of the gene present within the specimen, which is the case with HER2 FISH assays, as well as whether a genomic alteration exists within a specimen, which is the case with ALK fusion FISH assays.

DNA & RNA Sequencing

There are multiple DNA sequencing technologies being utilized for molecular diagnostics and personalized cancer therapy. These technologies range from the ability to sequence only select regions of one gene at a time, to the ability to obtain sequence and copy number of whole exomes of multiple genes or the entire genome. For the detection of single nucleotide variations within a single gene, polymerase chain reaction (PCR) followed by Sanger- or pyro-sequencing technologies are typically used. This strategy is only used when testing for known ‘driver’ mutations (mutations known to have tumor promoting and functional consequences). This has the advantage of identifying aberrations with known functional consequences but is limited in scope and ability to identify unexpected, but important abnormalities. In addition to this, real-time PCR strategies are now commonly used in companion diagnostic tests (Table 1) for the detection of multiple mutations within a gene, using primers that specifically recognize those mutations. This technique is limited to detecting alterations of known “hotspots”, recurrent mutations in cancer-related genes, or known chromosomal rearrangements.

NGS technologies are currently used for assessing inherited disease risk and are beginning to be more widely used for assessing acquired tumor-associated genetic driver abnormalities. Table 2 lists examples of NGS sequencing assays that are available for assessing cancer-related somatic and germline alterations. Several NGS assays are available for hotspot sequencing of multiple genes. Again, these assays are designed to test for known cancer-related mutations. For example, the Ion Ampliseq Cancer Hotspot Panel V2 available from Life Technologies Corporation (Grand Island, NY; Table 2) assesses around 2,800 hotspot mutations in 50 genes. Whole exome sequencing can be done for all genes or for a select gene panel using NGS technologies. Several of these approaches are now available in the CLIA environment. The advantage of sequencing the entire exomes of genes is the ability to detect rare and novel mutations that occur outside of specific, predefined regions. Whole genome sequencing is currently used for the detection of germline disease susceptibility abnormalities. However, this technology has the advantage of providing copy number as well as gene rearrangements in terms of somatic aberrations as well as germline events involved in cancer susceptibility suggesting that its use will likely increase.

Finally, RNA sequencing (RNAseq), or whole transcriptome sequencing, can provide information on gene expression, splicing patterns, RNA editing, gene rearrangements as well as gene mutations. RNAseq approaches have shown that many aberrations identified at the DNA level are not expressed at significant levels suggesting that they are not functionally relevant. RNAseq technologies are currently not frequently used for diagnostic or patient care purposes. However, it is conceivable that once RNA extraction from clinical diagnostic samples, particularly from formalin fixed paraffin embedded (FFPE) specimens, is optimized, this approach would be an ideal strategy to detect both losses and gains of multiple biomarkers as well as testing for multiple mutation biomarkers and rearrangements, all from a single sample.

Comparative Genomic Hybridization (CGH)

CGH is an oligonucleotide array where chromosomal abnormalities associated with over 270 genetic syndromes can be assessed (Ambry Genetics, Aliso Viejo, CA, and Baylor College of Medicine, Houston, TX). This technology uses multiple probes targeting genomic regions over the whole genome that are associated with genetic disorders traditionally assessed by cytogenetics or FISH. This approach can give copy number or chromosomal rearrangement alterations of large genomic regions over the entire genome, which can then be assessed further for genes located within these regions. This approach is currently designed for detecting inherited genomic abnormalities in patients with unexplained disorders. However, there likelihood that this technology will begin to bused for assessing global copy number changes and chromosomal rearrangements seen within sporadic tumors.

Transcriptional Profiling or Multigene Expression Panels

Transcriptional profiling with microarrays are assays in which complimentary DNA (cDNA) is amplified from mRNA, and is hybridized to a chip bound with single stranded complimentary DNA fragments. The hybridization intensity signifies the level of gene expression. Transcriptional profiling approaches MammaPrint (Agendia, Irvine, CA), 21 gene RNA panel Oncotype DX (Genomic Health, Carlsbad, CA), and 50 gene panel PAM50 (NanoString Technologies, Seattle, WA) are among the first multiplex diagnostic tests to be introduced into the CLIA environment. Similar approaches have been widely adapted for prognostication and prediction of chemotherapy benefit for breast, colon, and prostate cancers. Several other transcriptional profiles predictive of response to specific therapies have been proposed and are still undergoing clinical testing.

A Biomarker Has Been Detected, What Now?

Detecting a cancer-associated biomarker is only the first step in the process. If the biomarker is a common enough within a specific disease site and is associated with a Food and Drug Administration (FDA) approved targeted therapy, finding an appropriate therapeutic option is fairly straightforward. For example, *BRAF* V600E is the most common *BRAF* mutation in melanoma. In a recent retrospective study of 1112 cases of melanoma from The University of Texas MD Anderson Cancer Center, 45% of melanoma patients had a *BRAF* mutation, 75% of these were V600E mutations (Greaves *et al.*, 2013). Vemurafenib is currently FDA-approved for first-line treatment of *BRAF* V600E–mutant metastatic or unresectable melanoma. Therefore, in an instance where a *BRAF* V600E mutation is first detected within a metastatic melanoma patient, there is an FDA-approved targeted therapy strategy available. However, in cases where a *BRAF* V600 mutation is detected in a non-melanoma tumor, or if a novel *BRAF* gene mutation with unknown functional significance is detected within a melanoma, then therapy strategies begin to appear less straight forward. Although there may be a temptation to pursue off-label use of targeted therapies in some of these cases, the efficacy of drugs may be context dependent, as described below, thus therapy within relevant clinical trials would be preferable whenever possible.

Targeted Therapies

Targeted therapies are designed to interfere with specific molecules within cancer cells that are critical for cancer growth, survival, and/or invasion. Targeted therapy development is aimed at disruption of molecules that are selectively important for cancer cell biology but less critical for normal tissues. Therefore, the need for coupling molecular diagnostics to detect the presence of cancer-specific molecular alterations to assignment of appropriate targeted therapies becomes apparent. Table 3 describes FDA-approved targeted therapies and their disease indications within the United States. Interestingly, over half of these agents are associated with at least one molecular diagnostic test within indicated disease types.

The first step for determining a targeted therapy strategy is to determine if there is an approved targeted regimen applicable for a specific molecular alteration and/or patient disease type. There are currently around 16 targeted therapies that are FDA-approved to treat only about 9 molecular-subtype-specific diseases (Table 3). Off-label use of targeted therapies is defined by the use of an FDA-approved drug for an “off-label indication,” such as the use of the drug in a non-indicated disease type. However, even though there are well-established associations of molecular biomarkers to predict targeted therapy response in specific diseases, such as the association of *BRAF* V600E mutation to response to vemurafenib therapy in melanoma, it is preferable that it is demonstrated through clinical trials whether this biomarker- drug response association is scientifically applicable for other disease types. The cautionary use of this approach is illustrated by the finding that single agent vemurafenib has a less than 5% response rate in colorectal cancer patients with a *BRAF* V600E mutation (Kopetz *et al.*, 2010) .

Therefore, drug sensitivity to targeted therapies may be context dependent, influenced by underlying histology, other underlying genomic alterations or baseline activation of differing signaling networks. Thus, when a biomarker association is not proven for a given histology, it is preferable to treat patients on a clinical trial. Biomarker-drug sensitivity associations are increasingly tested across different tumor types in clinical trials. This includes proof-of-concept trials enrolling patients with specific biomarkers in histology independent trials, or “basket” trials enrolling patients from multiple tumor types with specific biomarkers, often with histology-specific cohorts as well as cohorts of “other tumor types.”

Functional Significance Tools

If an alteration of unknown clinical significance within a well-known cancer promoting gene is detected within a patient tumor, clinically applicable therapeutic options are less apparent. The first plausible scientific approach would be to determine if the detected alteration has a functional impact on protein function and/or tumorigenesis. There are several strategies currently used to obtain this information. The first strategy is the use of computational tools that can predict the functional significance of detected molecular alterations, namely single nucleotide variations. A second approach is a review of scientific literature for frequency and functional analysis that describe the *in vitro* and/or *in vivo* functional consequences of this variant. There are several databases that catalogue genetic mutations associated with cancer along with some predicted functional information (Table 4).

Computational Tools

Computational approaches can be used to identify mutations that alter protein structure and are more likely to be a driver mutation contributing to cancer initiation and progression, rather than a passenger mutation, with no significant oncological impact. The majority of the algorithms are based on the following features: (a) evolutionary conservation of the site, (b) physicochemical properties of the protein, (c) protein domains and (d) sequence context. Based on this understanding, a set of computational tools have been developed to represent these features and integrate them into scores that predict the functional impact of missense mutations. These algorithms have reasonable accuracy in predicting inactivating mutations but are challenged when faced with identifying activating mutations. Further, even the most predictive algorithms have significant false positive and negative rates making their use in patient management challenging.

SIFT—As one of the most widely used programs, SIFT (Sorting Intolerat From Tolerant) (Kumar et al., 2009; Sim et al., 2012) assumes that important positions in a protein sequence are conserved throughout evolution and substitutions at these positions may alter protein function. Thus, by measuring sequence homology among proteins across phylogeny or within families, SIFT can predict the effects of all possible substitutions at each position in any known protein sequences. The SIFT algorithm will predict if alteration is ‘deleterious’ (giving a score of 0–0.05) or ‘tolerated’ (a score of >0.05). When applied to a dataset of mutations found in affected individuals with disease (Ng & Henikoff, 2002), SIFT correctly predicted 69% of the substitutions associated with the disease to affect protein function. When applied to a second dataset consisting of non-synonymous SNPs (nsSNPs) in healthy humans, SIFT predicted only 19% of variants to affect protein function (Cargill et al., 1999). Thus, most of the variants in normal individuals are predicted to be tolerated. One limitation of the SIFT algorithm is that it does not use predictive protein structure features to assess the effect of an amino acid substitution. However, addition of a protein structure parameter only marginally improves the performance of prediction algorithms (Saunders & Baker, 2002).

Polyphen—Polyphen-2 (Polymorphism phenotyping version 2) (Adzhubei et al., 2010) includes 8 sequence-based and 3 structure-based predictive features in a naive Bayesian classification model, with parameters trained from the HumDiv and the HumVar datasets. The HumDiv dataset consists of 3,155 damaging alleles annotated in the UniProt database as causing human Mendelian diseases and affecting protein stability or function and 6,321 differences between human proteins and their closely related mammalian homologs, which were assumed to be non-damaging. The HumVar dataset consists of all the 13,032 human disease-causing mutations from UniProt and 8,946 non-damaging human nsSNPs without annotated involvement in disease. Polyphen reports if an alteration is predicted to be ‘probably damaging’ (0.85–1), ‘possibly damaging’ (0.2–0.85) or ‘benign’ (0–0.2). For a false positive rate of 20%, PolyPhen-2 achieved true positive prediction rates of 92% and 73% on training and test datasets, respectively.

MutationAssessor—MutationAssessor (Reva et al., 2011) was introduced to compute a functional impact score (FIS) for amino acid residue changes using evolutionary conservation patterns. It specifically addresses the distinction of driver versus passenger

mutations in human tumor tissues. Conservation patterns are derived from aligned families and sub-families of sequence homologs within and between species using combinatorial entropy formalism. The MutationAssessor score for an alteration can be 'high' (>3.5), 'medium' (1.9–3.5), 'neutral' (<0.8) or 'low' (0.8–1.9). The score performs well on a large set of human protein mutations in separating disease-associated variants (~19,200), assumed to be strongly functional, from common polymorphisms (~35,600), assumed to be weakly functional (area under the receiver operating characteristic curve of ~0.86). In cancer, using recurrence, multiplicity and annotation for ~10,000 mutations in the COSMIC database, the method assigns higher scores to more likely functional mutations, or drivers.

Condel—Condel (Consensus deleterious score) (Gonzalez-Perez & Lopez-Bigas, 2011), is an approach that integrates outputs from 5 tools (SIFT, Polyphen-2, Logre (Clifford et al., 2004), MAPP (Binkley et al., 2010), and MutationAssessor) into a unified classification model, based on a weighted average of normalized scores of each method. Condel reports whether an alteration is 'deleterious' or 'neutral' with score ranges of 0.468–1 and 0–0.468. Condel attains an accuracy of 88.2% for classifying HumVar and 89.6% for classifying HumDiv. It outperforms all individual tools and the other three integrated scores. This result suggests that integrating the output of multiple tools may improve the classification outcome of missense mutations.

CHASM—CHASM (Cancer-specific High-throughput Annotation of Somatic Mutations) (Carter et al., 2009) is the first approach that was designed to detect driver mutations in different types of cancer. It integrated 86 features using "Random Forests" (a machine learning algorithm) and achieved high sensitivity and specificity when discriminating between known driver missense mutations and randomly generated missense mutations. It outperformed previously described missense mutation function prediction methods at discriminating known oncogenic mutations in TP53 and EGFR.

CanDrA—CanDrA (Cancer-specific Driver missense mutation Annotation with optimized features) (Mao *et al.*, 2013) is a recent machine learning program that predicts cancer-type specific driver missense mutations based on 95 structural and evolutionary features computed by over 10 other functional prediction algorithms. CanDrA is trained based on the COSMIC database. For each missense mutation, it will predicts 'driver', 'no-call' or 'passenger' based on 2 scores: (1) CanDrA_GEN, representing how likely the mutation is a generic driver in all cancers and (2) CanDrA_CTS, representing how likely the mutation is a driver in a given cancer type. CanDrA has reported the lowest false discovery rate in annotating mutations from the cancer genome atlas (TCGA) and the cancer cell line encyclopedia (CCLE), compared to the methods described above.

Mutation Databases and Literature Mining

As mentioned, several existing databases provide the frequency of the variant within the population and give some predicted functional consequences of this variant based on similar computational tools listed above (Table 4). Unfortunately, many of these databases are limited by the lack of data for associations of gene variations with therapeutic response. Furthermore, these resources have rarely sufficiently mined the literature for scientific

support that the detected variation has proven functional consequences, and therefore, the impact of the gene variation may not be definitive. This, in fact, is a valid limitation due to the large effort needed to manually mine and research the scientific literature for this information.

Several commercial and independent entities have emerged over the last few years that function to manually curate literature describing both published functional consequence of variant as well as a review of the therapeutic literature linking gene variations to drug response (Table 5). Unfortunately, most of these services are not free publically accessible services, and gene-drug associations are not standardized across the field making literature interpretations from company to company somewhat subjective. Recently, My Cancer Genome released the DIRECT database cataloguing 188 primary EGFR mutations and 4 secondary mutations in lung cancer and their associations to clinical drug response (Table 4; Yeh et al., 2013), making it a valuable resource when assessing potential therapy options for EGFR-mutant lung cancer. This resource is currently limited to EGFR mutations in lung cancer, but has a goal to reach other gene mutations in other cancer types. Another resource worthy to mention is Free the Data browser (www.free-the-data.com). This resource is a consortium of organizations, managed by Genetic Alliance (Washington, DC), where patients and physicians can proactively share newly discovered *BRCA1/2* mutations along with patient clinical history, while protecting patient personal information. This information is then made publically available through the NCBI ClinVar Database (Table 4), where the information can help assess the deleterious nature of *BRCA1/2* alterations.

Nevertheless, given the speed at which new biomarker data and preclinical and clinical associations between biomarkers and therapeutic sensitivity is being generated, many of the existing database and manual curation approaches used are simply not robust enough to meet even the current clinical needs.

Tools for Finding Clinical Trials

At this time, only a select few molecular biomarkers are associated with FDA-approved targeted cancer therapy options (Table 3). Yet, current clinical gene panels sequence 50–400 genes or even the whole exome from patient samples (Table 2). Treatment of patients with genomic alterations in genes that do not have clear FDA-approved indications relies on selecting optimal investigational agents in clinical trials. How to identify and prioritize the most relevant clinical trials based on a patient's molecular profile is a problem that has yet to be solved.

To begin the prioritization process of identifying clinical trials, multiple alterations detected within the patient's tumors need to first be prioritized. In other words, each alteration needs to be attributed to some cancer promoting change in protein function. For example, mutations in codons 12, 13, and 61 of the *KRAS* gene have been well characterized as frequent tumor promoting alterations and would have much higher priority over mutations that are not in codons 12, 13, or 61, where cancer promoting effects are much less clear (Jancik *et al.*, 2010). Secondly, the altered gene needs to be associated with increased sensitivity/resistance to clinically applicable targeted therapies, where gene alteration to

drug response associations predicted by clinical studies have higher priority than those concluded from preclinical studies. For example, a recent phase II randomized study showed that the MEK inhibitor, selumetinib, and docetaxel combined therapy increased overall response rates and progression free survival in patients with *KRAS*-mutant NSCLC compared to the placebo control group (Janne *et al.*, 2013). However, only preclinical models are available showing increased sensitivity of *KRAS*-mutant NSCLC to mTOR inhibitor temsirolimus (Wislez *et al.*, 2005). Therefore, there should be a logical preference for matching a *KRAS*-mutant NSCLC patient to a trial using the MEK inhibitor, selumetinib, versus trials using mTOR inhibitors.

Once the molecular alterations and targeted therapies have been prioritized, these criteria can be used to query clinical trial databases for list of applicable clinical trials. Matching a patient with a specific tumor that harbors a specific molecular profile to an appropriate clinical trial can be done through: 1) searching for clinical trials that are enrolling patients with specific molecular alterations in a relevant tumor type, or 2) searching for clinical trials that use targeted therapies that demonstrate some increased drug response with the presence of gene alteration, and that are selecting for a relevant tumor type. Both of these strategies can be taxing and overwhelming for both a physician and the patient, especially the latter option which relies on sufficient expertise of the individual on all the potential gene alteration to drug response associations. There are several free and confidential clinical trial matching services that assist patients in finding applicable clinical trials based on their cancer type, location, and treatment history (Table 6). However, most of these services and databases are not designed to match tumors based on molecular profiles. An exception is the National Cancer Institute (NCI) Physician Data Query (PDQ) in which cancer type, location, and biomarker name/keywords can be added to the search criteria, allowing for the retrieval of only the clinical trials that mention a biomarker out of 12,000+ trials. Nevertheless, there is still quite a bit of personal curation that is left to the patient or physician.

Interestingly, several of the commercial and independent companies listed in Table 5 do provide a trial match service based on patients' cancer-type, treatment history, and molecular biomarkers. These services help patients and physicians to forgo the literature search necessary to prioritize molecular alterations and drug response associations, providing lists of relevant drugs and clinical trials that are customized for the patient's tumor type, treatment history, and molecular profile. However, as mentioned above, the limitation to these services is the lack of standardized prioritization of the alterations and drug response associations and subjective literature and study interpretations. In addition, these services may add to the already insurmountable cost of patient care. My Cancer Genome is a free service that does help to provide some of this customized therapy services by providing the possible functional consequences of the alterations as well as evidence on whether the alteration is associated with drug response. They also have curated lists of clinical trials that may be applicable for patients with specific cancer types harboring frequent molecular alterations. However, this browser is categorized based on disease types and frequent molecular alterations and not yet curated for rarer tumor types and less common genomic alterations.

Personalized Therapy: The Many Challenges That Remain

For optimal personalized therapy several challenges remain. Currently there are too few approved targeted therapies linked to specific biomarkers and further, the spectrum of targets being explored in preclinical studies is limited. Thus the majority of aberrations in patient tumors do not have a linked targeted therapeutic option. The increasing utilization of molecular testing is likely to tremendously expedite discovery of markers predictive of drug sensitivity and resistance as well as facilitate rapid enrollment into biomarker-driven trials. This suggests the growing clinical need for the design of basket trials, trials designed to assess the response of either a single genomic alteration in multiple tumor types or multiple genomic alterations in a single tumor type to targeted therapy, or N-of-one trials, where targeted therapies are given based on an individual tumor's genomic alteration(s) and individual therapy responses recorded. This will contribute to the larger consortia that are needed to conduct biomarker-driven trials for rarer alterations and in rarer diseases in order to expedite approval off effective drugs. Novel approaches are needed to facilitate access of patients treated outside of major academic centers to investigational therapies. Robust bioinformatics algorithms are needed not only to analyze large volumes of high-throughput data being generated on each patient, but to also make predictions on the functional impact for each alteration, classify drivers and passengers and prioritize different targets. Further, approaches are needed to determine the best approved or investigational therapy options that are available once putative drivers are identified and the level of evidence for their therapeutic relevance is weighed.

Conclusion

Within the past decade, personalized cancer therapy has become a powerful clinical strategy in oncology. A little less than a dozen molecular alterations have been correlated to clinical benefit from targeted therapies, leading to the FDA approval of targeted therapies for certain cancers harboring specific molecular alterations. With the development of NGS allowing for the detection of multiple genomic alterations, the need to scale and prioritize these gene alterations to drug response is pressing, especially when one tumor possesses multiple genomic alterations that have several therapy implications. Unfortunately, the current clinical infrastructure is not designed to support this massive and complex genomic- plus evidence- based medicine. Multiple tools have been or are being developed to help bridge these gaps. However, much work remains ahead in order to routinely implement personalized biomarker-driven therapy as the standard of care.

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Table 1

Selected Examples of Commercially Available Diagnostic Tests Associated Therapy Implication and Relevant Cancer Type.

Therapy Implications	Test	Cancer Type	Drug-Biomarker Clinical Association
IHC Assays			
Cetuximab; Panitumumab	EGFR	CRC	Established
Imatinib	C-KIT	GIST	Established
Trastuzumab	HER2	Breast Cancer; Gastric Cancer	Established
Resistance to PI3K, AKT, and MEK inhibitors	LKB1	NSCLC	Investigational (Mahoney *et al.*, 2009)
Crizotinib	C-MET	NSCLC	Investigational (Sadiq & Salgia, 2013)
Akt/mTOR Inhibitors; resistance to anti- EGFR therapies	PTEN	CRC, NSCLC	Investigational (Di Nicolantonio *et al.*, 2010; Sos *et al.*, 2009; Wang *et al.*, 2012)
***In Situ* Hybridization Assays**			
Crizotinib	ALK Fusion FISH	NSCLC	Established
Trastuzumab; Pertuzumab	HER2 FISH	Breast Cancer, Gastric Cancer	Established
Trastuzumab	HER2 CISH	Breast Cancer	Established
Trastuzumab	HER2 ISH	Breast Cancer	Established
Mutation Assays			
Cetuximab, Panitumumab	KRAS	CRC, NSCLC, Pancreatic Cancer	Established
Erlotinib, Gefitinib	EGFR	NSCLC, CRC	Established
Vemurafenib, Trametenib, Dabrafenib, Resistance to Anti-EGFR therapies	BRAF	CRC, Thyroid Cancer, Melanoma	Established
Imatinib; 2nd Generation TKIs	BCR-ABL	CML, Ph+ AML	Established
Crizotinib	ALK	NSCLC	Established
RAF and MEK inhibitors, resistance to anti- EGFR therapies,	NRAS	Melanoma, CRC, NSCLC	Investigational (Ascierto *et al.*, 2013; De Mattos-Arruda *et al.*, 2011; De Roock *et al.*, 2010; Huang *et al.*, 2013)
Imatinib	PDGFRA	GIST	Established
PI3K/mTOR Inhibitors	PIK3CA	Breast Cancer, CRC, Lung Cancer	Investigational (Di Nicolantonio *et al.*, 2010; Janku *et al.*, 2013)
Akt/mTOR Inhibitors; resistance to anti- EGFR therapies	PTEN	CRC, NSCLC, Breast	Investigational (Di Nicolantonio *et al.*, 2010; Jerusalem *et al.;* Sos *et al.*, 2009; Wang *et al.*, 2012)
Resistance to PI3K, AKT, and MEK inhibitors	LKB1	NSCLC	Investigational (Averette-Byers *et al.*, 2012)
Other			
Imatinib	BCR-ABL1 Quantitative Transcript Analysis	CML, Ph+ AML	Established
Resistance to Imatinib	BCR-ABL1 Copy Number	CML, Ph+ AML	Investigational (Hochhaus *et al.*, 2002)

Therapy Implications	Test	Cancer Type	Drug-Biomarker Clinical Association
PI3K Inhibitors	PIK3CA Amplification	Multiple Cancer Types	Investigational (Rodon *et al*.)
Erlotinib; Gefitinib; Cetuximab; Panitumumab	EGFR Amplification	NSCLC, CRC	Investigational (Gupta *et al.*, 2009)

The drug-biomarker clinical associations denoted ‘Established’ reflect well known drug FDA indications. The ones denoted ‘Investigational’ are associations that are hypothesized and demonstrated by scientific literature.

Table 2

Examples of Cancer Gene Panels.

Name	# Genes tested	Sequence Target	Company
Somatic			
Ion Ampliseq Cancer Hotspot Panel V2	50	Hotspot panel covering ~2,800 COSMIC mutations.	Life Technologies
Ion AmpliSeq™ Comprehensive Cancer Panel	406	Whole exons. Includes genes from the Cancer Hotspot Panel V2.	Life Technologies
OncoCarta Panel V1.0	19	Hotspot panel covering 238 mutations.	Sequenom Bioscience
Foundation One	255	Sequencing of 236 gene whole exons and 19 rearranged regions	Foundation Medicine
Engauge-cancer DLBCL Gene Panel	13	Hotspot panel of mutations in genes commonly associated with DLBCL disease.	Diagnovus
TruSeq Amplicon - Cancer Panel	48	Hotspot panel sequencing targeted with 212 amplicons.	Illumina
Human Comprehensive Cancer GeneRead DNAseq Gene Panel	124	Sequencing of whole exons of 124 cancer related genes and their 5' and 3' UTR regions.	Qiagen Manchester, Ltd.
Whole Exome Sequencing	Whole exome	Sequencing of the entire exome (~20,000), leaving out intronic and untranslated regions of the genome. This assay is used for assessing both germline and somatic mutations.	Baylor College of Medicine, Illumina, PGDx
Germline*			
Rapid Individual Sequencing Service	Whole genome	Sequencing of an individual's entire genome.	Illumina, PGDx
First –Tier and Clinical Diagnostic Exome Sequencing	Whole exome	First-Tier: sequencing of ~4,000 gene exomes; Clinical Diagnostic Exome: sequencing of ~20,000 gene exomes of family trios. This test is particularly designed for detection of genetic disorders to help explain clinical disease presentation.	Ambry Genetics
CancerNext	27	Genetic testing panel for hereditary cancers. Sequencing of whole exons plus 5 base pairs of 5'- and 3'-UTRs.	Ambry Genetics
BROCA	50	Cancer Risk panel of whole exons and flanking intronic regions.	University of Washington
ColoSeq	19	Genetic panel for hereditary colon cancer. Sequencing of whole exons and flanking intronic regions.	University of Washington
Hereditary Cancer Susceptibility Gene Panels	Variable	Customizable hotspot gene sequencing of ~31,000 clinically relevant variants associated with hereditary cancer-related/associated diseases	Invitae
Human Genotyping Array	23 chromosome pairs	Assays 1million reported SNPs that cover the entire genome on a DNA microarray BeadChip.	23andMe

DLBCL: Diffuse Large B-cell Lymphoma; UTR: Untranslated Regions; SNP: single nucleotide polymorphism; PGDx: Personal Genome Diagnostics Inc.

*Several other germline hereditary risk assays are currently clinically available than are listed in this table. However, the utility of many of these assays in assessing cancer-risk were not as apparent. Therefore, these assays were not further elaborated on.

Table 3

US FDA Approved Targeted Therapies and Indications.

Agent	Trade name	Target(s)	FDA-approved indication(s)	Company
Monoclonal Antibodies				
Ado-trastuzumab emtansine (T-DM1)*	Kadcyla	HER2	Breast cancer (HER2+)*	Genentech
Bevacizumab	Avastin	VEGF	CRC	Genentech
			GBM	
			NCLC	
			RCC	
Cetuximab*	Erbitux	EGFR	CRC (KRAS wild-type) *	Eli Lilly
			HNSCC	
Ipilimumab	Yervoy	CTLA-4	Melanoma	Bristol-Myers Squibb
Obinutuzumab	Gazyva	CD-20	CLL	Genentech
Panitumumab*	Vectibix	EGFR	CRC (KRAS wild-type) *	Amgen
Pertuzumab	Perjeta	HER2	Breast Cancer (HER2+)*	Genentech
Trastuzumab*	Herceptin	HER2	Breast cancer (HER2+)*	Genentech
			Gastric cancer (HER2+)*	
Small Molecule Inhibitors				
Afatinib*	Gilotrif	EGFR, HER2	NSCLC (with EGFR exon 19 deletions or L858R substitution)*	Boehringer Ingelheim
Axitinib	Inlyta	KIT, PDGFRβ, VEGFR1/2/3	RCC	Pfizer
Bosutinib*	Bosulif	ABL	CML (Philadelphia chromosome positive)*	Pfizer
Cabozantinib	Cometriq	FLT3, KIT, MET, RET, VEGFR2	Medullary thyroid cancer	Exelixis
Crizotinib*	Xalkori	ALK, MET	NSCLC (with ALK fusion)*	Pfizer
Dabrafenib*	Tafinlar	BRAF	Melanoma (with BRAF V600E mutation)*	GlaxoSmithKline
Dasatinib*	Sprycel	ABL	CML (Philadelphia chromosome positive)*	Bristol-Myers Squibb
			ALL (Philadelphia chromosome positive)*	
Denosumab	Xgeva	RANKL	Giant cell tumor of bone	Amgen
Erlotinib*	Tarceva	EGFR	NSCLC (with exon 19 deletions or L858R substitutions)*	Genentech & OSI
			Pancreatic cancer	
Everolimus*	Afinitor	mTOR	Pancreatic neuroendocrine tumor	Novartis
			RCC	
			Breast cancer (ER/PR+) in combination with exemestane*	
			Nonresectable subependymal giant cell astrocytoma associated with tuberous sclerosis	

Agent	Trade name	Target(s)	FDA-approved indication(s)	Company
Gefitinib	Iressa	EGFR	NSCLC with known prior benefit from gefitinib (limited approval)	Astrazeneca
Ibrutininb	Imbruvica	BTK	Mantle cell lymphoma	Pharmacyclics
Imatinib*	Gleevec	KIT, PDGFR, ABL	GI stromal tumor	Novartis
			Dermatofibrosarcoma protuberans	
			Multiple hematologic malignancies including Philadelphia chromosome-positive ALL and CML*	
Lapatinib*	Tykerb	HER2, EGFR	Breast cancer (HER2+)*	GlaxoSmithKline
Nilotinib*	Tasigna	ABL	CML (Philadelphia chromosome positive)?*	Novartis
Pazopanib	Votrient	VEGFR, PDGFR, KIT	RCC	GlaxoSmithKline
			Soft tissue sarcoma	
Regorafenib	Stivarga	KIT, PDGFRβ, RAF, RET, VEGFR1/2/3	CRC	Bayer
			Gastrointestinal stromal tumors	
Ruxolitinib	Jakafi	JAK1/2	Myelofibrosis	Incyte
Sorafenib	Nexavar	VEGFR, PDGFR, KIT, RAF	Hepatocellular carcinoma	Bayer
			RCC	
Sunitinib	Sutent	VEGFR, PDGFR, KIT, RET	GIST	Pfizer
			Pancreatic neuroendocrine tumor	
			RCC	
Temsirolimus	Torisel	mTOR	RCC	Wyeth
Trametinib*	Mekinist	MEK	Melanoma (with BRAF V600E or V600K mutations)*	GlaxoSmithKline
Vandetanib	Caprelsa	EGFR, RET, VEGFR2	Medullary thyroid cancer	Astrazeneca
Vemurafenib*	Zelboraf	BRAF	Melanoma (with BRAF V600 mutation)*	Roche

Acute lymphoblastic leukemia (ALL); Chronic myeloid leukemia (CML); Gastrointestinal stromal tumor (GIST); Estrogen receptor (ER); Progesterone receptor (PR); Non-small cell lung cancer (NSCLC); Colorectal cancer (CRC); Glioblastoma (GBM); Renal cell carcinoma (RCC); Head and neck squamous cell carcinoma (HNSCC); Chronic lymphoblastic leukemia (CLL); Bruton's tyrosine kinase (BTK)

*Targeted therapy that is associated with a molecular-specific cancer subtype alteration. There are approximately 17 targeted therapies that are associated with 10 molecular-specific subtypes of cancer.

Table 4

Databases Available for Assessing Functional Significance of Gene Mutations.

Name of Database	Description	Alteration Type	Web-link	Reference
The Roche Cancer Genome Database	A database that compiles somatic and germline variants from multiple databases including COSMIC and TCGA. This resource gives mainly frequency of variations and predicted functional consequences as determined by the SIFT functional annotation tool.	Somatic and Germline	http://rcgdb.bioinf.uni-sb.de/MutomeWeb/	(Kuntzer *et al.*, 2011)
Mutation of Kinases in Cancer (MoKCa)	A list of the commonly reported kinase mutations in cancer as reported in literature. Gives limited information on prevalence of variation, predicted functional consequence, and cancer types the mutation is commonly found.	Somatic	http://strubiol.icr.ac.uk/extra/mokca/	(Richardson *et al.*, 2009)
DNA-Mutation Inventory to Refine and Enhance Cancer Treatment (DIRECT)	Catalogue of clinically relevant mutations found in lung cancer, namely EGFR mutations. Program has goals to expand this to all known mutations.	Somatic	www.mycancergenome.org/about/direct	(Yeh *et al.*, 2013)
GEMINI: Integrative exploration of genetic variation and genome annotations.	A software program that integrates genetic variation information from multiple genome annotation databases into a unified database.	Somatic and Germline	No web interface	(Paila *et al.*, 2013)
My Cancer Genome	Freely available online resource for common molecular alterations within known cancer types Provides oncogenic properties of genomic alterations as well as potential therapeutic options.	Somatic	www.mycancergenome.org/	
The Human Gene Mutation Database	Catalogue for published gene lesions responsible for human inherited disease, including cancer-related disease. Data is obtained from scientific literature and analyzed by genetics experts. Broad database (>100,000 unique mutations in over 3,800 genes) that does provide predicted functional consequences of mutation. Catalogues only germline variants.	Germline	www.hgmd.org/	(Stenson *et al.*, 2013)
Human Genome Variation Society Locus-specific Databases (LSDBs)	The HGSV maintains a list of available LSDBs. This website allows you to search for your specific gene and refers you to the database which contains the mutational information. Data can include phenotype, functional data, family information, and frequency.	Somatic and Germline	www.hgvs.org/dblist/glsdb.html	(Johnston & Biesecker, 2013)

Name of Database	Description	Alteration Type	Web-link	Reference
	Catalogues both germline and somatic variants.			
Leiden Open Variation Database 3.0 (LOVD)	Contains information on 124,000 unique variants in 5175 genes. LOVD allows the user to search by either mutation type (substitution, insertion, deletion, or duplication) or by a specific variant. When looking up a specific variant, this database provides information regarding the codon, nucleotide change, amino acid change, link to PubMed reference, and prediction of either a deleterious or neutral effect.	Somatic and Germline	www.lovd.nl/3.0/home	(Fokkema *et al.*, 2011)
GeneReviews	Developed and maintained by the University of Washington. It is a collection of peer-reviewed inherited diseases. It contains phenotypic information and some clinical implications. Site focuses only on strongly implicated variations. Catalogues both germline and somatic variants.	Somatic and Germline	www.ncbi.nlm.nih.gov/books/NBK1116	(Johnston & Biesecker, 2013)
ClinVar	Archive of reports of the relationships among human variations and phenotypes. Catalogues both Germline and somatic variants.	Somatic and Germline	www.ncbi.nlm.nih.gov/clinvar/	(Johnston & Biesecker, 2013)
Online Mendelian Inheritance of Man (OMIM)	This database is curated at the Johns Hopkins University School of Medicine and began as a catalog of mendelian traits and disorders. This database includes 21,848 entries, 4926 with phenotypes of known molecular bases and 3003 genes with known causative mutations. Catalogues both germline and somatic variants.	Somatic and Germline	www.omim.org	(Johnston & Biesecker, 2013)
NHGRI: Breast Cancer Information Core	A central repository for BRCA1/2 mutations that are relevant for breast cancer risk.	Germline	http://research.nhgri.nih.gov/bic/	
The Universal Mutation Database	Contains frequency and predicted functional consequence data for 9 cancer-related genes involved in human genetic disease.	Germline	www.umd.be/	

Table 5

Examples of Commercial Resources Available for Genome-based Therapeutic Implications.

Commercial Services	Website
Molecular Health	www.molecularhealth.com/
Foundation Medicine	www.foundationmedicine.com
N-of-One	www.n-of-one.com/
Caris Life Sciences	www.carislifesciences.com
CollabRx	www.collabrx.com/
Next Bio	www.nextbio.com
Knome	/www.knome.com/

Table 6

Databases Available for Matching Patients to Cancer Therapy Related Clinical Trials.

Database	Description	Website
ClinicalTrials.gov	A database of publically and privately supported clinical trials conducted around the world.	clinicaltrials.gov
American Cancer Society Clinical Trials Matching Service	A free, confidential program that helps patients find cancer clinical trials appropriate for patients' personal situation. Site requires patient registration with a screening questionnaire to facilitate clinical trial retrieval. Patient is then responsible for contacting study coordinator or clinical trial specialists at ACS for further assistance.	www.cancer.org/index
Cancer Trials Help	Nonprofit organization for improving patient awareness, facilitating access, and promoting participation in clinical trials.	www.cancertrialshelp.org/
National Cancer Institute (NCI) Physician Data Query (PDQ)	Government funded database of 12,000+ clinical trials that are now accepting participants. Browser allows for searching by cancer type, subtype, location and specific cancer phrases that may assist in narrowing down appropriate trials. This feature may function to assist in finding biomarker-driven trials.	www.cancer.gov/clinicaltrials/search
Children's Oncology Group	A National Cancer Institute supported group devoted to helping pediatric cancer patients with finding clinical trials.	www.childrensoncologygroup.org/
Boehringer Ingelheim Clinical Trial Matching Services	A free, confidential service designed to help match patients to appropriate clinical trials. This database is specific for patients with Acute Myeloid Leukemia, Breast Cancer, Head and Neck Cancer, and Lung Cancer.	www.bicancertrials.com/
MolecularMatch	A free service that utilizes other patient response data to find best treatment options tumors with certain genomic alterations. Service also helps provide clinical trial support for appropriate treatment options.	www.molecularmatch.com