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Commentary

COMMENTARY

A Decision Support Framework for Genomically Informed Investigational Cancer Therapy

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Abstract

Rapidly improving understanding of molecular oncology, emerging novel therapeutics, and increasingly available and affordable next-generation sequencing have created an opportunity for delivering genomically informed personalized cancer therapy. However, to implement genomically informed therapy requires that a clinician interpret the patient's molecular profile, including molecular characterization of the tumor and the patient's germline DNA. In this Commentary, we review existing data and tools for precision oncology and present a framework for reviewing the available biomedical literature on therapeutic implications of genomic alterations. Genomic alterations, including mutations, insertions/deletions, fusions, and copy number changes, need to be curated in terms of the likelihood that they alter the function of a "cancer gene" at the level of a specific variant in order to discriminate so-called "drivers" from "passengers." Alterations that are targetable either directly or indirectly with approved or investigational therapies are potentially "actionable." At this time, evidence linking predictive biomarkers to therapies is strong for only a few genomic markers in the context of specific cancer types. For these genomic alterations in other diseases and for other genomic alterations, the clinical data are either absent or insufficient to support routine clinical implementation of biomarker-based therapy. However, there is great interest in optimally matching patients to early-phase clinical trials. Thus, we need accessible, comprehensive, and frequently updated knowledge bases that describe genomic changes and their clinical implications, as well as continued education of clinicians and patients.

Over the past decade, technologies for genomic profiling have rapidly evolved, making it possible to perform point-of-care next generation sequencing (NGS) in clinical laboratories compliant with Clinical Laboratory Improvement Amendments (CLIA) regulations. For the practicing oncologist, the emerging problem is not identifying genomic alterations, but rather how to best utilize the emerging information to select the optimum approved or investigational therapy. In spite of competing clinical productivity pressures, there is now an expectation that practicing oncologists will keep up-to-date on molecular therapeutics

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and ongoing clinical trials. However, a recent questionnaire study demonstrated that even oncologists at a leading cancer center express low confidence in their knowledge of genomics (1). This highlights the urgent need for a framework for genomically informed therapy, readily accessible genomic information focused on clinical relevance of specific alterations, as well as active decision support.

For genomically informed personalized therapy to become routine, several informational challenges must be addressed (Figure 1). First, the confidence in the next-generation sequencing (NGS) and genomic alteration calling must be assessed and the genomic profile of the patient's tumor must be determined, including mutations, copy number changes, and fusions. Second, clinical implications of the genomic profile must be determined. Third, relevant Food and Drug Administration (FDA)–approved drugs and clinical trials with relevant investigational agents must be identified. Fourth, the scientific evidence for each of these identified therapeutic agents in the context of the patient's specific genomic alterations must be weighed. This information must be incorporated into clinical decision-making, also taking into consideration the patient's clinical-pathologic characteristics, prior treatment, response to previous therapies, other treatment options, and personal preferences, including interest in clinical trial participation. In this Commentary, we review the major considerations for genomically informed cancer therapy and present a framework for clinical decision-making and investigational agent selection.

Determining Whether an Alteration Is "Actionable" and Its Therapeutic Implications

At this time, there is strong clinical evidence for only a few genomic predictive biomarkers in selected diseases, such as *HER2* amplification in breast and gastric cancers, *EGFR* mutations, and *ALK* fusions in non–small cell lung cancer (NSCLC), *BRAF* V600 mutations in melanoma, and *KRAS* mutations in colon cancer. Oncologic drugs that have FDA pharmacogenomic labels are listed in Supplementary Table 1 (available online); these include markers predictive of drug response, as well germline variants that play a role in drug metabolism. Treatment selection is relatively straightforward when the tumor has an actionable alteration and a therapy targeting that alteration is FDA-approved for that tumor type (eg, p.V600E BRAF mutation in metastatic melanoma). In such cases, the therapy is considered to be "standard of care." Even in that scenario, however, optimal therapy may differ based on other available therapy options, as seen with the evolution of melanoma treatment algorithms with the emergence of effective immunotherapeutic agents.

For these genomic alterations in other diseases and for other genomic alterations, the clinical data are either absent or insufficient to support routine clinical implementation of biomarker-driven therapy. In this scenario, there is great interest in optimally matching patients to clinical trials based on their genomic profile (2,3). These trials can be "genotype-selected" (ie, patients are required to a have a specific genomic alteration in their tumor to be eligible for a trial) or "genotype-relevant" (ie, trials that do not restrict enrollment based on a specific genomic alteration but that test agents that target a specific gene product or downstream signaling relevant to the molecular alteration in a patient's tumor).

A genomic alteration can be considered "actionable" if it:

1) predicts therapy response (sensitivity or resistance),
2) affects the function of a cancer-related gene and can be targeted directly or indirectly with approved or investigational therapies,
3) is a specific eligibility criterion for enrollment onto genotype-selected trials,

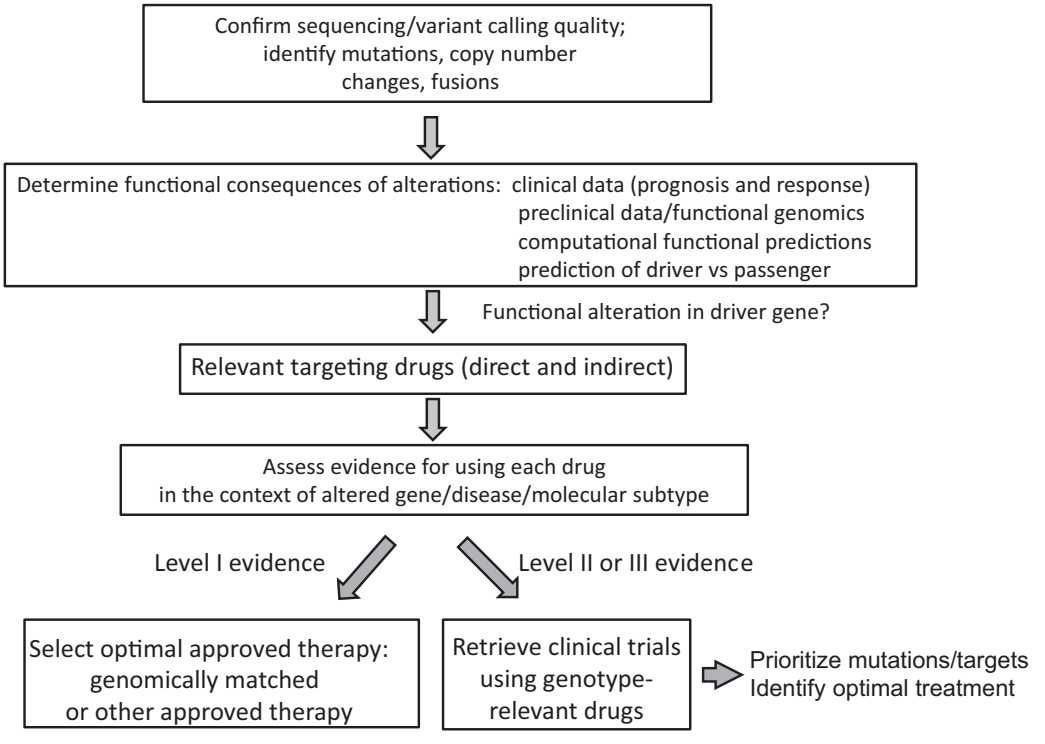

Figure 1. Information challenges associated with personalized cancer therapy.

4) has demonstrated the ability to establish diagnosis or influence prognosis,
5) is a germline alteration that predicts drug metabolism and/or adverse effects,
6) is a germline alteration that predicts future risk of cancer or other diseases (usually considered more "actionable" if prevention or screening with early treatment is feasible).

The first three categories are the focus of genomically informed therapy. In this context, an "actionable" alteration can be directly targeted with a drug or alternately can be indirectly targeted by targeting an activated protein downstream or another oncogenic process dysregulated by the alteration. In Supplementary Table 2 (available online), we list over 120 "potentially actionable" genes for genomically informed therapy. Genes were classified as potentially actionable if: 1) there is at least preclinical evidence or strong scientific rationale suggesting an alteration in a gene may impact protein function, malignant behavior (or patient prognosis, and/or therapeutic sensitivity/resistance), and this gene product can be targeted with an approved or investigational agent; or 2) the gene is being used as an enrollment criterion for ongoing genotype-selected trials. Notably, our actionable gene list was based on genes altered in cancer based on biomedical literature, with literature support for their therapeutic implications. Approximately two-thirds of the genes in our list overlap with the Tumor Alterations Relevant for Genomics-Driven Therapy (TARGET) gene list recently published by Van Allen et al. (4). The differences in the lists are mainly attributable to: 1) our focus on therapeutically actionable alterations, and 2) our inclusion of additional genes used for patient selection in clinical trials, as well as some alterations with preclinical data linking gene alterations to therapeutic sensitivity. These lists are dynamic and will continue to evolve based on novel observations as well as the development of new therapeutics. In fact, the TARGET list was made publically available online to encourage community contributions.

For genetic alterations where a therapy targeting the alteration has been FDA-approved for other tumor types, there is emerging interest in treating patients "off-label, off-protocol." Genes that are targeted by FDA-approved drugs are listed in Table 1 and Supplementary Table 3 (available online). Several of these drugs are tyrosine kinase inhibitors with multiple targets; it is often not known whether amplification or mutational activation of a particular gene confers sensitivity to these agents. Therefore, although it may be appealing to use a drug off-label, it

Table 1. Molecular targets of FDA-approved drugs*†

Targets	Name
ABL1‡ (BCR-ABL1)	Bosutinib‡, Dasatinib‡, Imatinib‡, Nilotinib‡, Sorafenib, Vandetinib
ABL2	Dasatinib, Nilotinib
ALK‡	Crizotinib*, Ceritinib*
BRAF‡	Dabrafenib*, Vemurafenib*, Regorafenib, Sorafenib
CSF1R	Sunitinib
DNMT	Azacitidine, Decitabine
EGFR‡	Afatinib†, Erlotinib†, Lapatinib, Cetuximab, Gefitinib, Panitumumab Vandetanib
EPHA2	Dasatinib
ERBB2‡	Lapatinib‡, Trastuzumab‡, Ado Trastuzumab, Emtansine‡, Pertuzumab‡, Afatinib
FGFR1	Pazopanib, Regorafenib, Sorafenib, Sunitinib
FGFR2	Pazopanib, Regorafenib, Sorafenib (mutant FGFR2)§, Sunitinib (mutant FGFR2)§
FGFR3	Pazopanib, Sorafenib, Sunitinib
FLT3	Cabozantinib, Pazopanib, Regorafenib, Sorafenib, Sunitinib, Vandetinib
FYN	Dasatinib
JAK1/2/3, TYK2	Ruxolitinib
KIT	Axitinib, Cabozantinib, Dasatinib, Imatinib, Nilotinib, Pozapanib, Regorafenib Sorafenib, Sunitinib
LCK	Dasatinib
MEK	Trametinib
MET	Cabozantinib, Crizotinib
MST1R	Cabozantinib
MTOR	Sirolimus, Everolimus, Temsirolimus
PDGFRA	Axitinib, Dasatinib, Imatinib, Nilotinib, Pozapanib, Sorafenib, Sunitinib
PDGFRB	Axitinib, Cabozantinib, Dasatinib, Imatinib, Nilotinib, Pozapanib, Ponatinib Regorafenib, Sunitinib
RAF1	Regorafenib, Sorafenib
RET	Cabozantinib, Pazopanib, Regorafenib, Sorafenib, Sunitinib, Vandetinib
SRC	Bosutinib, Dasatinib
TEK	Pazopanib
TIE2	Cabozantinib
VEGFR1/2	Axitinib, Vandetanib, Cabozantinib, Pazopanib, Regorafenib, Sorafenib Sunitinib, Bevacizumab (VEGFA)
VEGFR3	Axitinib, Vandetanib, Cabozantinib, Pazopanib, Sorafenib, Sunitinib
YES1	Dasatinib

* Many of these drugs are approved for a biomarker-driven indication that is different than its pharmacological target or for a disease without a biomarker-driven indication. FDA = US Food and Drug Administration.
† Please see Supplementary Table 2 (available online) for details.
‡ Target is linked to a biomarker-driven drug indication.
§ Sunitinib and Sorafenib are less potent against wild-type FGFR2, but have increased potency against mutant FGFR2.

is much preferable to treat such patients on a clinical trial where the response information can be formally captured and to ultimately pave the way for registration for additional indications.

Many trials now use genotype rather than histology (tumor type) as a selection strategy for study enrollment, and this is a potential path to FDA approval. However, drug efficacy may differ between tumor types with the same genomic alteration. For example, although vemurafenib is effective against BRAF p.V600E-mutant melanoma, single-agent vemurafenib has limited efficacy against BRAF p.V600E-mutant colorectal cancer (5). Gene-drug sensitivity may be context dependent, influenced by underlying histology and/or other genomic alterations. This is best captured when treatments are given on a trial, with formal assessment of coalterations and other variables.

Genotype-selected trials can be designed in several ways. Single-disease trials can select for or stratify for a specific alteration. Supplementary Table 4 (available online) lists the therapeutic implications for some of the genomic alterations relevant for novel therapeutics. Notably, the frequency of these alterations differs widely by tumor type (Table 2). In some tumor types, the alteration may be sufficiently rare to make it impossible to conduct a disease-specific trial, thus necessitating approaches such as "basket trials." Basket trials treat a variety of tumor types selected for one or more genomic alterations proposed to confer sensitivity to a specific agent. These trials may analyze all patients with a specific alteration as one cohort (eg in NCI-MATCH [National Cancer Institute- Molecular Analysis for Therapy Choice Program]) or analyze each disease type separately. To increase recruitment to genotype-selected clinical trials, efforts are ongoing to develop systems that automatically curate genotype-specific trials using natural language processing and machine learning (6,7). This information can be used to alert a clinician seeing a specific patient regarding trials for which the patient may be eligible.

Finally, in "n-of-one trials," each patient is prescribed an individualized treatment regimen with the endpoint being objective response or increased time-to-failure compared with the last regimen. Studies to demonstrate that these approaches benefit patients are sorely needed. These could include innovative trial designs and at a minimum a registry to aggregate data across centers to determine whether the process or specific pairs of biomarkers and drugs are effective. The American Society of Clinical Oncology is embarking on Targeted Agent and Profiling Utilization Registry (TAPUR), a large initiative that likely will address many of these issues.

Determining the Therapeutic Implications of a Specific Genomic Alteration

Determining Somatic vs Germline Status of Mutations

If matched normal germline DNA and tumor DNA are sequenced, mutations identified in both the tumor and normal would be

Table 2. Frequency (%) of selected somatic mutations and copy number changes in TCGA (April 2014)*

Gene	AML	Bladder	Breast	ccRCC	Colorectal	Head & neck	Lung (Adeno)	Lung (Squ)	Ovarian	Uterine
Selected somatic mutations										
AKT	0	0	2.4	0.5	0.9	0.7	0.9	0.6	0	1.6
BRAF	0	0.8	0.6	0.2	9.4	1.4	9.6	4.5	0.6	2.8
BRCA1	0	3.1	3	1.2	2.7	2.9	3	5.1	12	4.8
BRCA2	0	8.5	4.3	1.9	4.5	3.6	4.8	6.2	10.8	9.7
EGFR	1	1.5	0.8	1.7	4.5	4.7	14.3	3.9	1.9	3.2
FGFR1	0	3.1	0	0.9	1.3	0.4	0.9	1.7	0	3.2
FGFR2	0	2.3	0.8	0.2	1.3	0.7	2.2	3.9	0	12.5
FGFR3	0	14.6	0.2	1.2	0.9	2.2	0.4	2.2	0.3	2
HRAS	0	4.6	0	0.2	0	3.9	0.4	2.8	0	0.4
IDH1	9.5	2.3	0.2	0.5	1.3	0.7	1.3	1.1	0	1.6
IDH2	10	0	0	0	3.1	0	0.9	0.6	0	1.6
KIT	4	2.3	1	0.7	2.7	1.1	2.2	3.9	1.9	6.9
KRAS	4	0	0.8	0.2	42	0.4	32.6	1.1	0.6	21.4
NF1	1	9.2	2.8	1.7	3.6	2.2	11.3	11.2	3.8	8.1
NF2	0	1.5	0.4	0.9	1.3	1.4	0.4	1.1	0.3	2.4
NRAS	7.5	2	0	0	8.9	0.4	0.4	0	0.6	3.6
PIK3CA	0	20	35.1	2.6	20.1	20.8	6.5	15.7	0.6	53.2
PIK3R1	0	1.5	2.6	0.5	4	1.4	0.9	1.1	0.3	33.1
PTCH1	0.5	5.4	1.2	1.9	4	3.2	4.8	2.8	1.9	7.7
PTEN	0	3.1	3.6	4	3.6	1.8	1.3	7.9	0.6	64.9
SMO	0	1.5	0.4	0.7	0.4	0	2.6	0.6	0	2
TSC1	0	8.5	0.6	0.4	2.2	0	1.7	3.4	0.6	4
TSC2	0	2.3	0.4	0.9	0.9	1.1	2.2	3.4	0.6	4.8
Copy number changes										
ERBB2	0	6.3	12.9	NA	3.1	2.2	2.6	2.2	2.2	5.5
FGFR1	0.5	9.4	10.7	0.5	3.1	10	3.5	16.9	3.9	2.5
FGFR2	NA	0.8	1.7	0	NA	0.7	0.9	NA	2	0.8
FGFR3	NA	5.5	0.3	NA	0.4	0.7	1.3	0.6	3.5	2.2
MET	0.5	NA	NA	0.5	0.4	0.7	3.5	1.1	1.6	0.3
PIK3CA	NA	5.5	3.7	1.6	NA	21.1	2.6	38.2	18	6.1

* The cBIO data portal was used to download different disease data set (http://www.cbioportal.org/public-portal) (53). AML = acute myeloid leukemia; ccRCC = clear cell renal cell carcinoma; adeno = adenocarcinoma; squam = squamous cell carcinoma.

classified as germline, while those only in the tumor would be classified as somatic. In this scenario, somatic-only alterations can be reported. Sequencing of a tumor-normal pair allows for improved bioinformatics calling of somatic alterations. However, matched normal samples are not routinely obtained in clinical care. Therefore, some groups sequence tumor only (eg, Foundation One, Foundation Medicine) and have developed algorithms to predict the somatic status of alterations based on allelic frequency of somatic vs germline variants, factoring in tumor purity, copy number, and read depth (8).

Analysis of normal DNA creates an opportunity to also analyze germline alterations, which themselves may have clinical implications. Actionable germline alterations can also be discovered incidentally upon somatic tumor analysis or upon planned germline analysis; there is ongoing discussion about how to best handle these findings generated in the CLIA or research environment (9,10).

Assessing Clinical Implications of Genomic Alterations

Given the variability in quality control between sequencing facilties, the first step in genomically informed therapy is determining one's confidence in the sequencing and the analysis performed. There are substantial differences in variant calls based on software algorithms used for alignment and variant calling. For example, when 15 exomes were analyzed by five different alignment and variant calling algorithms, concordance between five SNV calls was 57.4%, and 0.5 to 5.1% of calls were unique to each pipeline (11). For each predicted variant, the overall coverage, as well as the number of supporting reads, average base quality, and number of strands observed for each allele needs to be assessed, if available. Sensitivity and specificity of variant calls are dependent upon the accuracy of the sequence alignments provided and coverage and allelic frequency of an alteration.

When assessing the clinical implications, the first step is to determine if the altered gene is involved in cancer prognosis or sensitivity or resistance to specific drugs. Because different genomic alterations in the same gene, and even different alterations in the same nucleotide, can have different effects, it is important to determine the effects of the specific alteration (12). Thus, we need to determine whether a mutation in an oncogene is an activating mutation, ie, mutations or gene fusions known to increase the activity of a gene already known to be involved in tumor promotion (for example, through increasing protein function such as activating kinase activity). In contrast, for tumor suppressing genes we must identify inactivating or dominant negative mutations or deletions that promote tumor cell growth or survival.

Ideally, the prognostic or predictive value of a biomarker would be demonstrated prospectively in a randomized clinical trial, providing the strongest evidence for the utility of a biomarker (13). Indeed, for biomarker-driven therapy selection, it is important to follow evidence-based medicine. Standards for assessing the level of evidence supporting information about tumor markers have been published (14) and are used by large groups such as the National Comprehensive Cancer Network. Thus, these standards can also be used while assessing the prognostic value of a genomic marker. A somewhat similar grading scale has been used by the Pharmacogenomics Knowledge Base (PharmGKB), a resource that collects, curates, and disseminates information about the impact of human genetic variation on drug responses (15,16).

To facilitate implementation of genomically informed therapy locally, we have adapted the basic principles in these grading schemes to create a three-tier scale for level of evidence for associations between genomic alterations and response (sensitivity/resistance) to therapy (Table 3). Level I associations require very strong clinical data, with Level IA data being FDA-approved agents in the context of a specific alteration in the same disease.

Level II and III data can determine strength of evidence of actionability of specific gene alterations to assist in clinical trial selection. Level II requires clinical data, which may or may not be from the same disease (Level IIA and B, respectively). Level II data can be a prospective trial where the biomarker study is the secondary objective, or an adequately powered retrospective study or a case-control study demonstrating a statistically significant association of a genomic alteration (or other biomarker) with objective response or clinical benefit. When retrospective analysis is performed to discover biomarkers associated with benefit, analysis should be statistically controlled for multiple testing, and validation studies should be performed in independent retrospective cohort studies, or preferably in biomarker-stratified prospective studies. Notably, to determine whether a marker is predictive of therapy benefit can only be definitely determined when patients with both biomarker-positive and biomarker-negative tumors are treated. If biomarker-selected trials are performed, the prognostic power (as opposed to predictive power) of a given biomarker must also be taken into consideration.

In a patient where effective standard of care options have been exhausted, it is not unreasonable to use Level III data to select the best genomically matched investigational agents in ongoing clinical trials. Level III associations may be based on limited case reports or small cohort studies suggesting response or clinical benefit from an agent in the context of a specific genomic alteration, with scientific rationale. Strong scientific rationale based on preclinical experiments demonstrating an association between specific genomic alterations with therapeutic sensitivity, preferably demonstrated in more than one study, may also be considered as Level III evidence. Although general classifications can be made at the gene level, decisions for individual patients should consider the variant, as levels of evidence may vary between specific variants of the same gene.

Assessing Preclinical Data for Effect of Genomic Alterations on Cancer Biology and Therapy Selection

When clinical data are lacking, the next step is to look for preclinical data to determine whether an alteration can affect protein function and/or tumor growth. Commonly, the mutated allele is expressed and its effects are compared in vitro to cells expressing the wild-type gene, small hairpin RNA (shRNA; to knock down expression) or a control vector. Transforming potential, cell proliferation, growth, and survival under selective pressure such as growth factor or nutrient deprivation is assessed. In vivo studies with transformed cells or genetically modified models increase the confidence that the alteration is indeed a driver.

Genomic alterations may affect sensitivity to specific drugs. One way to measure this is by determining the IC50 (inhibitory concentration 50; the concentration of an anticancer drug that inhibits the growth of cells by 50%, or GI50: growth inhibition 50 compared with baseline) of the drug either in cells that spontaneously express the mutation of interest vs the wild-type gene, or by comparing cells that are induced to express a mutant or wild-type gene. Genomic associations with therapeutic sensitivity

Table 3. Precision oncology decision support level of evidence classification: level of evidence for drug effectiveness in a specific tumor type harboring a specific biomarker*

Level 1	
1A	Drug is FDA-approved for the same tumor type harboring a specific biomarker.
1B	An adequately powered, prospective study with biomarker selection/stratification, or a meta-analysis/overview demonstrates a biomarker, predicts tumor response to a drug or that the drug is clinically effective in a biomarker-selected cohort in the same tumor type.
Level 2	
2A	Large-scale study demonstrates a biomarker is associated with tumor response to the drug in the same tumor type. This could be a prospective trial where biomarker study is the secondary objective or an adequately powered retrospective cohort study or a case-control study.
2B	Clinical data that the biomarker predicts tumor response to drug in a different tumor type.
Level 3	
3A	Single or few unusual responder(s), or case studies, show a biomarker is associated with response to drug, supported by scientific rationale.
3B	Preclinical data (in vitro or in vivo models or functional genomics) demonstrates that a biomarker predicts response of cells to drug treatment.

* Available from http://www.personalizedcancertherapy.org (54). FDA = US Food and Drug Administration.

can also be assessed by comparing genotype and therapeutic sensitivity across large cancer cell line panels (17–19). Although concerns have been raised regarding reproducibility of drug sensitivity data generated in large-scale pharmacogenomic screens (20), these approaches can help identify and confirm associations between genomic alterations and sensitivity to specific therapies.

The effect of specific alterations are easiest to determine when comparisons are done using isogenic models (cell lines that only differ in the gene of interest), with genes being knocked-in/knocked-out. New gene editing approaches such as CrispR/Cas and Talens facilitate the generation of knocked-in cell lines that complement the more time-consuming genetically modified animal models. One model being pursued as a high throughput approach to assess oncogenic variants is the introduction of a mutated or wild-type gene into cytokine-dependent BaF/3 cells to assess genetic complementation and survival upon cytokine withdrawal (18,21).

Genetically engineered mouse models, cell line–derived xenografts that vary in specific genomic alterations, and patient-derived xenografts of defined genomic backgrounds are used to assess effect on in vivo tumorigenicity, metastatic potential, and in vivo sensitivity.

Copy number alterations may be more difficult to interpret, as regions containing multiple genes are often amplified or deleted. The biological effect of copy number gain/loss may be interrogated preclinically with overexpression or siRNA/shRNA knockdown of specific genes to understand functional impact. However, substantial work is needed to determine the clinical relevance of copy number changes and to identify appropriate thresholds for delineating clinically relevant amplifications.

Predicting Functional Impact of Specific Variants

In the absence of preclinical data, computational tools for predicting the functional impact of a specific variant may give some insights. Actionable mutations usually are nonsynonymous mutations (ie, change the encoded protein). Mutations that change nucleotides but not the encoded protein are synonymous mutations and are unlikely to be functional. However, there is emerging data that these “silent” mutations may also contribute to cancer initiation or progression in selected scenarios (22). Further, somatic mutations in regulatory regions of the genome, such as the promoter region of telomerase, are also emerging as a tumorigenic mechanism (23,24).

Another important clue regarding the functional impact of a variant is the frequency at which it occurs in the same cancer type or other cancer types. This frequency can be found in databases such as Catalogue of Somatic Mutations in Cancer (COSMIC) (25), The Cancer Genome Atlas (TCGA) data portal (https://tcga-data.nci.nih.gov/tcga/), or the cBIOportal (http://www.cbioportal.org/public-portal/). While recurrent mutations at the same site in a cancer gene tend to indicate that the target is an oncogene, recurrent mutations can also indicate a dominant-negative effect for a tumor suppressor gene. Regardless, recurrent mutations are strong indications that a mutation is a driver event that warrants further evaluation. Thus, in the absence of functional data, frequency alone may be used to assess potential functional consequences of an alteration.

The functional impact of mutations can also be estimated using computational algorithms based on evolutionary conservation of the mutated site across species, protein structure, and functional protein domains. Several computational tools have been developed to integrate these features into scores that predict the functional impact of missense mutations. Relevant tools include Variant Effect Predictor (VEP) (26), Annotate Variation (Annovar) (27), Scale-Invariant Feature Transform (SIFT) (28), Polymorphism Phenotyping (Polyphen) (29), Consensus Deleteriousness (Condel) (30), Mutation Assessor (31), and cancer type–specific annotators Cancer-specific High-throughput Annotation of Somatic Mutations (CHASM) (32) and Cancer-specific Driver Missense Mutation Annotation (CanDrA) (33). The relative utility of these tools are reviewed in Bailey et al. (34). However, the predictive utility of these computational tools has not been established.

Reporting Actionability of Genomic Alterations

A recent survey by the National Institutes of Health Clinical Sequencing Exploratory Research Program demonstrated great variability in annotation tools as well as variant reporting across centers (35). Although there is some debate about the merit of returning somatic variants of unknown functional significance, given how rapidly our genomic knowledge is evolving, reporting all nonsynonymous somatic alterations is preferable. Presenting the alterations in tiers or categories, highlighting the alterations in actionable genes, may make genomic reports easier to interpret. Wagle et al. (36) proposed three categories: variants that predict sensitivity/resistance to

an FDA-approved agent in the same disease (Tier 1) or experimental therapies (Tier 2), with prognostic/diagnostic variants being the third category. MacConaill et al. proposed another three-tier presentation (37). Tier 1 alterations have well-established published evidence of clinical utility in the same tumor type in the context of predicting response to an FDA-approved drug, assessing prognosis, establishing diagnosis, or conferring inherited risk of cancer. Tier 2 alterations may have utility to select an investigational therapy, to provide limited evidence for prognosis, to be supportive of specific diagnosis, or to suggest an association with response in a different type of cancer, or are similar to a variant associated with response. Tier 3 alterations have uncertain clinical utility but may have a role because of association with response in preclinical data, alteration in biochemical pathway, or alteration in a highly conserved region of the protein.

Our Precision Oncology Decision Support (PODS) level of evidence (LOE) Classification (Table 3) is not a substitute for these classifications but rather provides a framework to critically assess the supporting evidence that a specific variant is actionable. Our level of evidence scheme differs from that recently proposed by Andre et al. (38), mainly as Level I evidence in our classification corresponds to a high enough level of evidence to warrant a clinical practice change. Alterations with Level I evidence based on PODS LOE would correspond to Tier I alterations in both the McCanaill (37) and Wagle (36) classifications. Alterations with Level II or III evidence would be Tier 2 in Wagle (36) classification, and 2 or 3 in McCanaill (37) classification. Reviewing what is known about each gene and its specific variants will allow for not only classification of LOE to assist in clinical trial selection, but can also assist in providing patients better information for informed consent, and can optimize target selection when multiple genomic alterations are concurrent.

Classifying Actionability of Specific Genomic Alterations

After the genomic alterations are identified, the functional significance of the alterations could be classified as: 1) activating, 2) inactivating, 3) likely benign, 4) unknown. Variants with Level I evidence for actionability in the same tumor type are actionable in standard of care. Variants/genes without Level I evidence for actionability can be considered actionable or potentially actionable in the context of investigational therapy, or alternately may not be actionable, or with "unknown" actionability. Actionability of a variant may be literature based or may be based on more limited preclinical data demonstrating variant function (such as with functional genomics). For selected alterations, the actionability may be inferred based on expected effect of a mutation on function of a mutation; for example, a mutation leading to early truncation of PTEN gene would be inferred to lead to loss function, even if the mutation has not been previously described. A mutation in a critical domain (eg, mutation in the kinase domain of an oncogene) in the absence of other literature supportive of impact of protein function may be considered a "potentially actionable variant." Examples for each category of variant classification are listed in Supplementary Table 5 (available online). Ideally only patients with mutations with known or suspected functional impact should be enrolled on genomically selected proof-of-principle targeted therapy trials, as enrolling variants of unknown significance that are nonfunctional is likely to dilute the therapeutic effect observed.

Identifying Genomically Relevant Clinical Trials

Because of the rapid growth in biomedical literature, tracking associations between genomic alterations and genotype-relevant drugs and clinical trials is difficult for treating oncologists and researchers alike. To address this need, health care institutions have embarked on genomic medicine units/clinics and many centers have set up "Molecular Tumor Boards." Commercial companies offer software tools, personalized testing services, and NGS accompanied by clinical reports that leverage the literature to describe published functional consequence of variants and link gene variations to drug response (34). Further, some institutions have created online resources for genomically informed treatment decisions, such as PersonalizedCancerTherapy.org (led by the UT MD Anderson Cancer Center), MyCancerGenome.org (led by Vanderbilt) (39,40), and the Drug Gene Interaction Database, dgidb.genome.wustl.edu (led by Washington University) (41). These resources have several differences that make them complementary. PersonalizedCancerTherapy.org and MyCancerGenome.org both have content on therapeutic implications that have undergone expert review, while Drug Gene Interaction Database automatically searches across several databases to find drug-gene interactions. PersonalizedCancerTherapy.org is organized in a gene-focused fashion, while MyCancerGenome.org is organized by disease. Thus the latter may be optimal to help review key drivers in selected diseases, while PersonalizedCancerTherapy.org is a knowledge base of therapeutic implications of genomic alterations across tumor types and provides decision support for histology-agnostic genomically selected trials, both for rare alterations in common diseases, and for common alterations in rare diseases.

Additional Considerations for Personalized Cancer Therapy

Implications of Intratumoral and Intertumoral Heterogeneity

There has been increasing recognition that tumor heterogeneity may impact genomic testing. Intratumoral heterogeneity refers to different alterations in different regions of the same tumor; this may be an especially important consideration when larger tumors are assessed with sequencing of only a small portion of the tumor. Intertumoral heterogeneity refers to differences between tumors, including differences between primary tumors and metastases, between metastases at different sites, and between different metastatic foci in the same organ. There is a growing concern that analysis of archived primary tumors may not reflect all changes in the metastases and analysis of a small biopsy or just a portion of tumor may not be reflective of the entire genomic complexity of that tumor. Although these are reasonable concerns, comparison of primary tumors with matched metastases have shown relatively high concordance in their mutational profiles (42), in many diseases including breast, colon, and lung cancers. Further, convergent evolution can lead to the activation of the same pathway by different mutations (43–45). At this time, the impact of tumor heterogeneity on therapeutic liabilities and patient outcomes is unclear. Heterogeneity may not only represent a challenge for biomarker assessment, but may suggest a greater propensity for the tumor to progress and to develop therapeutic resistance through multiple concurrent mechanisms.

Implications of Genomic Evolution

It is increasingly understood that the mutational landscape of tumors can change upon treatment with targeted therapies with both gain and loss of actionable alterations. When alterations not detected in the original biopsy are present in a subsequent biopsy, whether these represent new mutations or selection for rare subclones already present in the primary tumor remains unclear. Longitudinal biopsies of solid tumors have led to the recognition of a lot alterations that are selected for: 1) epidermal growth factor receptor (EGFR) mutation p.T790M, *MET*, or human epidermal growth factor receptor 2 (HER2) amplification in non–small cell lung cancer patients treated with EGFR inhibitors (46–48), 2) acquired EGFR ectodomain mutation (p.S492R) and *KRAS* mutations, or amplification in colon cancer patients treated with cetuximab (49,50), 3) *BRAF* amplifications, *BRAF* splice isoforms, *MITF* amplifications, and *NRAS MAP2K1*, *MAP2K2*, and *NF1* mutations in melanoma patients treated with Raf or MEK inhibitors (33,36,45), 4) loss of *HER2* amplification in HER2+ breast cancer patients treated with HER2-targeted therapy (51), and 5) acquired estrogen receptor 1 mutations in patients treated with adjuvant endocrine therapy (52). These studies emphasize that there's a variety of acquired genomic alterations associated with therapeutic resistance to targeted therapies.

Whether approaches such as targeted exome sequencing to high-depth or multiple single-cell sequencing runs can capture the heterogeneity in a tumor and the subclones that contribute to therapy resistance is unknown. However, it is clear that repeat biopsies and molecular profiling will be of greater value as the number of treatment options increases, especially in patients with acquired resistance after initial response, or in patients with mixed response (ie, some responding and some progressing lesions). "Liquid biopsies" with assessment of circulating tumor cells or circulating free DNA (cfDNA) are also being explored as alternate strategies for serial assessment of genomic evolution as well as for monitoring the efficacy of therapies.

Tumor Cellularity and Mutant Allelic Frequency

Tumor cellularity can influence the success of NGS, and subclonal mutations may be missed in lower cellularity samples even with high-depth sequencing. Thus, hematoxylin and eosin staining to confirm adequate tumor cellularity is critical, and tumor enrichment with macrodissection is another helpful step. In general, NGS requires a tumor nuclear cellularity of at least 20%, and higher is preferred.

While determining the actionability of a genomic alteration, the allelic frequency of the mutations is another consideration. Oncogenic drivers that are subclonal (eg, <10% allelic frequency) may not be as good of targets as those that are in the majority of cancer cells. In contrast, "resistance markers" that are subclonal theoretically may confer resistance. Further work is needed to determine whether patients with subclonal resistance mutations truly do not benefit from specific targeted therapies (eg, whether subclonal *KRAS* mutations confer resistance to EGFR-targeted therapy) or whether the patients more transiently benefit, with subsequent selection of the resistant clone.

Prioritizing Multiple Targets

As multiplex testing becomes more accessible, the likelihood of finding at least one genomic alteration in each patient increases. When more than one alteration is identified, we must prioritize alterations as potential therapeutic targets. Concurrent aberrations could represent both sensitivity and resistance markers, with a dominant resistance marker, such as co-occurrence of *EGFR* and *KRAS* mutations with *KRAS* mutations signaling dominant resistance. In other cases, a downstream lesion may bypass the effect of a sensitivity marker, for example *PTEN* mutations signaling resistance to HER2-targeted therapy. At this time, we have little data to help prioritize multiple targets. It is important to identify additional alterations downstream and parallel survival pathways that may confer therapeutic resistance. For example, for a patient with both an upstream and a downstream activating mutation, targeting downstream or with dual upstream/downstream blockade may be preferable. If more than one mutation or copy number alteration is actionable, the target with stronger evidence for actionability ("driverness" or therapeutic sensitization) should be pursued. In addition, higher allelic frequency of the mutations (ie, higher proportion of a particular mutation in DNA sequenced, usually expressed as % frequency), or higher copy number of the amplifications may make the targets more appealing. Ultimately, prospective validation of guidelines for target and agent selection is needed.

The Future of Personalized Cancer Therapy

The rapid evolution of genomic profiling and emergence of molecularly targeted therapies has made genomically informed therapy a reality. Although we have focused this manuscript on somatic genomic alterations, alterations can also occur through epigenetic regulation and RNA editing. The gene can also be regulated through changes in RNA stability, alternate splicing, altered protein translation or stability, and post-translational modifications of the protein. Thus, integrated analysis of the molecular profile of tumors by assessing DNA, RNA, and protein may provide information content that is not available from analysis of DNA alone. Further, the tumor microenvironment and immune system need to be incorporated into personalized therapy. The clinical history of an individual patient, including responsiveness/resistance to previous therapies, may also inform future treatment. Reimbursement of multiplex testing, the relevance of NGS platforms vs companion diagnostics for drug development, and the possible implementation of device approval for NGS platforms remain important issues. In addition, there is a great need to speed up discovery by supporting genomically selected trials through trial prioritization, greater trial awareness, information sharing, and interinstitutional collaborations. Approaches such as adaptive learning algorithms should be explored to more rapidly determine the impact of different genomic alterations on response to different investigational and standard-of-care therapies.

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