Published in final edited form as:

Clin Pharmacol Ther. 2018 August ; 104(2): 282–289. doi:10.1002/cpt.1041.

Considerations for Developing Targeted Therapies in Low-Frequency Molecular Subsets of a Disease

Robert N. Schuck¹, Janet Woodcock², Issam Zineh¹, Peter Stein³, Jonathan Jarow², Robert Temple², Thomas Permutt⁴, Lisa LaVange⁴, Julia A. Beaver³, Rosane Charlab¹, Gideon M. Blumenthal³, Sarah E. Dorff¹, Christopher Leptak³, Steven Lemery³, Hobart Rogers¹, Badrul Chowdhury³, David Litwack⁵, and Michael Pacanowski¹

¹Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA.

²Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA.

³Office of New Drugs, Center for Drug Evaluation and Research, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA.

⁴Office of Biostatistics, Office of Translational Sciences, Center for Drug Evaluation and Research, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, USA.

⁵Office of In Vitro Diagnostics and Radiological Health, Center for Devices and Radiological Health, U.S. Food and Drug Administration, Silver Spring, Maryland, USA.

Abstract

Advances in our understanding of the molecular underpinnings of disease have spurred the development of targeted therapies and the use of precision medicine approaches in patient care. While targeted therapies have improved our capability to provide effective treatments to patients, they also present additional challenges to drug development and benefit-risk assessment such as identifying the subset(s) of patients likely to respond to the drug, assessing heterogeneity in response across molecular subsets of a disease, and developing diagnostic tests to identify patients for treatment. These challenges are particularly difficult to address when targeted therapies are developed to treat diseases with multiple molecular subtypes that occur at low frequencies. To help address these challenges, FDA recently published a draft guidance entitled “Developing Targeted Therapies in Low-Frequency Molecular Subsets of a Disease”. Here, we provide additional information on specific aspects of targeted therapy development in diseases with low-frequency molecular subsets.

Address correspondence to: Michael Pacanowski, Pharm.D., M.P.H., Food and Drug Administration, Office of Clinical Pharmacology, 10903 New Hampshire Avenue, White Oak, Building 51, Room 2132, Silver Spring, MD 20993, Phone: 301-796-3919, Fax: 301-847-8720, Michael.pacanowski@fda.hhs.gov.

The authors declare no conflicts of interest related to this article. This article reflects the views of the authors and should not be construed to represent FDA’s views or policies.

Keywords

genomics; pharmacogenomics; precision medicine; drug development

INTRODUCTION

Precision medicine is an approach to tailoring disease prevention and treatment that considers differences in people's genes, environments, and lifestyles. Although many of the technologies driving the implementation of precision medicine are relatively new, major medical advances are already being made. One notable advance is the development of targeted therapies. Targeted therapies are drugs that, based on their mechanism of action, are designed to target subsets of patients with specific molecular alterations among the larger population of patients with a clinically-defined disease. FDA has sought to advance the application of precision medicine via several approaches, including guiding the development and approval of multiple targeted therapies with corresponding companion diagnostic devices, and updating the labeling of many drugs to include genetic information that helps prescribers choose the most appropriate drug and dose for each patient.¹ Precision medicine approaches may enhance the benefit/risk profile of drugs by identifying patients who are more likely to respond to a particular therapy and can provide effective treatments to patients who previously did not have approved therapies.² However, precision medicine and targeted therapies present additional challenges to drug development and benefit-risk assessment. These challenges include identifying the subset(s) of patients likely to respond to a drug, assessing heterogeneity in response across molecular subsets of a disease, and developing diagnostic tests to identify patients for treatment.^{3, 4}

Intrinsic (e.g., demographic, anthropometric factors) and extrinsic (e.g., concomitant drug regimen) factors may result in variable responses to a drug and have traditionally been assessed during drug development, sometimes resulting in altered dosage or limitations on the use of a drug in subsets of the patient population.⁵ Inter-individual variability in a drug's target (e.g., extent of expression of the target protein in tumor tissue, altered DNA sequence that affects protein expression or activity) can also be a source of heterogeneity of drug response.⁶ Recently, ascertainment of individual differences in molecular target status in the clinical setting has become feasible using methods such as genetic sequencing, and RNA and protein expression profiling, and has spurred the development of targeted therapies. Although molecular heterogeneity does not account for all the variability in response to targeted therapies (i.e., variable responses may be anticipated in different patients with the same molecular alteration), assessing molecular heterogeneity is critical to identifying the target population that is likely to benefit. As such, assessment of target status is now a common feature of targeted drug development programs.⁴

The mechanism of action of targeted therapies is often directed at the effects of specific molecular alterations that impact the drug target or a biological pathway relevant to the disease (e.g., changes in DNA, RNA, or protein, such as point mutations, gene fusions, mutational load, epigenetic changes, over/under expression).⁷ Most successes in targeted therapy development have been in cancer and monogenic diseases. In both these settings,

characterization of the molecular basis for disease has uncovered a large number of molecular subtypes, often with common pathogenic consequences, underlying many clinically-defined diseases (Figure 1).⁷ In the oncology setting, the identification of multiple molecular alterations underlying tumor development (or drug resistance) in some patients has led to the possibility of combining therapies in attempt to optimize clinical outcomes.⁸ In addition, targeted therapies have been developed for more common diseases that target a phenotypic or biomarker-defined subgroup, such as asthma with an “eosinophilic phenotype”. Because the pharmacological effect of a targeted therapy may be directly linked to the functional effect(s) of specific molecular alterations, patients who have different molecular alterations driving the development of a phenotypically similar disease may have different responses to a targeted therapy.

For many diseases diagnosed based on clinical features, there may be a range of different molecular alterations impacting a common target protein or pathway. In the case of monogenic diseases, a common phenotype may result from numerous different mutations within a gene that have distinct effects on the molecular target (e.g., absent protein, deficient protein, diminished binding of ligands, defective signaling), and some may occur at low frequencies within the clinical disease. For example, there are over 250 known disease-causing mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene that result in cystic fibrosis, ranging from ~90% prevalence to <1% prevalence among cystic fibrosis patients.^{9, 10} While cystic fibrosis is often categorized into five (or six) disease subtypes based on the functional impact of the mutation,¹¹ each mutation may have a different effect on protein function that may impact the responsiveness of a patient to a targeted therapy. For example, a targeted therapy that enhances CFTR protein function may not effectively treat patients with mutations that result in absent protein. Cancers are often more complicated because molecular alterations from multiple genes may activate (or silence) a biological pathway and result in a common phenotype, and molecular heterogeneity may be observed within an individual patient.^{12, 13} Moreover, the same molecular alteration may confer sensitivity to a drug in one cancer type but not in another due to resistance mechanisms acquired in specific tumor types.¹⁴

Many targeted therapies are effective in multiple molecular subtypes that make up a disease, for example, when multiple alterations have a common functional effect on the target that the drug modulates. However, the diversity in the molecular etiology of disease results in the potential for heterogeneity of drug effect across different molecular subtypes. The specific molecular alteration that leads to aberrant signaling may impact responsiveness to a targeted therapy, even within the targeted population in which the drug is hypothesized to work. This creates challenges generating evidence of effectiveness for targeted therapies in diseases with multiple molecular subtypes, particularly when some occur at low frequencies (Table 1).

DEVELOPING TARGETED THERAPIES IN DISEASES WITH LOW-FREQUENCY MOLECULAR ALTERATIONS

For diseases with low-frequency molecular subtypes, clinical trials will often enroll small numbers of patients (or no patients) with some of the specific molecular alterations that are eligible for inclusion in the trial. As such, it may be infeasible to assess drug benefits and risks for each specific molecular alteration in a controlled clinical trial, and doing so may draw incorrect conclusions from the limited amount of available data. For example, if the effect of an investigational drug is demonstrated in a trial of 100 patients with the clinically-defined disease, then a molecular alteration occurring at a prevalence of 1% in the general disease population would be anticipated in a very small number of patients. Thus, the information gained from the clinical trial would not be sufficient to conclude that the drug had (or did not have) its intended effect in patients with each specific molecular alteration (even if the response pattern in the one or two patients with the variant in the trial appeared to respond differently than the overall population).

Given these issues, many different drug development approaches can be considered, ranging from a very narrow drug development program focusing on a single molecular alteration to an “all comers” approach enrolling all patients meeting the clinically-defined entry criteria. Each of these approaches has advantages and disadvantages (Table 2); however, the diverse strategies for developing targeted therapies have led to varying approaches in drug approvals and labeling (Table 3).

In many cases, the strategy for narrow vs. broad enrollment and subsequent approval is dictated by the underlying molecular etiology of the disease and/or mechanism of action of the drug. For example, years of research into the pathological consequences of *BRCA1* and *BRCA2* mutations and the mechanism of action of poly (ADP-ribose) polymerase (PARP) inhibitors indicated that ovarian cancer patients with different deleterious *BRCA1* and *BRCA2* mutations would likely respond similarly to PARP inhibition.¹⁵ The clinical trials for these agents therefore enrolled a wide array of mutations (based on a pre-defined algorithm). The PARP inhibitors were subsequently approved broadly despite not all mutations being captured in the clinical trial.¹⁶ In contrast, knowledge of the varying functional consequences of specific *CFTR* mutations, the putative mechanism of action for ivacaftor, in vitro data, and preliminary clinical data all indicated that cystic fibrosis patients with different mutations would respond differently to ivacaftor.¹⁰ This led to a narrow initial focus for drug development and a narrow initial approval limited to the *CFTR* G551D mutation. Although in some cases, the molecular etiology of the disease and mechanism of action of the drug may guide the appropriate drug development strategy, in other cases the choice to enroll a narrow vs. broad population in clinical trials may hinge on practical or logistical considerations, such as the inability to enroll enough patients with a specific molecular alteration into clinical trials or having to screen large numbers of patients to capture the target population. In these cases, pragmatic drug development and regulatory strategies are needed to assess drug benefits and risks for all patients within the molecular subtypes hypothesized to benefit from the drug.

Considering the challenges of developing targeted therapies for diseases with multiple low-frequency molecular subsets, FDA recently published a draft guidance entitled “Developing Targeted Therapies in Low-Frequency Molecular Subsets of a Disease”.^{17, 18} The draft guidance outlines FDA’s current thinking on developing targeted therapies for diseases where one or more molecular subsets occur at low frequencies. Here, we provide additional information on specific aspects of targeted therapy development in diseases with low-frequency molecular subsets, including factors that may be important when deciding whether to restrict development to a molecular subset of a clinical disease, data that can support grouping of molecular alterations for clinical trial enrollment, and strategies outside of the traditional controlled clinical trial paradigm that can be useful for generating data to support efficacy in a molecular subtype. The perspectives here are meant to provide additional background for the above-mentioned draft guidance document and should not be considered final official FDA guidance.

PURSUING A TARGETED DRUG DEVELOPMENT STRATEGY

The appropriateness of pursuing an indication in a molecular subtype of a disease depends on the strength of the evidence supporting the hypothesis that patients with the subtype of interest will be more likely to respond to the targeted therapy than the remaining patients with the same clinically-defined disease. A targeted drug development program is generally appropriate when heterogeneity in response (1) is anticipated based on the drug’s mechanism of action in the context of the disease etiology or (2) is observed in nonclinical or clinical studies. Although a drug may be developed for use in a molecular subtype of a disease to address an unmet medical need or because the subtype has a particularly poor prognosis, in these cases drug effects are not expected to differ across subgroups for mechanistic reasons. Therefore, the principles below may not be applicable.

Mechanistic Evidence

In some cases, a drug is intentionally designed to address a specific functional effect of a molecular alteration or group of molecular alterations that may be present in only a subset of the clinical disease. In these cases, heterogeneity of response across the molecular subtypes that make up the clinical disease would obviously be anticipated based on the mechanism of action, and a targeted drug development strategy would be appropriate when clinical benefit is not anticipated for some molecular subtypes. In addition, drugs that directly target and modulate a specific molecular defect (e.g., increase expression of an ion channel in a patient with reduced channel expression, or inhibit signaling from a receptor in a patient with a variant that results in excessive receptor signaling) may only show response in individuals with the clinical disease who have that particular molecular alteration. Individuals with a similar clinical phenotype but with a distinctly different molecular etiology would not be expected to respond to the drug. In contrast, drugs acting downstream from the targeted molecular alteration may be less sensitive to subtle differences in the functional effects because the different molecular alterations that contribute to disease pathology may converge to result in a common phenotype that is targeted by the drug. For example, drugs that increase insulin release work in patients with multiple monogenic and polygenic causes of type 2 diabetes mellitus. Similarly, the mechanism of PARP inhibitors suggests they

would be effective in ovarian cancer patients with numerous different “deleterious” *BRCA1* and *BRCA2* mutations as well as patients with other molecular alterations underlying their disease pathology.⁷ Of note, even when a drug’s mechanism of action makes it unlikely that heterogeneity in response will be observed across molecular subtypes, as with any drug, variability in response is still anticipated based on other biological and environmental factors.

If heterogeneity in drug effect is anticipated based on the mechanism of action, molecular subtypes of a disease for which the drug is active may be informed by using nonclinical or clinical studies. These studies can be useful both to establish proof-of-concept that certain molecular subtypes will be responsive to the drug and to help identify the appropriate population for enrollment in clinical trials. In some cases, the mechanism of the drug may make it counterintuitive to conduct trials in certain subtypes of disease; for example, in oncology when a patient’s tumor does not express the target protein. When a drug is established to have differential responsiveness across molecular alterations based on strong mechanistic rationale or in experimental models, targeted development approaches are generally appropriate to enrich the patient population for individuals more likely to respond to the drug.

Empirical Evidence

Drug response heterogeneity may be observed directly in nonclinical or early clinical studies. While response heterogeneity is anticipated in all clinical trials as a result of biological variability (independent of the impact of molecular factors), clinical studies may show well-defined and consistent differences in response across molecular subtypes, with evidence of non-responders and clear responders, that appear to be beyond usual range of variability. Often this heterogeneity is anticipated based on the mechanism of the drug, and future studies can enroll an enriched patient population consisting of only the molecular subtype(s) considered likely responsive. When heterogeneity across molecular subtypes is not anticipated based on mechanism but is observed in nonclinical studies or early clinical trials, additional nonclinical or clinical studies may be useful to determine the underlying cause and inform appropriate grouping of molecular alterations for clinical trials and clinical use of the drug. In either case, when substantial empirical data exist indicating that the drug will only be effective in a subset of the clinical disease, a targeted development program is generally appropriate.

CONSIDERATIONS FOR CLINICAL TRIALS

Determining who to enroll

Grouping patients based on similarity of molecular features for enrollment in clinical trials may allow evaluation of molecular subtypes that could not reasonably be studied individually. The primary target population for a targeted therapy may be defined by grouping putatively similar molecular alterations based on mechanistic or bioinformatic information, or empirical evidence from nonclinical or clinical studies. Once the primary target population has been identified, it may be appropriate to utilize a traditional randomized controlled trial design, or non-traditional clinical trial designs such as master

protocols¹⁹ or adaptive design trials²⁰ may be advantageous in some cases to facilitate enrollment or assessment in certain subgroups.

The process of grouping to evaluate a therapeutic product should consider both the biological (or pathological) similarity among the molecular alterations and the mechanism of the drug. The validity of a circumscribed molecular subtype of a disease and evidence of therapeutic effectiveness may be drawn from many clinical and nonclinical sources. Evidence from *in silico* assessments (e.g., 3D structure of drug binding site), nonclinical data, clinical data, and other data sources (including evidence from other drugs in the same pharmacological class) that suggest responses within the grouped molecular subtype will be similar can be useful to support baseline grouping of alterations (or exclusion of certain alterations) for trial enrollment and/or primary hypothesis testing. Multiple lines of evidence (e.g., nonclinical studies combined with preliminary clinical data) may increase confidence that the grouping strategy is appropriate.

It may not be feasible to perform nonclinical experiments or conduct clinical studies across the spectrum of molecular alterations prior to initiating pivotal trials. One strategy that can obviate the need to collect data on all molecular alterations within a disease is to generate data that supports responsiveness in a broad, categorically-defined population (e.g., “activating” or “sensitizing” mutations) based on data from a representative group of the molecular alterations within the category. This strategy may support enrollment of a broad group of molecular alterations in clinical trials (in contrast to highly selected trials where only a single variant is enrolled). However, when grouping patients into functional categories, it is important to define the category in a way that is objective and clear to avoid misclassification. Moreover, in the case of continuous biomarkers (e.g., gene expression, protein expression), cut-offs above and below which response is expected or not expected should be carefully chosen based on data from exploratory studies.

In most cases, it is appropriate to include some “marker-negative” patients (those not in the molecular subtype predicted to benefit from the targeted therapy) in trials intended to provide support for primary effectiveness unless it is already established that these patients do not respond, the drug’s mechanism makes it clear that they are unlikely to respond, or there is a safety concern associated with enrolling marker-negative patients.²¹ In general, greater uncertainty of response in marker-negative patients makes it more important to include a reasonable sample of marker-negative patients in clinical trials. In contrast, when convincing evidence indicates marker-negative patients will not respond, inclusion of these patients may raise ethical issues. When there is uncertainty as to whether a molecular alteration is predictive (i.e., can select a population in which treatment is effective), the primary endpoint of a clinical trial could be the effect in the subgroup of patients with the molecular alteration(s) of interest (while enrolling additional subgroups to generate preliminary clinical data) or the study alpha could be divided between the two groups (overall population and the molecular subgroup of interest).²¹ Although the target defect, its role in disease pathogenesis, and the drug’s mechanism of action on its intended target may be considered fully characterized, unexpected responses may be seen that can change the hypotheses of disease pathogenesis or drug responsiveness. Such unexpected observations can be of great importance in early development—and drug developers should maintain an

open-mind when examining experimental results. For example, although a drug may work by modulating protein function not expression, patients with a gene variant reducing trafficking of the protein to the membrane might respond to the drug when a low level of protein is made to “hyperfunction” and thereby provide some benefit in the disease (even if the drug was expected to only work in patients with protein dysfunction not deficiency).

Interpreting results

A basic question in the interpretation of clinical trials when the drug demonstrates a positive effect in the total population of the clinical trial, is whether an average effect in a wider population can be taken as representative of the effect within subgroups. The answer depends on how different the subtypes are, but there will rarely be enough evidence within the study itself to draw a reliable statistical conclusion about heterogeneity or homogeneity. Thus, interpretation of the results will need to rely on a priori scientific judgment about the similarity of various subtypes. As with all drugs, it is important to consider the totality of evidence when weighing the benefits and risks of targeted therapies to determine the population for which the drug has demonstrated safety and efficacy. For example, if no effect or a harmful effect is observed in a clinical trial for a certain subgroup and other nonclinical or clinical data support this finding, it may be appropriate to restrict use of the drug in that subgroup.

Even when it is believed that subtypes might differ meaningfully, it will often be impractical to estimate the effect in a subtype from results in that subtype alone. The sample sizes for individual subtypes will often be too small to provide reliable estimates. It then becomes necessary to combine estimates from the subtype, which are most directly relevant but highly variable, with information from a larger group, which is less directly relevant but more precise. Consideration should thus be given to the use of methods that involve borrowing of information from other subtypes (e.g., Empirical Bayes methods, shrinkage estimation). In the statistical literature, these methods have been developed most extensively in the context of small-area estimation in sample surveys.^{22, 23} Adjustment for baseline covariates through modeling or stratification may enhance the comparability of treatment effects across subtypes and therefore make the information from outside the target subtype more relevant.

GENERATING ADDITIONAL DATA TO SUPPORT EFFECTIVENESS IN LOW-FREQUENCY MOLECULAR SUBSETS

Data obtained from sources outside randomized controlled clinical trials may enhance understanding of the therapeutic risks and benefits of the drug. These data may provide further support that the original grouping strategy was appropriate, or support responsiveness of the drug in patients with molecular alterations that were not included in the initial grouping strategy but for whom the drug might be effective. When a drug has demonstrated efficacy and safety in other variants by other means (e.g., in clinical trials demonstrating a clinical benefit in a similar disease subtype), additional nonclinical and/or clinical data such as a pharmacodynamic biomarker demonstrating that the molecular

subtype responds to the drug can be supportive of substantiating or extrapolating efficacy to subtypes where benefit-risk is uncertain.²⁴

Experimental Approaches

If substantial clinical evidence exists demonstrating molecular alterations belonging to a certain category (e.g., “deleterious” or “activating” mutations) in a given gene or pathway result in sensitivity to a drug, then a pre-defined algorithm or set of rules may be used to generate evidence that newly discovered variants are similar to the known variants in that category and are also likely responsive to that drug. In circumstances where variants cannot be categorized using an algorithm or set of rules, mechanistic studies, presuming availability of an established model, can be used to support responsiveness of low-frequency molecular alterations or subtypes. In vitro or animal experiments may be appropriate, depending on the availability of assays and animal models for the drug and disease being studied.

Clinical Approaches

Several options are available to generate data to support responsiveness in low-frequency molecular subtypes of interest. The most appropriate study design for collecting clinical data in low-frequency molecular subtypes is context-dependent, and may be impacted by factors including the type and quantity of nonclinical and clinical data previously collected to demonstrate the drug’s efficacy, the rarity of the molecular alterations being studied, the ability to monitor response (e.g., with a biomarker), reversibility of the outcome, need to minimize time on control therapy, and other factors.

Clinical trial designs that may utilize smaller sample sizes include randomized withdrawal, crossover, baseline control, and N-of-1. Each of these may be appropriate trial designs depending on the information that is desired. Observational studies, patient registries, and collection of real world evidence (e.g., using electronic health records) can also be useful to further enhance understanding of therapeutic benefits and risks of the drug, particularly in the post-market setting and when effect sizes are large.

Ideally, clinical trials should incorporate appropriate patient evaluations and sample collections to analyze exposure-response, pharmacodynamic endpoints, and pharmacokinetic/pharmacodynamic relationships. Although a surrogate or pharmacodynamic biomarker may in some circumstances independently support approval in low-frequency molecular subtypes, these endpoints can be particularly useful to support extrapolation of a pharmacologic effect. When a targeted therapy has previously demonstrated safety and efficacy based on clinical endpoints in a more broadly defined patient population or phenotypically similar population with different disease-causing variants, a pharmacodynamic biomarker or surrogate endpoint may be used to extrapolate efficacy to additional putatively similar subtypes.

DIAGNOSTIC TEST DEVELOPMENT AND USE

Identifying patients with specific molecular alterations relies on the use of in vitro diagnostic devices (IVDs). Reliable IVDs that can accurately detect one or many biomarkers are essential for enrollment of patients into clinical trials and to identify appropriate patients for

use of a drug in the clinical setting once a drug has been approved. In the case of genotyping assays, some are designed to detect only a few specific variants (which may or may not include low frequency variants of interest), while others may take advantage of next-generation sequencing technologies and use targeted panels or whole-genome or whole-exome sequencing approaches. Assays quantifying gene or protein expression can be even more complex, using multi-analyte gene expression profiles, protein based profiles, or immune signatures.^{23, 25} Therefore, the choice of assay for use in clinical trials and its performance could clearly affect the ability to enroll patients with different molecular alterations, and thus the population for which the drug demonstrates evidence of effectiveness. As such, it is important to use an assay that has been analytically validated to detect the molecular alterations of interest when enrolling patients into clinical trials. This will help ensure the population enrolled in the trial(s), to the extent possible, is representative of the inclusion criteria and of the population the drug will be indicated for in the clinical setting. In addition, assays that report the specific molecular alteration (rather than “mutation positive” or “mutation negative”) can facilitate better understanding of the benefit/risk profile across the spectrum of molecular alterations represented in the trial and identification of all patients eligible to receive the drug in the clinical setting.

When a specific IVD is used to enroll or stratify patients, the trial will provide evidence for the safety and effectiveness of both the drug and the IVD. A consideration for approval of drugs indicated for patients with specific molecular alterations is the clinical availability of an FDA-cleared or -approved IVD that can identify the same patient population as those studied in the trial. In some cases, those tests may already exist in clinical practice. However, in their absence, developers may need to codevelop an IVD with the drug. When it is determined that an IVD is essential for the safety and effectiveness of a drug, that IVD is considered a companion diagnostic, and FDA may require co-approval of the assay with the drug.^{26, 27}

If a drug is approved, accurately interpreting test results is essential to identify the appropriate molecular subtypes for which the drug is indicated. Therefore, if a drug is approved for a broad, categorical group of molecular alterations such as “sensitizing mutations”, then clear rules need to be developed to determine if a specific molecular alteration identified by the IVD fits into that category and thus is eligible to receive the drug. In some cases, if clear rules cannot be established, it may be necessary to list all eligible molecular alterations in labeling. Alternatively, mechanisms could be developed to continually update the list of molecular alterations that are amenable to treatment with the drug.

FDA PERSPECTIVE ON ADVANCING PRECISION MEDICINE

The advent of next generation sequencing and other advanced genomic technologies has facilitated the development and use of targeted therapies for many diseases. While targeted therapies have resulted in medical breakthroughs and provided treatments to patients who previously lacked effective drugs, they have also created new challenges for both drug developers and regulatory agencies.

Sources of variability in drug response have long been of interest in clinical drug development of traditional “non-targeted” drugs. In some cases, patients with differences that are related to disease status (e.g., disease severity, success of prior treatments) have been studied as different populations (e.g., severity of heart failure, prior myocardial infarction vs. no prior myocardial infarction). In other cases, sources of variability (e.g., sex, age, presence of co-morbidities) are explored via post-hoc analyses of the development program. When the drug is clearly beneficial for some subsets but uncertain for others, alternative prescribing recommendations or restrictions on use based on certain patient factors are sometimes utilized to ensure that benefits are balanced against potential risks.

In the case of targeted therapies, variability in drug response may be anticipated across patients with different underlying molecular alterations because the mechanism of action of the drug is often directly linked to a specific molecular alteration or group of molecular alterations that are observed within the clinical disease. As such, targeted therapy development programs must be well-planned to identify and evaluate the molecular subgroups of the disease that are likely to benefit from the drug. It can be beneficial to group the molecular alterations according to the likely biologic impact, using evidence from multiple data sources. This evidence may further enhance understanding of drug responsiveness and help inform the indicated patient population.

In the recently released draft guidance “Developing Targeted Therapies in Low-Frequency Molecular Subsets of a Disease,”¹⁷ FDA outlines methods for generating evidence to demonstrate efficacy of targeted therapies across different molecular subtypes and general approaches for evaluating the benefits and risks of targeted therapies. This guidance details science-based strategies allowing use of multiple data sources to support efficacy of a drug in low-frequency molecular subsets of diseases where use of the traditional randomized controlled clinical trial is impractical or inadequate. However, additional framework is needed to more effectively evaluate the benefits and risks of targeted therapies across molecular subsets of a disease. Further research is needed to establish computational methods that accurately determine pathological relevance of molecular alterations, non-clinical model systems that are predictive of clinical drug efficacy, clinical trial designs for evaluating small patient subsets, and learning health systems to enable collection of real world evidence that is robust and informative.

As genomic technologies have evolved and enabled the development and utilization of targeted therapies and other precision medicine strategies, FDA has used a regulatory science based approach to develop policies that help bring safe and effective therapies to patients.²⁸ The FDA has created units with expertise in precision medicine to promote the effective utilization of genomic strategies in drug development and regulatory evaluation, established inter-center working groups to address emerging issues in drug and device development, and developed research programs to address gaps in regulatory science. This has led to the publication of multiple guidance documents to outline FDA’s current thinking on precision medicine related issues such as clinical trial enrichment, in vitro companion diagnostic devices, codevelopment of a companion diagnostic device with a therapeutic product, pharmacogenomic studies, and now, developing targeted therapies in low-frequency molecular subsets.^{17, 21, 26, 27, 29} Future advances in genomic technologies will inevitably

create new challenges for the traditional drug development paradigm, and FDA will continue to use innovative and science-based strategies to advance precision medicine and improve health outcomes.

REFERENCES

1. U.S. Food and Drug Administration. Table of Pharmacogenomic Biomarkers in Drug Labeling <http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>. Accessed November, 2017.
2. Zineh I & Pacanowski MA Pharmacogenomics in the assessment of therapeutic risks versus benefits: Inside the united states food and drug administration. *Pharmacotherapy* 8: 729–735. (2011).
3. Schuck RN, Charlab R & Blumenthal GM Leveraging genomic factors to improve benefit-risk. *Clin. Transl. Sci* 2: 78–83. (2017).
4. Pacanowski MA, Leptak C & Zineh I Next-generation medicines: Past regulatory experience and considerations for the future. *Clin. Pharmacol. Ther* 3: 247–249. (2014).
5. Zineh I & Woodcock J Clinical pharmacology and the catalysis of regulatory science: Opportunities for the advancement of drug development and evaluation. *Clin. Pharmacol. Ther* 6: 515–525. (2013).
6. Wang L, McLeod HL & Weinshilboum RM Genomics and drug response. *N. Engl. J. Med* 12: 1144–1153. (2011).
7. Biankin AV, Piantadosi S & Hollingsworth SJ Patient-centric trials for therapeutic development in precision oncology. *Nature* 7573: 361–370. (2015).
8. Sharma P & Allison JP Immune checkpoint targeting in cancer therapy: Toward combination strategies with curative potential. *Cell* 2: 205–214. (2015).
9. Clinical and Functional Translation of CFTR (CFTR2). https://cftr2.org/mutations_history. Accessed August 2017.
10. Okiyonedo T & Lukacs GL Fixing cystic fibrosis by correcting cftr domain assembly. *J. Cell Biol* 2: 199–204. (2012).
11. Cutting GR Cystic fibrosis genetics: From molecular understanding to clinical application. *Nat. Rev. Genet* 1: 45–56. (2015).
12. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Jr. & Kinzler KW Cancer genome landscapes. *Science* 6127: 1546–1558. (2013).
13. McGranahan N & Swanton C Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell* 1: 15–26. (2015).
14. Prahallad A, et al. Unresponsiveness of colon cancer to braf(v600e) inhibition through feedback activation of egfr. *Nature* 7387: 100–103. (2012).
15. Drew Y The development of parp inhibitors in ovarian cancer: From bench to bedside. *Br. J. Cancer* S3–9. (2015). [PubMed: 26669452]
16. George A, Kaye S & Banerjee S Delivering widespread brca testing and parp inhibition to patients with ovarian cancer. *Nat. Rev. Clin. Oncol* 5: 284–296. (2017).
17. U.S. Food and Drug Administration. Guidance for Industry - Developing Targeted Therapies in Low-Frequency Molecular Subsets of a Disease Accessed from <https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm588884.pdf>. (2017).
18. Schuck RN, Pacanowski M, Woodcock J & Zineh I Regulatory policy for the development of targeted therapies for low-frequency molecular subtypes of disease. *Nat. Rev. Drug Discov* (2017).
19. Woodcock J & LaVange LM Master protocols to study multiple therapies, multiple diseases, or both. *New Engl. J. Med* 1: 62–70. (2017).
20. U.S. Food and Drug Administration. Guidance for Industry - Adaptive Design Clinical Trials for Drugs and Biologics Accessed from <https://www.fda.gov/downloads/drugs/guidances/ucm201790.pdf> (2010).
21. U.S. Food and Drug Administration. Draft Guidance for Industry and Food and Drug Administration Staff - Enrichment Strategies for Clinical Trials to Support Approval of Human

Drugs and Biological Products Accessed from <http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm332181.pdf> (2012).

22. Alosch M, Huque MF, Bretz F & D'Agostino RB, Sr. Tutorial on statistical considerations on subgroup analysis in confirmatory clinical trials. *Stat. Med* 8: 1334–1360. (2017).
23. Rao JNK and Molina I (2015) Direct Domain Estimation, in *Small Area Estimation*, John Wiley & Sons, Inc, Hoboken, NJ, USA. doi: 10.1002/9781118735855.ch2.
24. Durmowicz AG, Lim R, Rogers H, Rosebraugh CJ & Chowdhury BA The fda's experience with ivacaftor in cystic fibrosis: Establishing efficacy using in vitro data in lieu of a clinical trial. *Ann Am Thorac Soc* (2017).
25. Beaver JA, et al. An fda perspective on the regulatory implications of complex signatures to predict response to targeted therapies. *Clin. Cancer. Res* 6: 1368–1372. (2017).
26. U.S. Food and Drug Administration. Guidance for Industry and Food and Drug Administration Staff - In Vitro Companion Diagnostic Devices Accessed from <https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327> (2014).
27. U.S. Food and Drug Administration. Draft Guidance for Industry and Food and Drug Administration Staff - Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product Accessed from <https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM510824> (2016).
28. U.S. Food and Drug Administration. Paving the way for personalized medicine Accessed from <http://www.fda.gov/downloads/scienceresearch/specialtopics/personalizedmedicine/ucm372421.pdf>. (2013).
29. U.S. Food and Drug Administration. Guidance for Industry and Drug Administration Staff - Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling Accessed from <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM337169.pdf>. (2013).

Figure 1.

Types of molecular heterogeneity.

A clinically-defined disease may have varying degrees of diversity in the molecular etiology underlying the clinical phenotype including A) uncharacterized molecular etiology, B) known molecular etiology in most patients with a single pathological variant, C) known molecular etiology in most patients with multiple variants, some of which occur at low frequencies, and which in some cases can be grouped into categories based on functional effect or likelihood that they are amenable to treatment with a targeted therapy that has a specific mechanism of action (categories are denoted by shading) and D) known molecular etiology in most patients with multiple variants, all of which occur at low frequencies but may be amenable to grouping.

Table 1.
Challenges in developing targeted therapies for low-frequency molecular subtypes within disease.

Drug Development Issue	Review/Policy Issue
Patient selection for clinical trial enrollment	Balancing use of molecular enrichment criteria against need for enrolling patients without the enrichment factor for benefit/risk assessment
Interpretation of clinical trial results	Determining benefit/risk in patients who have molecular alterations that were poorly represented in clinical trials
Generalizability of findings from clinical trials	Extrapolating overall findings from clinical trials to putatively similar molecular subtypes
Drug Labeling	Determining population for whom the drug should be indicated and how safety and efficacy data should be communicated in labeling

Table 2.

Potential drug development approaches for evaluating targeted therapies.

Approach	Advantages	Disadvantages
Evaluating a single molecular alteration	Ideal if the drug is expected to be effective in only a single alteration Straight forward evaluation of drug effect in the studied subset Effective if a single alteration underlies disease in all or nearly all patients Simplified diagnostic development	No data in other alterations Approval likely limited to a single molecular alteration when the drug may be effective in other alterations May screen large numbers of patients who are not eligible
Evaluating each molecular alteration individually in separate trials	Straight forward evaluation of drug effect in each alteration studied Simplified diagnostic development	Difficult enrolling adequate numbers of patients for trials evaluating low-frequency alterations (and large numbers screened and not eligible) Limits the generalizability of study results to other alterations Does not generate evidence of efficacy in all patients hypothesized to benefit from the drug
Grouping putatively similar alterations for evaluation in a single trial	Allows assessment of efficacy in all patients who hypothesized to benefit from the drug Fewer ineligible patients will be screened	Requires thorough understanding of the functional effects of molecular alterations (and may enroll patients who are unlikely to respond if grouping strategy is not appropriate) More complex diagnostic development
Enrolling all patients with the clinically-defined disease	Allows for evaluation across all molecular alterations	Trial is not enriched for patients likely to respond and enrolling patients who are unlikely to respond may lower the benefit/risk ratio or result in an unsuccessful trial

Table 3.

Selected examples of FDA-approved targeted therapies in diseases with low-frequency molecular alterations.

Disease/ Mechanism	Drug(s)	Indicated Subset in FDA-Approved Labeling	Low-Frequency molecular alteration(s) within disease
NSCLC/ EGFR inhibitor	Erlotinib	EGFR exon 19 deletions or exon 21 L858R substitution	Additional <i>EGFR</i> mutations including L861Q, G719A/C/S, and T790M
	Gefitinib		
	Afatinib	Non-resistant <i>EGFR</i> mutations [*]	
Melanoma/ BRAF inhibitor	Vemurafenib	<i>BRAF</i> V600E mutation [†]	<i>BRAF</i> V600K/D/R
	Dabrafenib		
Melanoma/ MEK inhibitor	Trametinib	<i>BRAF</i> V600E/K mutations	<i>BRAF</i> V600D/R
Ovarian cancer/ PARP inhibitor	Olaparib	Deleterious or suspected deleterious germline <i>BRCA</i> mutations	Most individual <i>BRCA1</i> and <i>BRCA2</i> mutations occur at low frequency in ovarian cancer
	Rucaparib	Deleterious germline and/or somatic <i>BRCA</i> mutations	
Colorectal cancer/ EGFR inhibitor	Cetuximab	<i>KRAS</i> wild-type (not <i>RAS</i> mutations) [‡]	Low-frequency <i>KRAS</i> mutations outside of exon 2, other <i>RAS</i> family mutations
	Panitumumab	<i>KRAS</i> wild-type (exon 2) (not <i>RAS</i> mutations) [‡]	
Cystic fibrosis/ CFTR potentiator	Ivacaftor	<i>CFTR</i> mutations that are responsive to ivacaftor based on clinical and/or in vitro assay data [*]	
Duchenne muscular dystrophy/ exon skipper	Eteplirsen	<i>DMD</i> exon 51 skipping amenable	All individual mutations occur at low frequencies

^{*} Refers to current labeling at the time of publication; the indicated population was expanded to include additional mutations after the initial approval

[†] Initially approved indication that has since been expanded to include Erdheim-Chester Disease with *BRAF*V600 mutation

[‡] Refers to current labeling at the time of publication; labeling at the time of approval did not have information on *KRAS*

Definitions: NSCLC: non-small cell lung cancer, EGFR: epidermal growth factor receptor, BRAF: serine/threonine-protein kinase B-raf, PARP: poly (ADP-ribose) polymerase, MEK: mitogen-activated protein kinase kinase, CFTR: cystic fibrosis transmembrane regulator