**Opportunities and Challenges in Oncology Targeted Drug Development: An Assessment of the Use of Prevalence and Companion Diagnostic Performance Thresholds to Guide Clinical Trial Strategies**

by

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## Abstract

Targeted, especially stratified or biomarker-guided, therapies offer significant advantages over traditional oncology therapies in certain settings. Selecting patients most likely to respond to a drug increases the therapeutic efficacy while reducing toxicities and may accelerate regulatory approval since smaller clinical trials are needed to demonstrate benefit. Several drugs, including vemurafenib and crizotinib have demonstrated these benefits along with commercial success. However, significant risk exists for the drug developer since approval may be threatened if they fail to meet unclear and differing yet parallel requirements for both the drug and the required companion diagnostic. Tumor biology is also increasingly complex since recent studies suggest that there are limited numbers of individual driver mutations, complicated interactions throughout signaling pathways as well as extensive tumor heterogeneity, all of which will challenge the effectiveness of targeted therapies.

Clinical trial strategy decisions can greatly impact the success of a targeted therapy due to these challenges. While therapeutic efficacy is considered important, biomarker prevalence and companion diagnostic performance have been shown to be as important, yet more informative at the time decisions are made. I hypothesized that common prevalence and companion diagnostic performance thresholds are being used to guide biomarker-guided clinical trial strategy decisions for targeted oncology therapies. Seventeen interviews with preclinical, clinical or translational leads were conducted across a focused set of ten "pathway-modifying" cancer drug programs (CDK4/6, MDM2 and PI3Kβ inhibitors) that reflect the biological complexity of future targeted therapies. These interviews provided empirical data as to how biomarkers are being incorporated into current clinical trial decisions.

All respondents were planning to use a companion diagnostic for their program, however, the use of biomarkers varied significantly. For those programs with ongoing clinical trials in phase I and II, 54% (n=7/13) were pursuing a biomarker-guided strategy while 46% (n=6/13) were using an initial all-comers strategy. This fairly equal split separated when compared by phase where trials in phase I and I/II, 60% (n=6/10) were using an all-comers strategy but for those trials in phase II (n=3), all were using biomarker-guided strategies. A key finding of the interviews was that 66.7% (n=4/6) were planning biomarker enrichment as part of expansion plans. Disproving my hypothesis, however, common thresholds for neither biomarker prevalence nor companion diagnostic performance were being used to guide these decisions. Biomarker prevalences of 50-100% were stated as potentially appropriate for an all-comers strategy. Companion diagnostic performance thresholds were even less influential as only a few respondents provided a general range of desired sensitivity and specificity. This study found that actions of drug developers are not necessarily following the emerging recommendations for targeted therapies due to the significant challenges of biomarker and companion diagnostic development.

Thesis Supervisors: Ernst R. Berndt, PhD – MIT Sloan School of Management
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# 1. Introduction

Several successful stratified therapies have emerged over the past decade with the evolution of "personalized" medicine. Targeted therapies have been developed with many of them being restricted to populations that were likely to respond using a biomarker. These biomarker-guided therapies provide substantial advantages over traditional cancer drug development. Therapeutic efficacy can be improved and the toxicities reduced with a biomarker-selected population and smaller clinical trial sizes may be needed therefore accelerating the approval timeline with potentially less cost. While a biomarker-selected population is smaller than the overall market, the improved therapeutic window and efficacy allows for a dynamic in which adoption can be maximized and administered in a chronic setting, thereby increasing the commercial value to a desired level. This has led to different clinical trial designs, which while useful for stratified therapies, are not universally applicable across targeted therapies and serious consideration needs to be given to choosing between a biomarker-selected population or leveraging the strengths of a traditional all-comers approach in early stage clinical trials.

Several disadvantages exist for using smaller clinical trials, such as not having a diverse or large enough patient population to detect responses, toxicities and perhaps even the correct biomarker. The use of a selection biomarker requires a certain level of validation and that level is yet to be defined, which adds confusion and possibly impacts the success of the therapeutic. Additionally, to have a drug apply to only a biomarker-selected population, the patients must be reliably identified by a diagnostic test, which has its own significant challenges. Recent US Food and Drug Administration (FDA) guidelines are also recommending that the diagnostic, termed a companion diagnostic, would be approved in parallel with the drug. These requirements are still evolving and create uncertainty as to not only the technical specifications of the diagnostic but also how best to incorporate the biomarker development into the

drug process. Significant risk exists for drug developers of biomarker-guided targeted drugs since there is a lack of companion diagnostic expertise within most companies and the technology is constantly evolving. Another critical challenge is that as our knowledge of tumor biology increases, the landscape for significant drug targets and the ability to inhibit them, similar to those that contributed to the success of imatinib, vemurafenib and crizotinib, decreases. Reduced molecular aberrations that drive tumorigenesis, interaction and signaling throughout the biological pathway and tumor heterogeneity all contribute to this increased biological complexity.

There are a multitude of factors that contribute to the eventual commercial success of a therapy from the clinical trial assumptions (size, cost, time, etc.), to the probability of technical and regulatory success (therapeutic efficacy, toxicities, approval, etc.), and the overall market (size, prevalence, share, price, adoption, etc.). Targeted therapies using a biomarker-guided strategy add factors relating to the biomarker definition and companion diagnostic performance. However, the factors that are believed to have the most impact for stratified medicines are therapeutic efficacy, biomarker prevalence, and the companion diagnostic performance. Unfortunately, when making critical clinical trial decisions, true therapeutic efficacy is not known due to the biological complexity and lack of representative preclinical models.

Prevalence and companion diagnostic performance are therefore the key inputs to determine the clinical trial strategy for a targeted oncology therapy. Through interviews with leads of drug development programs, in this pilot study I assess the use of and hypothesize that common prevalence and companion diagnostic thresholds are being used to help guide early-stage biomarker-guided clinical trial strategy decisions. To evaluate this hypothesis empirically, I examined several molecular targets of

drugs in development, described here as "pathway-modifying" targeted therapies that illustrate the challenges of increased biological complexity.

This thesis is organized in the following manner. The Background section will provide an overview of not only the factors contributing to the success of targeted oncology and stratified therapies but also the critical challenges they may face that minimize future successes. Targeted therapies will be defined in terms of mechanisms but also by their use within a biomarker selected patient population. Case studies of trastuzumab, imatinib, and more recent examples, vemurafenib, and crizotinib, illustrate the benefits that stratified medicine can have on the development of oncology therapeutics. The challenges surrounding regulations and scientific biological complexity will also be discussed to provide perspective on the concerns that threaten future success for targeted therapies. As part of this thesis, drug targets were chosen to represent the level of biological complexity that is expected to continue for targeted drug developers. A section provides a scientific overview of these targets to help illustrate how key findings of scientific investigation are impacted and the potential implications on developing therapies. The Study Purpose and Methodology section will provide an overview of the resources used in the course of this study and the particular dataset that was used for qualitative investigation. The interview guide is also shared to provide a view into the discussions held with interviewees. Relevant interview responses are discussed and summarized in the Results section. The Discussion section will provide a summary of the findings and insights on the context and concerns for drug developers. There are several areas for future research that will be suggested, but the thesis will conclude with a summary of the analysis and implications for biomarker-guided targeted drug development.

## 2. Background

### Emergence of Targeted Therapies

Targeted oncology therapies, or "personalized"/"stratified" medicines for those targeted agents being used in biomarker-selected populations, have grown in prevalence and importance in medical treatments since the emergence of endocrine receptor positive breast cancer. To illustrate the increase across therapeutic areas, in 2006 there were only 13 examples of personalized medicine drugs, treatments and diagnostics products while in 2011 that number grew to 72 (Personalized Medicine Coalition, 2011). Growth in this area will continue since while only 30% of all treatments in late clinical development rely on biomarker data, 50% of those in early clinical development and 60% of all treatments in preclinical development do (Tufts Center for the Study of Drug Development, 2010). The growth is likely a result of the clinical, regulatory and commercial success of the types of therapies, especially in oncology indications.

Traditional cancer chemotherapy works by inhibiting cell division through several mechanisms. Alkylating agents interfere with DNA base pairing which prevents DNA replication, antimetabolites also prevent DNA replication by blocking the use of essential nucleic acids, while topoisomerase inhibitors block DNA uncoiling and taxanes and vinca alkaloids interfere with mictotubule function (Gerber, 2008). Since many normal rapidly dividing cells are also affected by these therapies, significant toxicities can result. Unfortunately, in addition to the severe side effects patients experience with traditional chemotherapy, the level of efficacy has been limited. Curative outcomes in patients with advanced cancers have been restricted to the subpopulations with testicular cancer, leukemia, and lymphoma, which account for fewer than 10% of all cancer cases (DeVita, 2011). Only a modest life-prolonging or

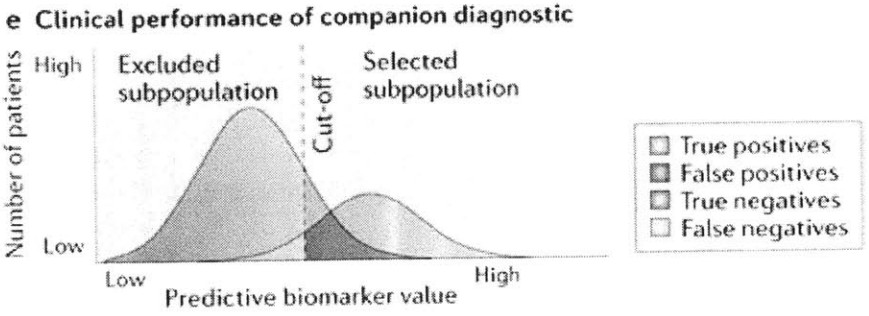
palliative benefit has been observed in several more common cancers. Additionally, there is only a 25% rate of efficacy with standard drug treatment across cancer types (Aspinall, 2007).

Targeted therapies, on the other hand, prevent the proliferation of tumor cells by blocking or interfering with specific molecules that have been implicated in tumor formation, proliferation, and metastasis. Often, these molecular mediators are mutated or overexpressed in tumor cells. In theory, increased efficacy compared to traditional chemotherapy would be anticipated by targeting molecules that are fundamental in the formation or dissemination of a patient's tumor. Toxicities would also likely be reduced by targeting molecules and pathways that cancers have "selected" as conferring a growth or survival advantage, whereas normal tissues take advantage of the redundancy of proliferative and survival pathways and are less harmed by inhibition of a specific signaling molecule or pathway. Another benefit to targeted therapies is that with reduced toxicities, treatment options become available for those that would be unable to tolerate traditional chemotherapy (Gerber, 2008) and they can be administered over many years in a chronic setting.

Targeted therapies may or may not utilize a biomarker to help identify which patients would most likely respond to the drug. The term biomarker can be used broadly and includes those markers used to inform target and pathway modulation and decisions surrounding pharmacokinetics (PK), pharmacodynamics (PD), and pharmacogenomics. For the purposes of this thesis, the term biomarker is one that is predictive and used to stratify or select a patient population for those "associated with response to or lack of response to a particular therapy" (Yap, 2010). Biomarkers also can be characterized differently as dichotomous or continuous. A dichotomous biomarker is one in which there is a binary yes or no decision as to whether a patient has a given biomarker. A continuous biomarker is one where a "cutoff" point for being considered biomarker positive needs to be made. Essentially there

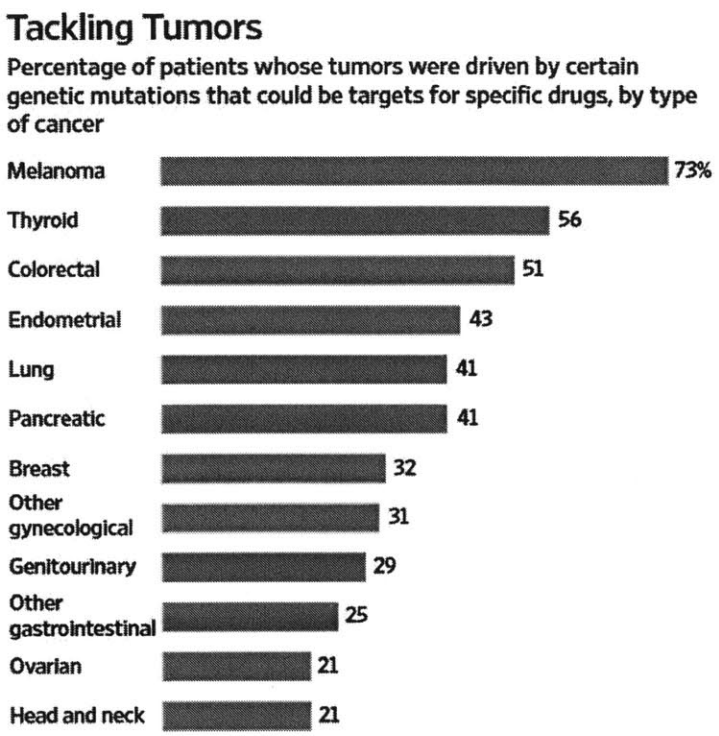
is a range of biomarker positive and negative testing responses that overlap and represent a potential continuum of response to any given therapy (see Figure 1 for example).

**Figure 1. Continuous biomarker cut-off example** (Trusheim, 2011)




Some of the most visible early examples of stratified or biomarker-guided targeted cancer therapies were trastuzumab (Herceptin, Genentech/Roche) for breast cancer in 1998 (a continuous biomarker) and imatinib (Gleevec, Novartis) (a dichotomous biomarker) for chronic myelogenous leukemia (CML) in 2001 (FDA). In metastatic breast cancer, the overall response rate for Herceptin was 45% as compared to 29% in the control group and median time to progression increased from 2.7 months with the control group to 7.2 months with Herceptin for the approximately 25% of breast cancer patients with overexpression of HER2 (Trusheim, 2011). Imatinib dramatically changed the landscape of CML treatment by targeting what was found to be the driving mutation in over 90% of patients, the BCR-ABL gene. The five-year survival rate once imatinib was introduced as a first-line therapy for CML increased from approximately 50% to 89% (Druker, 2006). Since the emergence of these successes, it has become clear that targeted therapies have applications in a wide range of cancer types (See Figure 2). Both companies and patients have been able to benefit from the shift to stratified medicine.

**Figure 2. Frequency of drug targets by indication** (Winslow, 2011)




## Benefits of Stratified Medicine in Oncology

Several case studies have demonstrated significant benefits to developing targeted therapeutics using a stratified patient population strategy. Favorable risk/benefit profiles have been documented across dozens of therapeutics through limiting the clinical trial patient population to those expected to benefit from the drug or not experience adverse events. An accelerated approval timeline has also emerged, especially with the approval of two recent compounds and commercial value has been proven despite the typically unfavorable smaller applicable patient populations.

The cases of trastuzumab and imatinib provide clear benefits for efficacy as compared to traditional chemotherapy as mentioned above. Subsequent targeted therapies also provided improved response rates as shown in the table below. Trials utilizing these stratified medicines were conducted with biomarker-guided strategies where patient selection was limited to those having the hypothesized target aberrations. Limiting the patients to those likely to experience a benefit theoretically increased the therapeutic efficacy.

**Table 1. Examples of early clinical trial response rates** (adapted from Dienstmann, 2012)

| Marker/Population | Agent | Mechanism of Action | Response |
|---|---|---|---|
| CD117-overexpressed GIST | Imatinib | c-KIT, PDGFR inhibitor | 54% |
| BRCA1/2 mutant breast, ovarian and prostate cancer | Olaparib | PARP inhibitor | 47% |
| BRAF V600E-mutant melanoma | Vemurafenib | BRAF inhibitor | 75% |
| | Dabrafenib | BRAF inhibitor | 60% |
| ALK-rearranged NSCLC | Crizotinib | ALK, MET inhibitor | 57% |
| Basal cell carcinomas (majority have inactivating mutations in PTCH1 or activation of SMO) | Vismodegib | SMO inhibitor (Hedgehog pathway) | 58% |

The increased therapeutic efficacy with a biomarker-guided targeted therapy allows for significantly smaller clinical trials to demonstrate a clinical benefit and accelerates the path to approval. Vemurafenib (Zelboraf, Roche) and crizotinib (Xalkori, Pfizer) were approved in August 2011 with a corresponding companion diagnostic (Roche's cobas 4800 BRAF V600 Mutation Test and Abbott's Vysis ALK Break-Apart FISH Probe Kit, respectively) to reliably identify those patients that would benefit from the treatment (FDA). Both drugs received accelerated approval under the FDA's priority review program for those therapies addressing critical unmet needs (Chabner, 2012). Vemurafenib was able to achieve approval with a single arm phase II study and a randomized (but with crossover) phase III trial totaling 807 patients. An early phase I study was able to show an 81% response rate with only 32 patients.

Clinical development was clearly accelerated as approval was achieved only four years after the first patient entered the phase I trial. Crizotinib achieved approval in only three years after the first patient entered the phase I trial. The pivotal trials were an 82 patient expansion cohort of a phase I trial and a confirmatory phase II trial with 173 patients for non-small cell lung cancer. These therapies illustrate that drug development timelines can be shortened by using biomarker-guided clinical trial strategies where the subpopulation constitutes an unmet need and where smaller sample sizes are required to show significantly greater therapeutic efficacy over standard therapy.

Further clinical success of targeted therapies over traditional chemotherapy was reflected in regulatory approvals as well. From 2000 to 2008 only five traditional chemotherapeutic agents were approved as new anticancer therapies by the FDA while in that same timeframe, 15 targeted therapies were approved (Gerber, 2008). As of this year, 2012, almost two-dozen targeted therapies (both first in class and follow-on molecules) have been approved. A breakdown of those therapies, their targets and indications is listed in the table below.

**Table 2. FDA approved targeted therapies for cancer treatment** (Li, 2012)

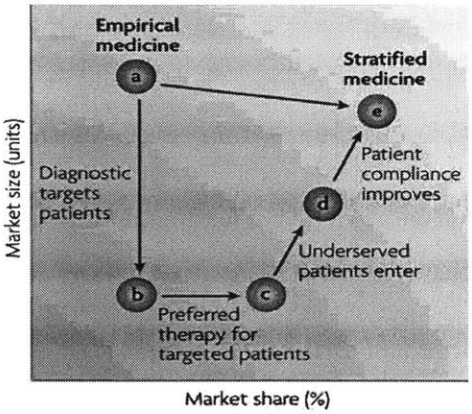
| Name | Targets | Oncology uses |
|---|---|---|
| Small molecule inhibitors for cancer | | |
| Dasatinib | BCR-ABL, SRC family, c-KIT, PDGFR | Chronic myeloid leukemia (CML), acute lymphocytic leukemia |
| Erlotinib | EGFR | Non-small cell lung cancer(NSCLC), pancreatic cancer |
| Gefitinib | EGFR | NSCLC |
| Imatinib | BCR-ABL, c-KIT, PDGFR | Acute lymphocytic leukemia, CML, Gastrointestinal stromal tumor |
| Lapatinib | HER2/neu, EGFR | Breast cancer |
| Sorafenib | BRAF, VEGFR, EGFR, PDGFR | Renal cell carcinoma(RCC), Hepatocellular carcinoma |
| Sunitinib | VEGFR, PDGFR, c-KIT, FLT3 | RCC, gastrointestinal stromal tumor |
| Temsirolimus | mTOR, VEGF | RCC |
| Pazopaniba | VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-a/β, and c-kit | RCC |
| Nilotinib | BCR-ABL | CML |
| Crizotinib | ALK, HGFR | NSCLC |

| | | |
|---|---|---|
| Vemurafenib | BRAF | Late-stage melanoma |
| Monoclonal antibodies for cancer | | |
| Alemtuzumab | CD52 | Chronic lymphocytic leukemia |
| Bevacizumaba | VEGF | Colorectal cancer, NSCLC, RCC |
| Cetuximab | EGFR | Colorectal cancer, head and neck cancer |
| Gemtuzumab Ozogamicin | CD33 | Relapsed acute myeloid leukemia |
| Ibritumomab Tiuxetan | CD20 | Non-Hodgkin's lymphoma (NHL) (with yttrium-90 or indium-111) |
| Panitumumab | EGFR | Colorectal cancer |
| Rituximab | CD20 | NHL |
| Tositumomab | CD20 | NHL (with Iodine-131) |
| Trastuzumab | HER2/neu | Breast cancer with HER2/neu overexpression |

Commercial success has also been achieved despite initial concerns that smaller patient populations would limit revenues. The biomarker prevalence for each therapy varies widely from >90% for BCR-ABL and imatinib, 40-60% for BRAF and vemurafenib to 4-6% for ALK and crizotinib. Clearly, pre-selecting patients can severely reduce a potential patient population. However, a number of factors contribute to each therapy's overall success thereby compensating for the smaller market. As mentioned previously, by including only those patients likely to respond to the drug through biomarker-selection, smaller numbers of patients are needed in clinical trials to establish clinical evidence of benefit. The design of these targeted therapies also allows the drug to be taken chronically, which extends the overall market. This has led to the concept of patient-years being a better measurement for an oncology market opportunity. Imatinib is a clear example of this in CML where the drug is administered chronically as a maintenance therapy for years and not a single administration regimen as in the cases of traditional chemotherapy. Trastuzumab also utilizes the same principle where patients will typically receive the drug in combination with multiple lines of other therapies (Flaherty, 2012). The positive risk/benefit profiles help to drive stronger adoption by physicians and better compliance by patients, but also helped

to set a new standard for oncologic agent pricing; over $100,000 for a year of treatment (National Cancer Institute, 2011). An example of this dynamic is illustrated in the figure below.

**Figure 3. Factors contributing to commercial value of stratified therapies** (Trusheim, 2007)




Imatinib (and its follow-on nilotinib) brings in over five billion in revenue per year (Novartis, 2011). This represents a clear commercial success for Novartis that initially was applicable to only 9,000 patients worldwide (Novartis, 2001).

## Stratified Medicine Regulatory Environment

Evidence-based medicine and the regulations structured to support it require a novel therapy to prove superiority (or in many cases non-inferiority) over the current standard of care that ideally is accomplished through large, prospective, randomized, and multi-centre studies. With the increased approvals of targeted agents, the FDA has adapted (as seen by the targeted agents discussed previously) and is attempting to provide stronger guidance on the requirements for drugs being used in smaller (often orphan) indications and only in molecularly-stratified patients. Last year in July 2011 the FDA issued its first draft guidance (final issuance date is unknown) for the use of in vitro companion diagnostic devices (IVD). It was meant for not only the device manufacturers but also those sponsors

"who are planning to develop a therapeutic product that depends on the use of an in vitro companion diagnostic device (or test) for its safe and effective use". It recommends that for a novel therapeutic product, an IVD should be developed and approved "contemporaneously". The FDA plans to ensure that the IVD is properly validated and meets acceptable safety/efficacy standards before approving the therapeutic product (FDA, 2011). It is important to note that the regulatory processes for each have their own nuances and requirements as they are reviewed by two separate branches; Center for Drug Evaluation and Research (CDRH)/Center for Biologics Evaluation and Research (CBER) for drugs and Center for Devices and Radiological Health (CDRH) for devices. The most recent approvals of vemurafenib and crizotinib followed this strategy ensuring that the commercial assay to be used was validated as part of the clinical trials. Interestingly, this guidance follows the strategy several other targeted oncology products took as well; Herceptin and its companion diagnostic by Dako achieved joint approval in 1998, as did Dako with its EGFR pharmDx for Erbitux and Vectibix in 2004 and 2006 respectively (FDA).

In order to comply with these regulations drug makers will need to adopt practices to develop a reliable method of identifying those patients likely to benefit from the therapeutic during clinical trials and essentially validate the drug, novel biomarkers and an associated IVD for approval. The significant risks can be disproportionate with biomarker-guided clinical trials since if the companion diagnostic is not able to meet its technical or scientific specifications through registration trials, the approval of the drug is also threatened. Therefore, specific activities related to the companion diagnostic must begin very early in the clinical trials process to ensure that a reliable assay is available for pivotal trials (potentially as early as phase II).

## Challenges for Stratified Medicine in Oncology

The decision whether to stratify the patient population based on a predictive biomarker remains challenging despite recent successes of therapies using predictive biomarker based strategies. The frequent practice of limiting the patient population, especially in early clinical trials has significant drawbacks. Phase I trials are typically used to ensure safety and find potential doses for the next set of clinical trials (Yap, 2010). By limiting the patient enrollment it is possible that important off-target effects, that could mediate efficacy and broader market potential, could be missed. There is also the possibility that target inhibition could be dose-dependent and by not including a range of patients with varying target levels potential benefits to a wider patient population could be missed. For example, expression of proteins is a common biomarker and while high expression may respond at the highest levels, by not including lower levels of expression responses (as occurred with Herceptin) would not be detected and developed further. The biological complexity and heterogeneity issues noted above can contribute to the fact that a target and its biomarker may not be validated to the extent necessary. With small and restricted patient populations the drug could actually impact other targets or not be the primary marker of response sensitivity. The success of crizotinib actually began as a MET inhibitor and only after further investigation was its effect on ALK mutations found. Sorafenib is another example where it was originally thought to be a targeted inhibitor of RAF but in fact was a potent VEGF2 inhibitor (Carden, 2010). The complicated EGFR history provides an example of how the true marker can be missed as well (Janku, 2011). Although developers and regulatory authorities focused on EGFR mutations, KRAS proved to be the primary (albeit negative) predictor of response to cetuximab and panitumumab. The subsequent validation of KRAS went so far as to remove EGFR as a biomarker since the NCCN states in its guidelines "EGFR testing has no demonstrated predictive value, and therefore routine EGFR testing is not recommended" (National Comprehensive Cancer Network, 2009). These

primary scientific and clinical challenges directly reflect the biological complexity. Clearly, they can impact the success of choosing a stratified medicine strategy for a novel anticancer agent.

Additional regulatory and cost challenges still exist despite the FDA's commitment to improving the process and collaboration to bring biomarker-stratified medicines to the market. There is no consensus as to how a targeted therapy should be studied in a selected population. The FDA Oncologic Drugs Advisory Committee initially suggested that therapies should be evaluated in both biomarker-positive and biomarker-negative populations, which would require extremely large trials and negate the benefit of smaller, faster and cheaper development for targeted therapies. The therapy that should serve as a comparator is also unclear as some biomarkers confer a tumor growth advantage (or disadvantage in terms of clinical outcomes) and may identify a subpopulation of patients who fare poorly with current standard of care treatments (Schilsky, 2011). The FDA also desires insight regarding the natural history of the biomarker selected patient populations and not just the response to a therapy. This can be challenging for companies that do not have access to this type of information or the tumor samples needed to produce this type of information (Flaherty, 2012). Dr. Hakan Sakul, who was responsible for the diagnostic associated with crizotinib on the Pfizer side, stated that a key learning from their experience was to meet with the FDA and CDRH early to understand the diagnostic pathway (Sakul, 2011).

Finding the appropriate patients and the time and cost to do so also adds additional considerations (Schilsky, 2011). A total of 2107 patients were screened to enroll 675 for vemfuramib's pivotal trial. Each of those screenings incurred the cost of the diagnostic and added time to identifying those patients (Roche/Genentech, 2011). A more drastic example is that for crizotinib, Pfizer/Abott screened approximately 1,500 patients in order to identify the 82 patients enrolled in the phase I clinical trial

(Kwak, 2010). To make the diagnostic validation and clinical trial process more efficient, recent targeted therapies have used the drug clinical trials as evidence for both the drug and diagnostic approvals. While this approach can be efficient, the sponsors and investigators must be diligent to include all relevant and differing requirements for approvals from separate regulatory agencies (Phillips, 2006).

The success of companion diagnostics associated with approved targeted therapies and the regulatory requirements around using the therapies in a selected population has likely helped to drive the surge in drug and diagnostic company co-development deals (PricewaterhouseCoopers, 2011). There are many challenges to these co-development deals, however, and it may be more prudent to avoid the process through not limiting or selecting the patient population. The relationship of drug and diagnostic developers is complicated due to mismatched timelines and incentives. During a discussion at the 2011 Personalized Medicine Conference, Dr. Stafford O'Kelly from Abbott stressed that the two-year timeline (due to crizotinib's accelerated approval) to develop a commercializable companion diagnostic was difficult and Pfizer/Abbott were only able to achieve it because there were other sources of data to support a modular submission. If a companion diagnostic is required, he stressed that an IVD should be developed as soon as possible and be available for phase II studies, which is difficult given the biological complexity as discussed below (O'Kelly, 2011). In addition to the fact that diagnostics have their own regulatory and phase I through III testing as mentioned previously, the product development cycle of diagnostic devices is slightly different from that of drugs in that there is typically an iterative process during which subsequent refinements will be made. For example, HER2 testing has undergone considerable changes and improvements since its first introduction, with a new test even being approved this year, more than a decade after its development (FDA). The lack of investment in diagnostics and the limited reimbursement rates may also make it difficult to attract a diagnostic

partner and ensure their commitment for something that is absolutely critical to the drug and its significantly greater commercial value (Phillips, 2006).

## Increasing Biological Complexity

Advancements in genomics and other diagnostic technologies have enabled a stronger understanding of tumor drivers. Unfortunately, recent investigations have shown an Increasing biological complexity that will be a key consideration for future targeted drug developers. Projects such as The Cancer Genome Atlas (TCGA) have shown remarkably few genes that are mutated at a high frequency across cancer indications (Yauch, 2012) suggesting that clear drivers such as BRAF and ALK will be difficult to come by in the future.

Another key challenge is that not all mutations or aberrations are druggable. Oncogenes relate to activated molecules that can be targeted and inhibited with drugs. Oncogenes can transform the cells to drive cellular proliferation. The BRAF V600E mutation and ALK-EML4 fusion are examples of these types of events (Flaherty, 2012). "Oncogene addiction" is a concept where one or a few genes are responsible for both the maintenance of the malignant phenotype and cell survival (Weinstein, 2006). The BCR-ABL gene for CML is a prime example of this type of cancer and perhaps the driving rationale for targeted therapies. Tumor suppressor genes, on the other hand, are those molecules where their function, controlling cell proliferation, is disabled. Inactivation of a tumor suppressor leads to an increased likelihood of cancer development (Weinberg, 2007). Unfortunately, current technology does not allow the restoration of tumor suppressor function (Flaherty, 2012). When tumor suppressor function is compromised it is critical to consider the overall signaling pathway in developing therapeutic

strategies and due to the additional scientific challenges there is the risk of sacrificing the benefits of a targeted therapy approach.

As sensitivity of testing for mutations improves, distinguishing the key driver mutations from "bystander" or "passenger" mutations becomes increasingly difficult, as there are a large number of mutations that occur in any given cancer cell. For example, a study of 33 lung neoplasm samples and lung cancer cell lines discovered 188 somatic mutations across 141 genes. Most of them were passenger mutations that were not causal in tumor development (Davies, 2005). Melanoma also confers a high passenger mutation rate due to carcinogenic UV light exposure (Hodis, 2012). Intense analysis is required to understand what actually constitutes as a "driver" of tumor growth. The first TCGA project examined glioblastoma and found eight genes mutated with sufficient frequency to act as drivers, including alterations in the tumor suppressor NF1, PARK2, and amplifications in AKT3 which were previously unreported in glioblastoma. (The Cancer Genome Atlas Research Network, 2008). A recent melanoma study identified six novel genes as potential driver mutations (Hodis, 2012).

Analyses have also shown a high level of interaction amongst potential drivers thereby increasing the challenge to actually generate a response to an individual targeted agent. Of the glioblastoma samples, 74% of tumor samples contained aberrations in three (Rb, TP53 and RTK) separate pathways (The Cancer Genome Atlas Research Network, 2008). Within melanoma, almost 44% of those with highly recurrent mutations in BRAF also harbored a PTEN aberration in addition to several unique characteristics of p53 mutations (Hodis, 2012). While such observations point toward the rationale for developing combination regimens of multiple targeted therapies, the success of an individual targeted therapy is in jeopardy.

The multiple levels of heterogeneity that exist in each tumor add another later of challenges, making truly understanding the biology of any given tumor extremely difficult. Genomic profiles differ between cancer types (or origin of the tumor) but also within the same cancer lineage (within the tumor origin) and even the cells in the same tumor (Yauch, 2012). A key study, recently published in the New England Journal, that revealed the extent of intratumor heterogeneity. Of all the somatic mutations found in four separate tumors, 63% to 69% were not detectable in every sequenced region; therefore a majority of mutations were not found across all sites of disease within a single patient (Gerlinger, 2012). Resistance mutations contribute to a tumor's biological complexity, heterogeneity, and possibly limited impact of a targeted therapy as well. The glioblastoma study found samples of previously treated tumors were "hypermutated" as compared to those samples that were untreated (The Cancer Genome Atlas Research Network, 2008). Resistance emerges due to mutations within the same driver present in a subpopulation of tumor cells, due to acquired mutations or activation of other pathways. Resistance is perhaps best exemplified by imatinib where soon after its introduction, mutations within the BCR-ABL gene prevented the drug's inhibition of ABL signaling (Gorre, 2001). Secondary mutations for the EML4-ALK gene have been reported which contribute to resistance to crizotinib as well (Choi, 2010). Therefore, any given sample may not inform a tumor's biology and restrict understanding of any given therapeutics' impact on the tumor and its molecular aberrations.

## Scientific Overview for Examples of Complex Biological Targets

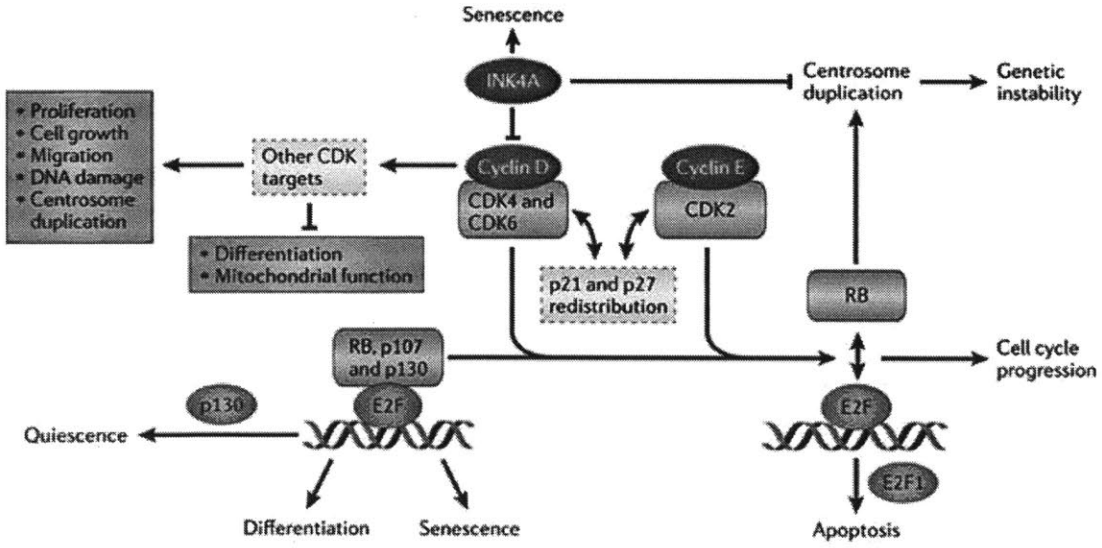
Drug targets CDK4/6, MDM2 and PI3Kβ represent examples of emerging biological complexities involved in regulating known tumor suppressor genes, p16, p53 and PTEN respectively. With deletion of the entire gene, inactivating mutations in key functional domains, or decreased protein expression, it is the absence of these molecules that allows others to function in an unopposed fashion. Currently, it is not

possible to restore the lost function or expression of these molecules and therapeutic approaches are left to focus on targeting the molecules that are overactive as a consequence of these lost tumor suppressor genes/protein (Flaherty, 2012). Among other targets, these tumor suppressor genes can act as biomarkers for inhibition of the protein targets. This class of therapies can be categorized as "pathway modifying" since the overall pathway is involved in determining sensitivity and a patient's response to the drug.

**CDK4/6 and p16**

Activation of specific kinases at various cell cycle states coordinates cell proliferation and halt progression in response to DNA damage. These cyclin-dependent kinases (CDKs) include CDK4 and CDK6, which are activated by their regulatory partners, cyclin Ds, and phosphorylate the tumor suppressor Rb thereby promoting the activation of the E2F genes required for DNA synthesis (Musgrove, 2011).

**Figure 4. Cyclin-dependent pathway and function** (Musgrove, 2011)



INK4A, also known as CDKN2A and p16, acts as an inhibitory protein to CDK4/6 as it competes for binding with cyclin D and prevents the catalytic activity required to drive the cell cycle (Knudsen, 2010). p16 arrests the cell cycle during senescence when cyclin Ds are released and p16 therefore acts as a negative regulator of CDK4/6 and overall cell cycle progression (Shapiro, 2006).

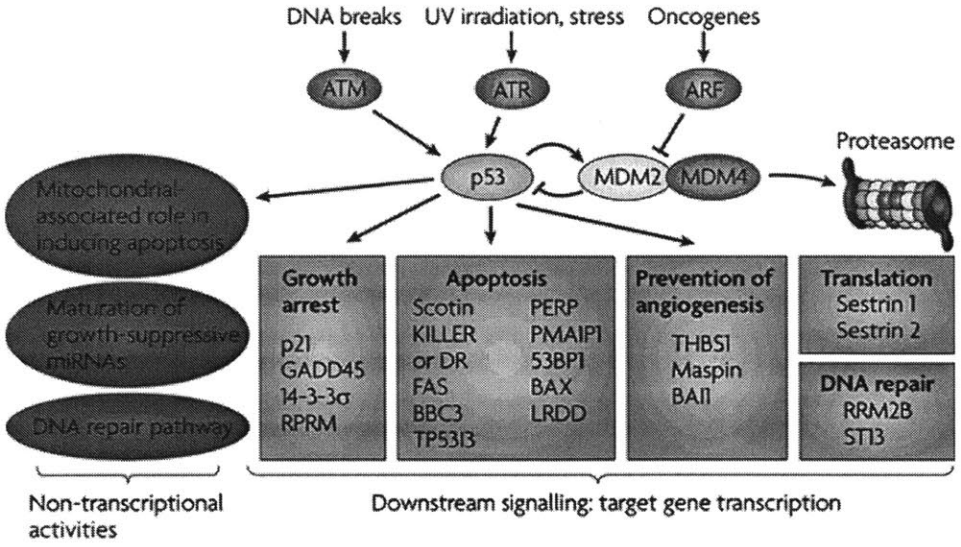
The cyclin D-CDK4/6-p16-Rb pathway is disrupted throughout cancer types by differing lesions. Although Rb loss occurs frequently in some cancer types the majority of tumors increase the activity of CDKs and retain wild-type Rb. Increased CDK activity can occur through a variety of mechanisms but the most common is p16 inactivation. Loss of p16 can be achieved through several mechanisms including mutations, deletion, allelic loss, and epigenetic silencing of the gene, etc (Shapiro, 2006). Since p16 normally inhibits CDK4/6 function and helps to control progression through the Rb pathway, the loss of p16 expression or function allows CDK4 to signal in an unopposed fashion and drives aberrant cell proliferation (Witkiewicz, 2011) and is functionally equivalent to inactivating Rb (Knudsen, 2010). Other disruptions in this pathway leading to tumorigenesis include overexpression of cyclin D1 and CDK4/6. Cyclin D1 overexpression can occur through a variety of mechanisms such as gene rearrangement, translocations, amplification, and mutations although none are required (Shapiro, 2006). CDK4, itself, can have activation mutations and amplifications similar to the HER2 protein (Flaherty, 2012). The differing lesions complicate the understanding of what aberrations are critical in any given tumor. For example, a study of 206 glioblastoma samples found that the Rb-pathway was altered in 78%. However, only 52% and 11% were due to homozygous mutations and deletions in p16 and Rb, respectively while 18% had amplifications of CDK4 (Kundsen, 2010). Further understanding of the driver of any given tumor will need to specified, not only to the tumor type, but also the specific lesion driving cell proliferation in the cyclin D-CDK4/6-p16-Rb pathway.

A key consideration for targeting this pathway beyond the varying lesions is which one acts as the biomarker for denoting sensitivity to a targeted therapeutic. Since p16 normally negatively regulates CDK4/6 it is hypothesized that p16 loss, which is common in many cancers including pancreatic and melanoma, would denote sensitivity to CDK4 inhibition. However, p16 can also be overexpressed in several cancers, including breast and small cell lung cancer. The overexpression can be either the cause or result of Rb loss leading to the need for additional markers (such as Ki67 for proliferation) to understand the functional state of the pathway. (Witkiewicz, 2011). There have also been conflicting reports as to the interaction among the aberrations. Earlier studies yielded that p16 loss is mutually exclusive with Rb loss or cyclin D1 amplification (Aagaard, 1995) while others have found cyclin D1 overexpression commonly occurs with p16 loss (Shapiro, 2006). To understand each lesion and its associated biomarker will require significant investigation. Several CDK4/6 inhibitor programs are now in the clinic and evaluating several of the markers within the pathway to better understand the inhibitors potential as a therapeutic target and what best confers sensitivity (see Results section).

**MDM2 and p53**

The p53 protein is a key regulator of the cell cycle where if there is too much stress or damage to a cell's genome or metabolism, it can induce apoptosis, DNA repair and silencing, thereby controlling the growth of aberrant cells. p53 is affected by upstream signals as a result of stress and is critical in directing a cell's fate through various downstream molecules (Weinberg, 2007).

**Figure 5. p53 signaling pathway and function** (Brown, 2009)

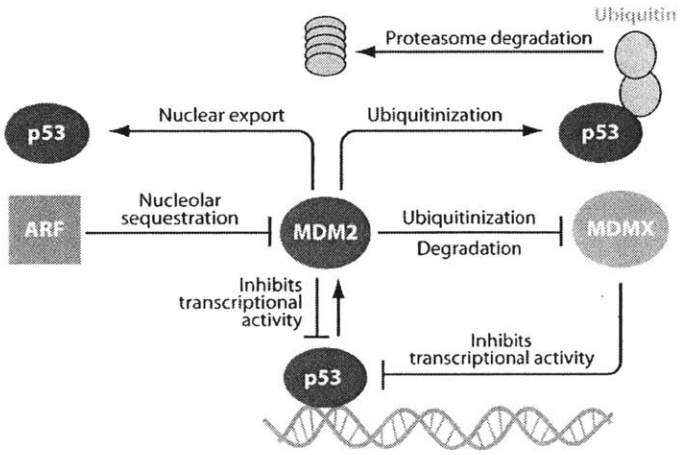



With this role, the p53 protein acts as a tumor suppressor gene for cancer cells (Weinberg, 2007). In addition to acting as a tumor suppressor, studies have shown that activation of the p53 response can be curative in some animal model tumors (Lane, 2010). It is also one of the most mutated proteins in human cancers with about 50% of tumors having alterations, mostly mutations and deletions, resulting in inactivation or loss of p53 proteins (Shangary, 2009). With such a potent role and frequency, the targeting of p53 using many approaches, including small molecules, has emerged as a key potential therapy in cancer treatment.

MDM2 (also known as HDM2) acts as a negative regulator of p53 by direct protein-protein interaction. The MDM2 protein binds to p53 and blocks its activity through multiple pathways: including blocking its transcriptional activity, exporting the protein into the cytoplasm and/or promoting its degradation (Alarcon-Vargas, 2002). MDM2 is an E3 ubiquitin ligase that binds to p53 and enables ubiquitin-dependent degradation, which in turn inhibits p53 transcription since the binding domains overlap

(Tovar, 2006). The direct protein-protein binding interaction acts through an autoregulatory feedback loop where their cellular levels are mutually controlled (illustrated by Figure 6).

**Figure 6. Autoregulatory Feedback Loop of p53 by MDM2** (Shangary, 2009)




By inhibiting the MDM2 protein it is thought possible to activate the p53 pathway through blocking the p53-MDM2 binding site, which leads to stabilization of p53 and its activation (Tovar, 2006). Importantly, p53 must be genetically normal and expressed for MDM2 antagonist to have an impact on p53 function. One of the more important observations for this target was the development of small molecule inhibitors (named Nutlins) of the p53-MDM2 protein interaction. The Nutlins were able to show activity against human xenografts in preclinical models (Vassilev, 2004), and in a preclinical study all 10 cell lines shown to express wild-type p53 had their cell cycle progression arrested (Tovar, 2006). The activity is due to the fact that some tumors retain wild type p53 function and when activated can induce tumor cell death.

Studies using MDM2 inhibitors have shown the potential biomarker utility of p53 and MDM2. Preclinical studies and findings from the Nutlin series of MDM2 inhibitors indicate that wild-type p53 is required for

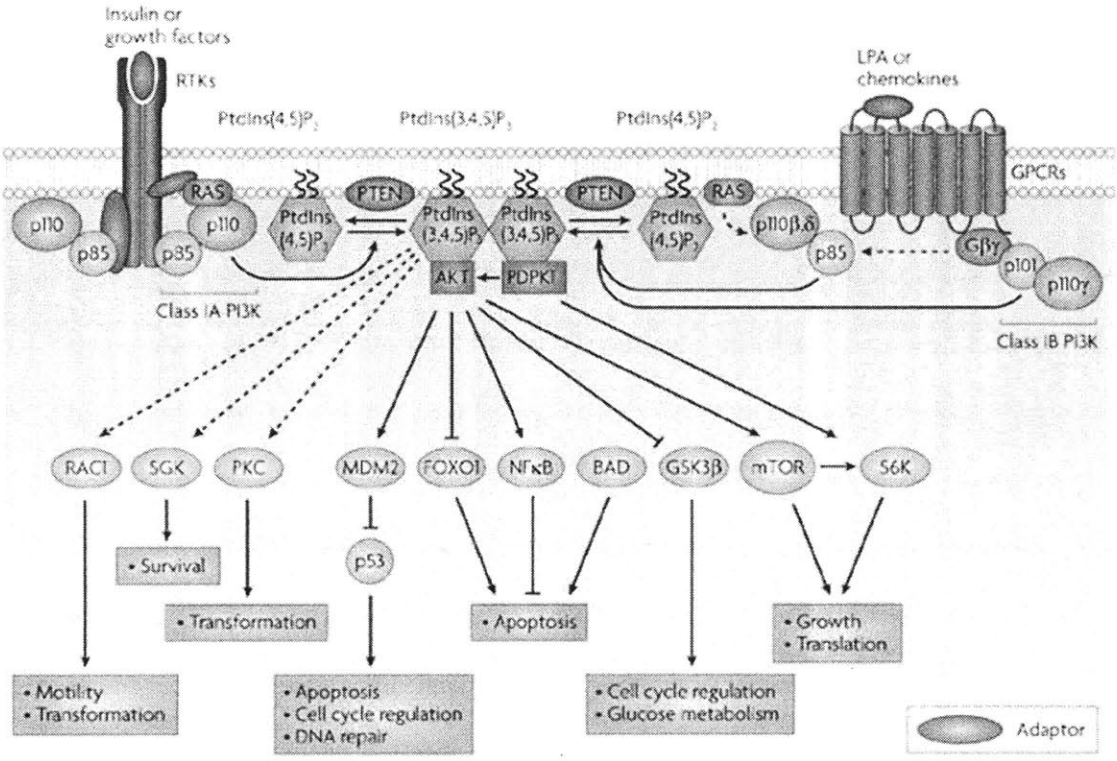
anti-tumor activity, thereby providing convincing evidence for using p53 mutational status as a biomarker for patient selection (Vassilev, 2004). It is important to note however, that although 50% of tumors express wild-type p53, there are likely defects in the overall signaling pathway. Of these additional defects, MDM2 inhibitors can bypass only upstream signaling molecules. The downstream signaling molecules may impact the overall effectiveness of the p53 pathway and limit re-activation by MDM2 inhibitors (Vassilev, 2007). Cells may also retain wild-type p53 because they have found another way to disable the pathway, which includes overproduction of MDM2. In the preclinical study mentioned above, one cell line had ~25 fold greater MDM2 amplification and the small molecule inhibitor was able to arrest the cell cycle, signaling reactivation of p53. MDM2 was further investigated and results suggested that if MDM2 were amplified or overexpressed, it was the only abnormality in the p53 pathway and that overall signaling was intact (Tovar, 2006) thus conferring sensitivity to an MDM2 inhibitor (Shangary, 2009). These observations illustrate the complicated biology of the p53 pathway, its regulators and role in cancer, which needs to be better understood to select those patients who would respond to such a targeted therapy. While one can reasonably conclude that MDM2 antagonist should only be developed in p53 wildtype cancers, there are likely other, unidentified biomarkers that would confer resistance to such a therapy and limit therapeutic impact.

**PI3Kβ and PTEN**

The family of lipid kinases known as phosphoinositide 3-kinases (PI3Ks) play a key regulatory role in many cellular processes including cell survival, proliferation, and differentiation. PI3K are part of a complex signaling pathway in which they transmit signals from various growth factors and cytokines into intracellular messages. PTEN, a tumor suppressor gene, is the key negative regulator that reverses the

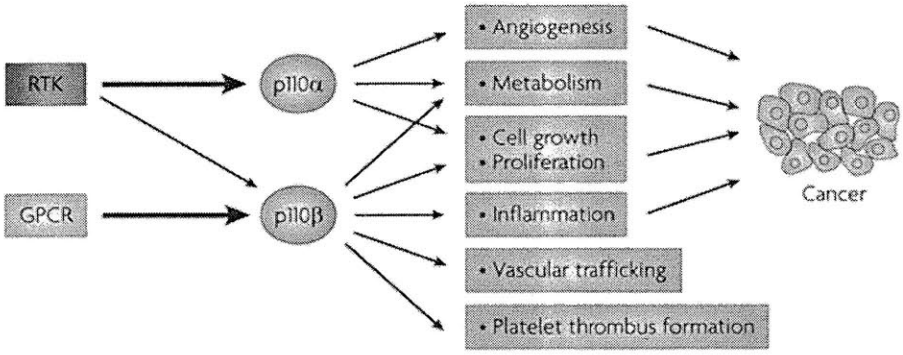
effects of PI3K by dephosphorylating the same site that PI3K phosphorylates on the membrane (Stemke-Hale, 2008).

**Figure 7. PI3K (Class I) signaling pathway** (Liu, 2009)




PI3Ks are divided into classes based on their structural characteristics and their specificity (Fruman, 1998). PI3Kβ is an isoform of Class IA which is a heterodimer consisting of a p110 catalytic subunit (p110β) and a p85 regulatory subunit. Each isoform has a slightly different functional role in tumor growth (illustrated by Figure 8). PI3Kβ is an effector for both receptor tyrosine kinases (RTKs) and G protein coupled receptors (the p110α isoform is the main effector for RTKs only). Both Class IA isoforms are expressed in almost all tissue and cell types but PI3Kβ has a role in integrin-mediated platelet adhesion, arterial thrombosis and certain kinase-independent functions (Liu, 2009).

**Figure 8. Differential function and role in cancer for PI3K Class IA p110 isoforms** (Liu, 2009)




Several cancer genomic studies indicate that mutations frequently occur within the PI3K pathway and may be involved in a range of tumor types, suggesting they constitute an attractive target for targeted therapies. Interestingly, while mutations of the p110α isoform gene, PIK3CA, have been found in many of the major (27% breast, 15% colon, 3% lung) and other cancer types, only increased activity and overexpression, not mutations, have not found for PI3Kβ. Loss of PTEN allows unrestricted signaling by the PI3K pathway and contributes to its role in tumorigenesis in many tumor types. Loss of heterozygosity of PTEN is detected in greater than 25% of gastric, breast, melanoma, prostate and glioblastoma cancers and mutated in 38% of endometrial cancer and to a lesser extent in several other indications (Liu, 2009).

Pan-PI3K inhibitors are in development, however, Isoform specific inhibitors are particularly desirable to avoid the expected toxicity to the immune system, which is driven by p110δ and p110γ (Liu, 2009) and normal tissues, which predominantly rely on p110α signaling. PI3Kβ inhibition, in particular, has shown to be promising in PTEN-deficient cancers. Several studies have shown PI3K signaling inhibition with p110β knockdowns and blocked neoplasia formation when p110α was unable to show inhibition of the

pathway (Engelman, 2009). These results suggest a strong relationship amongst the pathways and are important to understand further for therapy development.

Initially PTEN mutations were thought to be the primary driver of deregulation in the PI3K pathway but the varying impact across tumor types leads to some questions of PTEN's utility as a biomarker (Cully, 2006). Complicating its use as a biomarker, PTEN loss can occur not only through mutations and deletions but also truncated proteins, epigenetic silencing of the gene, complete genetic loss when starting with one allele, etc. similar to p16 (Flaherty, 2012). Unfortunately although positive results have been seen with PI3Kβ inhibitors within PTEN deficient tumors, the mechanism is not known, thereby limiting the ability to understand how the response will translate into the clinic. The effect may be only for a portion of PTEN deficient tumors since it is only one member of the pathway. Some PTEN deficient tumors may be less reliant on p110β through a p110α gain of function mutation, for example, or mutations in downstream signaling molecules (Ni, 2012). These complex interactions still require extensive investigations to understand each player's role in any one tumor type.

## Key Factors for Biomarker Clinical Trial Decisions

The challenges associated with developing a biomarker directed anticancer therapy can limit the clinical and commercial success of a drug program to the point where a more traditional all-comers approach for early-stage clinical trials is more appropriate. Among the multitude of factors influencing commercial success, three are especially important: therapeutic effect within the selected population, prevalence of the predictive biomarker and performance of the companion diagnostic. These factors can be used to help guide the clinical trial strategy decisions since other analyses suspect alternative clinical trial strategies may be more appropriate for targeted therapies.

Simulation studies have revealed quantitatively that targeted therapies have a significant benefit in situations where there is a clear risk/benefit profile. For example, if there is a clear scientific rationale with a true predictive biomarker and the cutoff point for determining marker status is well established, significant toxicities would be experienced by the biomarker-negative patients (even if modest drug benefit) or when it would be ethically irresponsible to use an all-comers approach based on prior studies. Several cases have been documented, however, in which an all-comers approach followed by additional testing to understand subgroup treatment effect is more appropriate, particularly in those situations where there is increased uncertainty due to biological complexity, where the cutoff point for marker status is not well-established, and the treatment has the potential to benefit both biomarker (positive and negative) populations (Mandrekar, 2009).

Dr. Keith Flaherty of Massachusetts General Hospital has noted that targeted drug developers need to consider the sliding scales of biomarker prevalence, assay validity/confidence and therapeutic efficacy when determining their biomarker strategy (Flaherty, 2012). These three key factors were also confirmed through a modeling study performed through the Massachusetts Institute of Technology (MIT) Center for Biomedical Innovation. The team, which included participants from MIT, FDA, pharmaceutical industry, diagnostics industry, and international academicians, performed modeling simulations and analyses on over 90 factors across two case studies to understand the factors influencing the success of stratified medicine therapies. A key element of the study was to evaluate and compare the expected net present value of each drug depending on the clinical trial design. Four different pivotal (late-stage) clinical trial design strategies were used: all comers, all comers followed by retrospective biomarker analysis and a confirmatory biomarker-positive trial, biomarker-positive only enrollment, and a dual strategy incorporating biomarker arms in an all-comers context (Trusheim, 2011).

The case studies of trastuzumab and panitumumab are especially relevant from this study where the modeling documents the benefit of prospectively using a biomarker-positive only enrollment strategy. While panitumumab would have added only 9.8% to the economic value with a biomarker-positive only strategy, trastuzumab would have had only 29% of the eNPV with a traditional all-comers trial. This difference in value illuminates how critical biomarker validation can be as trastuzumab had a clear biomarker hypothesis before phase II development began and panitumumab began with EGFR overexpression only to realize that KRAS status was the key determinant of response. While all four scenarios found a biomarker-positive only enrollment approach superior, the authors stated that if there were weaker predictive biomarkers or a lower therapeutic efficacy the clinical trials would have required more patients and impact on the overall benefit of a stratified approach. The need to screen patients with a companion diagnostic also increases the complexity and duration of the clinical trials, again affecting the overall eNPV value. Additionally, the authors specifically noted that those projects where there is limited prior knowledge for the clinical performance of the predictive biomarker or complexity of a novel companion diagnostic may actually prefer an all-comers clinical trial design strategy due to the lower probability of clinical and technical success of a stratified approach (Trusheim, 2011).

Sensitivity analyses revealed the same three factors mentioned by Dr. Flaherty interacted "particularly closely to affect economic value". Therapeutic efficacy within the selected population, prevalence of the predictive biomarker and the performance of the companion diagnostic were further interrogated to understand the tradeoffs involved. The retrospective modeling analysis found that in determining commercial success there was no specific threshold required for either prevalence of the biomarker or performance of the companion diagnostic but shortcomings in either area needed to be compensated by the other along with and an improved therapeutic effect (Trusheim, 2011). A key question still

remains however, as to how and on what basis to make these critical decisions early in the drug development process with the scientific knowledge gap that exists.

# 3. Study Purpose and Methodology

## Study Purpose

Despite and perhaps even because of the multitude of challenges and tradeoffs associated with developing targeted therapeutics as discussed above, drug developers must still use the information at hand to guide clinical trial stratification decisions. Three key factors have been shown to be especially significant in this decision process: therapeutic efficacy, biomarker prevalence and companion diagnostic performance as discussed above. Unfortunately, at the time these decisions are typically made, true clinical therapeutic efficacy is not known, given how complex and heterogeneous tumor biology can be. The two factors drug developers can consider are the biomarker prevalence and the sensitivity and specificity of a planned companion diagnostic, however, these can be widely variable and thresholds could be used for each factor to support the rationale for early stage clinical trial strategy decisions.

Using a sample of drug targets that represent the increasing tumor biological complexity (CDK4/6, MDM2 and PI3Kβ), in this thesis I hypothesize that early stage oncology drug developers do, in fact, establish minimal thresholds for both biomarker prevalence and companion diagnostic sensitivity/specificity when determining strategies of all-comers versus biomarker stratified clinical trials for targeted therapies. To evaluate this hypothesis, I examine several programs that are in the

preclinical stages or have clinical trials in progress, which provides me with empirical data on how these key factors are being incorporated into the implementation of the strategic clinical trial design decisions.

## Literature Review and Information Interviews

An extensive literature review was carried out to understand the key business and scientific drivers and challenges for targeted oncology therapeutics. This review was conducted across scientific, clinical, regulatory, statistical, economic, and mass media sources. Additionally, several interviews were conducted to understand challenges for both the drug and diagnostic industries. Key insights acquired during these conversations are incorporated into the Background and Discussion sections. Finally, discussions held at conferences on personalized medicine and clinical oncology I attended helped to understand the key challenges clinicians and drug developers are grappling with and their perspectives on resolutions to move forward. The findings informed what types of inquiries would be needed to answer the questions posed and placed the responses in the context of the overall development of targeted therapies. This enables me to gain insights on key implications for future decisions on guiding clinical trial decisions for targeted oncology therapies.

## Target Selection

The selection of targets used as the focus of this thesis was chosen after speaking with Dr. Keith Flaherty, both an advisor to this thesis and a well-known principal investigator in oncology studies. He has a unique perspective for targeted oncology therapeutics, since he is involved with a multitude of selective compounds and has had success with the BRAF inhibitors, specifically serving as principal investigator for several vemurafenib clinical trials.

Selective inhibitors that have negative regulators are a relatively new area of drug development and there are several programs that allow for a focused study. Several sources were used to identify the key programs from which to draw interview participants, including expert investigators and drug development databases/data aggregators. Dr. Flaherty knew of several key programs and this information was verified and augmented through searching Thomson Reuters Pharma. Thomson Reuters Pharma lists programs based on a target and extensive information on a compound's development. Additionally, this information was cross-checked with Adis, a R&D pipeline database.

This approach yielded ten programs across the three targets as the example dataset. A summary of these targets, programs and associated progress is provided below. The programs exist across nine different large pharmaceutical companies with only one company having more than one program. The programs were initiated both internally and in-licensed from smaller firms.

**Table 3: Summary of Relevant Programs**

| Inhibitor Target | Development Company | Originator Company | Development Phase |
|---|---|---|---|
| CDK4 | Eli Lilly and Company | Eli Lilly and Company | Phase II |
| CDK4 | Novartis AG | Astex Therapeutics Ltd | Phase I |
| CDK4 | Pfizer Inc | Onyx Pharmaceuticals | Phase II |
| MDM2 | Amgen | Amgen | Preclinical |
| MDM2 | Boehringer Ingelheim | Nexus Pharma/Priaxon | Preclinical |
| MDM2 | Roche Holding AG | Roche Holding AG | Phase I |
| MDM2 | Sanofi | Sanofi | Preclinical |
| PIK3-beta | Bayer | Bayer | Preclinical |
| PIK3-beta | GlaxoSmithKline | GlaxoSmithKline | Phase I/II |
| PIK3-beta | Sanofi | Sanofi | Preclinical |

## Interview Guides

An interview guide was created to evaluate the hypothesis that drug developers have established thresholds for biomarker prevalence and companion diagnostic performance for their "pathway modifying" programs. Additionally, several questions were aimed at gaining insight into the larger drug development strategy for such programs. Initially, there was some concern as to whether interview participants would be willing to provide quantitative information on very early stage programs. In order to mitigate this challenge, additional follow up questions were used to ascertain an operating range for a given threshold.

Each interview was formulated as a discussion around drug biomarkers and companion diagnostic drug development that addressed each of the following questions:

1. What biomarkers are you currently using to direct development and/or select patients for your program?
    a. Would the biomarkers be considered dichotomous or continuous?

        i.e. would the "operating characteristics" of the assay require cutoff points established between positive and negative results?
2. Are you currently planning to pursue a companion diagnostic for your program?
    a. What types of platforms are you evaluating for a companion diagnostic?

        i.e. genotypes, proteins, IHC, immunoassays, next generation sequencing
    b. If yes, what is the expected sensitivity and specificity of your planned assay? Is there a specific range in which you expect the companion diagnostic to perform?
        i. If the sensitivity/specificity was found to be lower would you still pursue a companion diagnostic approach?

1. At what degree of performance would you no longer consider using the companion diagnostic or abandon the biomarker?
2. In the absence of a companion diagnostic, would you still pursue the program?

c. If no, why and is there any situation in which you would reconsider the approach?

i. What level of confidence would you need to see in an assay to move forward with a companion diagnostic strategy?

i.e. what efficacy in biomarker positive population versus lack of efficacy in biomarker negative population?

3. What is the prevalence of the biomarker within the targeted population or indication?
   a. If the prevalence were higher or lower would that change your development approach?
4. Would you expect to include all methods of negative regulator loss in one assay?

   i.e. would you expect to capture all populations of potential patients or only the group that can be identified through a single assay?

   a. Which would you expect to use: multi-marker assays using one method (such as sequencing all exons of PTEN/p53/cyclin D/p16) versus a multi-modality assay that incorporates genetic and protein-based information? Why?

## Interview Participants

In order to capture the development strategy from several perspectives for each identified program, the preclinical, clinical and translational leaders were targeted for interviews. Each role has its own rationale including them in the dataset. Insight from the Clinical Lead is critical as they are the individuals ultimately responsible for the strategy and decisions required for clinical testing. Understanding the impact of companion diagnostics and patient selection is absolutely critical to moving

a program through the development path and will ultimately impact the drug's approval decision. Since these programs are in the early stages of development the Preclinical Lead provides valuable information regarding biomarker use and assay development/validation, which is a key analysis point for this thesis. A Translational Lead is responsible for understanding how the assay performance impacts compound development and therefore provides insight surrounding challenges in developing a reliable companion diagnostic and its impact on therapeutic development. While not all three roles may be able to completely answer all questions discussed above, this collection of perspectives provides both verification for an individual compound strategy and a view of the challenges for biomarker based drug development.

Points of contact for each program were identified through personal professional network contacts and thesis advisors. Additional interviewees were identified through preceding interviews. A total of 17 interviews were conducted across the different programs and roles. Interviews were conducted across nine of the ten programs. There was equal representation across the different programs with six, five and six interviews conducted for the CDK4, MDM2 and PI3Kβ programs respectively. A total of six clinical leads were interviewed while five interviewees were preclinical leads and five were translational leads (one interviewee was both the clinical and translational lead).

To maintain confidentiality, the responses collected from the interviews will be reported in aggregate. Clinical development strategies and the information used to support that process are proprietary and therefore both the identify of the interviewee and the associated company will not be disclosed.

## Clinical Trial Designs

The published clinical trial protocols can provide insight on the strategies and decisions for those programs that have already entered the clinic. These protocols were found through searching the public database clinicaltrials.gov on the particular target (CDK4/6, MDM2, PI3Kβ) or the compound name if previously identified. While the extensiveness of the protocol details vary depending on the sponsor, any molecular stratification would be included in the description or inclusion/exclusion criteria and therefore can provide valuable insight on actual strategy decisions.

# 4. Results

## Biomarker Descriptions

The biomarkers being investigated by the drug programs and their characteristics were similar to what was expected and detailed in the background section of this thesis. However, one of the key differences from expectations involved the fact that several of the programs were using the most conservative or simplistic definition of the biomarker.

### CDK4/6 Inhibitors

CDK4/6 inhibtor programs are investigating the use of several biomarkers including p16 loss but also (and in some cases more importantly) Rb status, cyclin D amplification and CDK4/6 amplification. While p16 loss is one way the tumors proliferate, there is essentially a parallel process where cyclin D levels rise and activate which then activates CDK4/6 and that complex inhibits Rb and allows cell cycle progression when it should not, as is the case in tumorigenesis.

Rb status was considered the most important biomarker by the interviewees since preclinical data indicates that a CDK4/6 inhibitor would not work within a tumor where Rb was non-functional (or had a negative status). Therefore, Rb was considered a dichotomous biomarker; it was either positive or not. Rb was considered by one interviewee to be the direct target while the other biomarkers were more of a mechanistic question where they were only a part of the pathway, not the desired measurement of response. The other biomarkers were considered continuous but interestingly there was less clarity about testing methodologies and all were being considered in different contexts as compared to biomarkers for the MDM2 and PI3Kβ programs.

The interviewees expressed concerns that since there were multiple modalities for achieving p16 loss and overexpression of cyclin D and CDK4/6 they were unsure as to what method would be most appropriate. Loss of p16 would include protein expression assays (IHC) where a level of expression would need to be defined as sufficient "loss". One interviewee, however, indicated that comparative genomics would be useful for p16 (as well as Rb) that would make the categorization more dichotomous. Expression levels of cyclin D and CDK4/6 would need to be defined as low or high and characterized by the level at which it contributed to tumor growth and patient response with a similar protein expression assay. Adding to the confusion, the interviewees raised the possibility that overexpression was not necessarily the same as gene amplification, with this still needing further investigation. Similar to p16, methods of loss exist that would make cyclin D and CDK4/6 more dichotomous. Cyclin D can have translocations, which simplifies the categorization, and CDK4/6 can have mutations, again classifying it as a dichotomous biomarker.

Additionally, the biomarkers could be indication-specific as an interviewee illustrated substantial evidence exists suspecting that p16 loss in melanoma is a driving event, while in liposarcoma almost all patients have CDK4 amplification.

**MDM2 Inhibitors**

For MDM2 inhibitor programs the identified biomarkers were p53 mutation status and MDM2 amplification. p53 mutation status was considered dichotomous by all interviewees but several nuances were raised. For example, while having a p53 mutation may be a yes/no categorization, once you know the gene is wild-type there could be a more continuous response to consider. For example, there may be downstream mutations or signaling issues that would impact the level of response to an MDM2 inhibitor. This is where the retrospective studies may help indentify and describe sub-stratifications that could become useful in understanding a patient's response. Additionally, one interviewee raised a larger issue that while mutation status may be dichotomous now, it will be important to consider the sensitivity of the detection test in making patient treatment decisions. His/her concern was that the sequencing was now so sensitive that it could pick up p53 mutations that did not have an impact on function and therefore would exclude a segment of patients that may benefit from an MDM2 inhibitor. It was stressed that much still needs to be learned on the impact of specific mutations on functional status. Despite these concerns, however, the interviewees envisioned p53 status as the first line of patient stratification.

MDM2 amplification was less defined and although interviewees felt that it was characteristically more like a continuous curve, there was the possibility to fit it into a dichotomous decision. It comes down to whether or not there is amplification. The fact that there is already a defined algorithm for what is amplification helps make the decision more clear in one interviewee's opinion. Several added layers of

considerations include how much amplification is needed for patient response and the issue of overexpression vs. amplification. It is possible for a patient to have overexpression of MDM2 without amplification and whether or not the drug would benefit those patients is an unresolved issue.

### PI3Kβ Inhibitors

PTEN loss was the primary stratification measure and was considered to be a continuous biomarker by the interviewees, since it is most frequently determined by protein expression assays (IHC). It is necessary however to create a cutoff to move forward with clinical development. One interviewee stated that his/her program was using the most stringent cutoff in the literature and putting it where they expected to start seeing noise in the assay. In this case, it was 10% staining or less as a defined cutoff. The program considered putting it at 100% absence of staining but they were afraid that they would be unable to find patients. Without knowing the distribution of IHC results in the population it was difficult to know at what point was it the most appropriate and feasible to conduct a clinical trial. Another interviewee stated that there is no hard number as to what is PTEN loss but he/she hoped that a binary curve would appear in the clinical data. Another interviewee stated, however, it is too early to tell. The confusion stems from the fact that although there may be staining it is important to know at what point does the protein become non-functional. This determination will require further investigation and may allow the patient population to expand as more is known about the biomarker.

## Companion Diagnostic Performance

Not surprisingly given the recent successes and regulatory environment for targeted oncology therapeutics mentioned in earlier sections, all programs interviewed are at least planning to use a companion diagnostic as part of their strategy. When asked about the targeted companion diagnostic

performance (sensitivity and specificity), however, only a few interviewees responded with a desired range. One interviewee referenced that past IHC performance characteristics included about 20% of a "gray zone" and therefore a companion diagnostic would need to perform at least at an 80% level. Another interviewee stated that the FDA requires 90% concordance but a level of around 85% could possibly be acceptable. Two other interviewees stated that the diagnostic would need to be at least 90% sensitivity and specificity. Finally, another interviewee referenced the current performance of sequencing companies, such as Foundation Medicine who presented at the American Society of Clinical Oncology (ASCO) meeting this year, where they could detect approximately 5% mutants 100% of the time and less reliably for mutants at lower frequencies.

Several of the interviewees stated that it was too early to understand the performance they could achieve and as one stated, they would need the expertise of a diagnostic company and regulatory authorities to better understand the requirements. It is known that a certain level of rigor is required but the program leads did not state the desired level.

The technical performance levels were also considered to some extent variable depending on the type of activity or response in patients. One interviewee was more concerned with at what level would you see a difference in activity as opposed to a certain specificity. Another mentioned that it was more of a consideration between efficacy and safety. For example, if there were some response in the biomarker negative population then the companion diagnostic technical specifications would be more focused on the safety of those patients being exposed to toxicities. Two interviewees considered a threshold of companion diagnostic performance to be too simplistic to grapple with the tradeoffs of sensitivity and the number of factors that would need to be considered.

The interview participants were asked whether the drug program would continue if it were unable to validate an assay and reliably identify the appropriate patients in a clinical setting. Stated another way, they were asked whether the program would continue if the companion diagnostic could not achieve a technical performance level to be defined at a later date. The interview participants responses ranged in confidence but almost 60% (n=9 of 17) said that they did not expect the program to continue without a companion diagnostic. The 40% that felt the program could continue in some way varied in their reasoning. Several interviewees were unable to say that the program would be discontinued but did mention it would be difficult for it to continue but might be deprioritized. An example would be the program could continue in an indication where the biomarker prevalence was extremely high but most likely it would be an ultra-small indication (less than 3,000 patients). This market size would be difficult to support a business case. One of the interviewees who stated the program would not continue specifically mentioned that if the market were large enough for a business case then it would need a companion diagnostic. Interestingly, a few interviewees discussed the possibility of the program continuing as long as there is drug activity (patient response), perhaps being used in a combination setting or as an immunoadjuvant.

## Biomarker Prevalence

Thresholds for biomarker prevalence in guiding clinical trial stratification decisions were also highly variable. Responses for at what level of biomarker prevalence would allow an all comers trial to be appropriate ranged from 50% to examples as high as 99%. One interviewee stated that thresholds were being used but the levels that were considered proprietary. The remaining interviewees expressed concerns that an all-comers trial would never be appropriate.

On the lower end of thresholds, three interviewees established 50% as a possible threshold for an all-comers strategy if the drug activity were significant enough. As one of the interviewees stated, you could take all patients, but you would not expect full activity. That same interviewee cautioned however, that even if the biomarker prevalence were at 80% it would be important to consider the biological complexity. If there is known interaction between targets it may not be possible to trust patient responses will behave in a similar manner. On the high end of the thresholds, two interviewees used the example that if only 1% of any given patient population were excluded (i.e. a biomarker prevalence of 99%) that a companion diagnostic would not likely be developed. It was important to note however, that if the drug was applicable across multiple cancer types that the companion diagnostic would again need to be considered. Two interviewees fell into a more intermediate range. One interviewee stated that if the biomarker prevalence was above 70-80% then he/she would consider not developing a companion diagnostic. The other interviewee was a bit more conservative, stating that if the prevalence were greater than 80-90% they would feel comfortable without patient selection. The final interviewee stated that thresholds were being used for selecting patients onto a trial, but the actual range was considered proprietary.

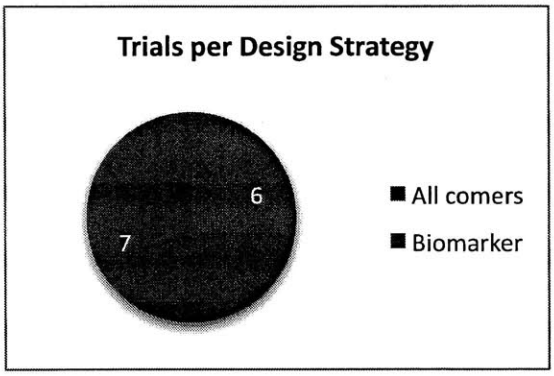
A few interviewees were unable to provide a range in which they thought an all comers trial would become appropriate due to ethical considerations. One interviewee thought that a companion diagnostic was critical regardless of the biomarker prevalence but conceded that it depended on how clear the stratification was. For example, if some biomarker negative patients received a benefit, it would be more difficult to require only biomarker positive patients. With a high prevalence of 50-60% it could be possible to use an all comers strategy, stated one interviewee, but the ethics of harming patients that may not receive a benefit "trumped" including them in the trial.

Most of the interviewees used this question as a springboard to also discuss whether there was a minimum biomarker prevalence to encourage program development. The responses ranged from no required minimum to indications being prioritized above 20% biomarker prevalence. A few interviewees mentioned that there was not a minimum requirement for prevalence or incidence; if there were an unmet need and the company had a potential drug then it would be pursued. One interviewee mentioned that a minimum threshold depended on the attitude of the company. Past successes and failures can impact the minimum requirement in either direction. For example, one company had a prior failure with a less selective molecule and therefore would most likely not move a more selective one forward if it were only a small applicable patient population (the previous failure "poisoned the probability of success"). However, another company's success with a small indication could allow it to consider other small indications with high biomarker frequencies. This interviewee mentioned however, that an indication with a small patient population and only moderate biomarker frequency would most likely not go far in development. Four interviewees provided a threshold of biomarker frequencies below 5% would most likely not be investigated within their organizations. The highest minimum prevalence given by one interviewee was that the company wanted prevalence of at least 20%. Indication decisions for one company (per an interviewee) involved stack ranking the indications by biomarker frequency and choosing to keep the top ten as indication options. The size of the indication mattered however. An indication that would have been discarded due to the frequency was included in the final list due to the indication size being very large ("big 4" cancer types). Interestingly, another interviewee used the example of the "big 4" where the company would tolerate lower frequencies of biomarker prevalence at 10-15% while for ultra-small indications (<3,000 patients) then a prevalence of at least 75% would be desired. One of the key considerations for one interviewee was whether it would be possible to accrue a clinical trial both from overall patient numbers but also what potentially would still be available after taking into account competitor compounds.

## Clinical Trial Designs

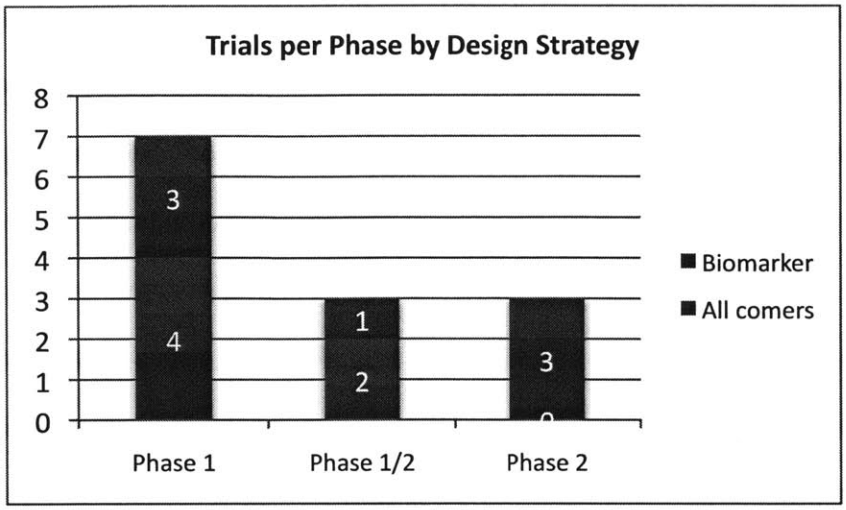
A total of thirteen clinical trials were in progress or completed across the ten programs (clinicaltrials.gov). Twelve were company sponsored while only one was investigator sponsored. Of those thirteen trials, 54% (n=7) had a molecular biomarker inclusion or exclusion criteria included in the protocol that signaled biomarker stratification while 46% (n=6) were enrolling on an all-comers basis. The lone investigator sponsored trial was a phase II trial that included biomarker-based enrollment.

**Figure 9. Published clinical trial strategy decisions**




Biomarker based enrollment increased as the development phase advanced, however, there were still almost as many biomarker based enrollment strategies in the phase I trials. For those trials in phase II all were using a biomarker restricted enrollment strategy.

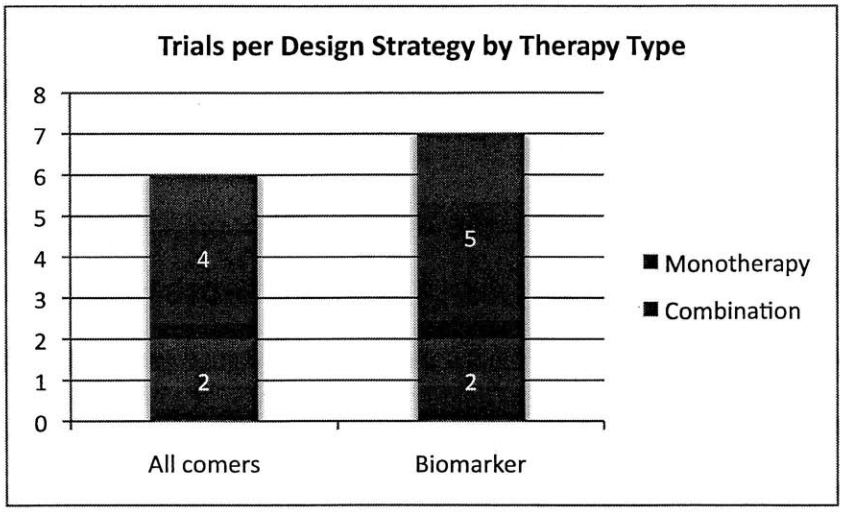
**Figure 10. Published clinical trial strategy decisions by phase**




Interestingly of those six trials enrolling first as all-comers, three included biomarker enrichment or correlation as part of expansion plans (one in phase I and two in phase I/II). An additional trial was also being planned to require a mutation in the pathway as part of expansion per an interviewee (although not detailed in the published protocol).

Despite interviewee responses regarding using the drug in combination therapy without a biomarker, the therapy type did not appear to affect the stratification decision. Each strategy had around 50% of their trials as each therapy type – combination or mono therapy.

**Figure 11. Published clinical trial strategy decisions by therapy type**




Interviewees also provided information on the plans for programs still in the preclinical stage. Two programs planned to start enrolling as all comers in phase I. However, expansion plans would include enrolling a specific proportion of indentified biomarker patients in those indications with high prevalence of the desired biomarker.

## 5. Discussion

In this pilot study I have examined the factors of prevalence and companion diagnostic performance and their impact on biomarker-guided clinical trial strategy decisions. In addition, I have evaluated the key challenges facing drug developers of complex biological targets using the representative "pathway-modifying" drug programs. Interviews with leads of drug programs for CDK4/6, MDM2, and PI3Kβ inhibitors were conducted to understand these factors and their implications for overall development for targeted oncology therapies. The thresholds of prevalence that would lead to using an all-comers clinical trial strategy as opposed to a biomarker-guided strategy varied widely from approaching 100%

down to 50% depending on several factors including drug activity, toxicities, biomarker separation and ethical concerns. Companion diagnostic thresholds were even less common where the majority of responses implied that additional expertise and investigation was needed to guide those decisions. While the hypothesis that common prevalence and companion diagnostic thresholds were being used to guide these clinical trial decisions was not supported, several valuable insights were gathered from interviews with drug development experts.

## Limitations

This study was meant to act as a pilot and therefore is informative of trends and considerations in developing stratified therapies but small sample size prohibited my examining any possible statistically significance. The molecular targets and drug programs were chosen to represent the expected biological complexity of targeted therapies, and therefore are certainly not representative of all targeted agents in development. Although the drug program dataset was not meant to be this, all ten programs were limited to large pharmaceutical companies. Different considerations and factors may be considered by smaller or biotechnology companies.

Additionally, while three interviewees (Preclinical lead, Clinical lead, and Translational lead) were targeted from each program, the response rate overall was 58.6% (n=17/29) due to time constraints and challenges in identifying the appropriate individuals and scheduling time for an interview. Therefore, conclusions are limited by potential biases that exist in the respondents despite having an almost equal number of responses across the roles. Another limitation is that some responses were incomplete due to the interviewee's desire to keep proprietary information confidential. The level of knowledge of each discussion item (prevalence, companion diagnostics, diagnostic methodologies, etc.) also varied across

the different respondents. Some interviewees were hesitant to answer questions outside of their knowledge base or responsibilities, and in some cases stated that they were speaking speculatively or hypothetically. Importantly, each of these responses in the interviewee's opinion or perspective is not a quantitative datapoint and can therefore limit hard conclusions. However, these perspectives do provide valuable information on the factors considered and the direction of activities as stratified medicines face considerable challenges ahead.

## Extent of Uncertainty for Companion Diagnostics

A key unexpected theme of the interview discussions was the extent of uncertainty surrounding companion diagnostic utility and performance. Only a few interviewees were willing to suggest a range in which a companion diagnostic should perform and even one of those used past diagnostic approval examples (HER2 testing) as a reference. No interviewee responded with regulatory requirements, which is not unexpected since there is still a great deal of ambiguity for companion diagnostics development and biomarker validation. The response is somewhat surprising, however, since in order to support approval, the company will need to show the regulatory authorities, including the FDA, that it can reliably identify those patients who are expected to respond to the targeted therapy. Without understanding the ability of what is needed to detect those patients, late-stage clinical trials could be more technically challenging and more costly. Omacetaxine (Omapro, ChemGenex) is a recent example of the risks associated with not achieving strong enough analytical performance. The drug, for CML, was tested with different biomarker diagnostic testing protocols at different sites but they could not reliably demonstrate what population was actually enrolled in their trial. The drug was submitted in Sept 2009 and is still not approved since they have not presented a test that selects the same population they did in their trials (Mansfield, 2012). The respondents did seem to recognize this type of risk since the

majority of them (almost 75%) stated that they thought their drug program would either not move forward or be deprioritized if the companion diagnostic could not be developed.

Companion diagnostics technology is also continually evolving, which can limit the establishment of a standard. Despite its being approved over a decade ago, there is still no gold standard for HER2 testing and quality of the assay performance was considered a major issue in the later stage randomized clinical trials (Carden, 2010). Both IHC and FISH methodologies are used and while several test kits are FDA approved, a CLIA laboratory can also perform testing with various reagents and protocols. Surprisingly, the concordance between the two tests can be as low as 25% as a result of the various inconsistencies (Bilous, 2003). Additionally, Dr. Stephen Spielberg of the FDA at the 2011 Personalized Medicine Conference said that the technology for companion diagnostic is changing faster than that for therapeutics (Spielberg, 2011). A key example is how whole genome or next generation sequencing will impact IVD testing. It was clear from the interviews that these drug developers were grappling with these same issues. Several respondents noted that the role of sequencing was still to be determined but there were concerns that it is not the "be all end all" for companion diagnostics. Not only are some aberrations (such as epigenetic silencing) not detected through sequencing, but the function of each mutation still needed to be determined. As previously mentioned, one interviewee stated that the sequencing was becoming so sensitive that it was becoming difficult to understand which mutations were important for function and therefore relevant for targeted therapies. When asked about the assays being used in developing a companion diagnostic, interviewees stated that several methodologies were still being considered, even in those compounds already in clinical trials. IHC versus FISH was a common consideration but new methodologies such as chromogenic in-situ hybridization (CISH) and sequencing were also options for companion diagnostic assays. Only one program was using a validated assay but it was still unclear whether it would serve as the companion diagnostic to support

pivotal trials. Therefore, while companion diagnostic technology may be changing more rapidly than therapeutics, the process of assay and biomarker validation is actually lagging behind the drug development process (Carden, 2009).

## Conservative Biomarker Cutoffs

Another key topic emerging from the study was the fact that while interviewees recognized biomarkers could be continuous, in almost all instances the drug programs would move forward using conservative biomarker cutoffs. This could be in fact a way of drug developers compensating for the biomarker validation uncertainty. For example, in those programs where tumor suppression loss could occur through multiple mechanisms (p16 and PTEN), respondents asserted that the cutoff for selecting patients would be complete loss, as opposed to varying levels of expression. Dr. Elizabeth Mansfield, Director of Personalized Medicine staff of the FDA's Office of IVD/CDRH suggested that the FDA would like to gather information the extent various populations benefit from a therapy whether it be lower protein expression levels or rare genetic mutations (for example a BRAF mutation that was not V600E) and in order to gain approval for these "other" subpopulations, some data would have to be seen on them. However, she conceded that the companies did not know how to anticipate what the FDA would do if their data comes back lower than with a more conservative cutoff. These companies are therefore reducing the risk on a regulatory level as well. There are several examples of companies using a more conservative cutoff and then using anecdotal evidence from physicians on a treatment effect to drive further trials to expand the overall market to new subpopulations or indications (Mansfield, 2012).

## Variation in Prevalence Thresholds

The level of variation in the suggested biomarker prevalence thresholds, from 50% to 100% required for an all-comers trial may illustrate the effects of complex biological targets and the impact of not being able to estimate potential therapeutic efficacy. If strong drug activity and responses are seen, lower levels of biomarker prevalence would be needed in order to demonstrate clinical benefit. The levels of uncertainty are so great that the early-stage trials are merely providing information rather than validation of a biomarker. Several interviewees also suggested this when they considered drug activity the primary objective and biomarker correlation only secondary. In a discussion of using biomarker-based patient selection in phase I trials, a group of authors state that "to date, many of the biomarkers used in clinical trials of novel agents have been exploratory and not well validated, leading to inefficient or unsuccessful deployment" and often introduced too late to have an impact on early clinical trials (Carden, 2010). This particular sentiment seems also true for the programs that represent complex biological targets and served as this study dataset. It is also possible that for such targets where it is not possible to understand potential therapeutic efficacy that prevalence of the biomarker should not impact clinical trial decisions; a phase I all-comers trial may be more appropriate. Further evaluation and modeling will be required in order to understand what prevalence levels are appropriate for varying clinical design strategies and importantly, at what levels will drug developers undertake the challenge.

Dr. Mansfield stated that the FDA had addressed prevalence a couple times where the biomarker is a diagnostic determinant of disease (similar to BCR-ABL CML, Philadelphia-positive acute leukemias). In these cases, the diagnostic is not considered a companion and would not affect approval. In her opinion, there was not a particular number at which using an all-comers trial throughout clinical trials was appropriate but if the biomarker prevalence was 90-95% then in many cases a companion

diagnostic would not be needed. It would depend on how much harm comes to the other subpopulation, however (Mansfield, 2012).

An unexpected yet fascinating finding of this study was the levels of prevalence drug developers thought would continue through the drug development process. Although some interviewees stated that as long as there was an unmet need they would pursue the target, several used the example of crizotinib and ALK as a threshold where the biomarker prevalence was only around 5% of non-small cell lung cancer. Although no longer possible, it would have been interesting to understand what perspectives would have been without such a visible example of success. As a contrasting example, while CML was a small indication, the biomarker effectively defines the disease and therefore approaches 100% prevalence. Interviewees stated that it was not just the prevalence of the biomarker but also the size of the overall indication in which the subpopulation would reside that would influence their decision to pursue that target. The opposite was also true where although a given ultra-small indication (<3,000 patients) was close to 100% biomarker prevalence, several interviewees felt that the target would not be pursued unless it was applicable in a wider range of cancer types. Further clinical studies and which indications are included would shed light on what levels of prevalence and indication size are needed to justify targeted drug development.

## Emerging Alternative Clinical Trial Strategies

Several alternative clinical trial designs and recommendations are now being considered to potentially deal with the high levels of ambiguity. The use of expansion trials was a key finding through the study interviews that echoes recommendations put forth in the literature and elsewhere. Four of the six clinical trials using an all-comers strategy to start phase I or phase I/II studies were planning to enrich an

expansion cohort. This was also a strategy undertaken by several of the successful targeted therapies, including vemurafenib and crizotinib. It is important to ensure safety and gather appropriate PK/PD data in a large enough patient cohort during a dose-escalation phase. Donna Neuberg, a biostatistician at the Dana Farber Cancer Institute mentioned it will be difficult to understand any off target effects in only single arm trials. She recommended starting with all-comers and then differentiating prognostic factors in subpopulations (Neuberg, 2012). Several sources suggested that casting wide nets in early-stage clinical trials allowed for identifying possible predictive biomarkers, which can then be followed by integration of the biomarker into further trials to correlate the clinical benefit. Testing many hypotheses could be accomplished by using smaller and more seamless, adaptive designs in phase I/II (Bradley, 2012). Dr. Anthony Tolcher during a presentation at the American Society for Clinical Oncology (ASCO) 2012 meeting recommended a unselected phase I, then expansion cohorts and phase II should include cohorts of molecular "hot sites" to deal with the disadvantages of pre-selecting early stage trials, including the level of molecular complexity (Tolcher, 2012). Through using an adaptive approach and allowing cohort enrichment it is possible to accelerate development by performing hypothesis testing studies in phase I expansion cohorts (Yap, 2010).

It is interesting to note however, that 40% of the clinical trials already in progress for the study drug programs were using a biomarker-selected only population in phase I and I/II. These trials are forgoing the benefits of wider PK/PD data due to what they believe is a strong scientific hypothesis despite the challenges in identifying the targeted patients. A common statement in the literature is that for those compounds with unknown mechanisms of action, multiple potential targets or broad-spectrum inhibitors, biomarker-based evaluation may not be appropriate (Carden, 2010, Yap, 2010). A critical risk of using biomarker-selected only populations is that data is not collected on the responses of the biomarker-negative population in early-stage clinical trials. Dr. Robert Temple of the FDA's CDER

department stated that the FDA believes you have to "know something" about the "other group" but there is uncertainty as to what extent is necessary (Temple, 2008), which could threaten the approval of the agent. Dr. Mansfield mentioned that initially the FDA thought drug/diagnostic developers would pursue a predictive claim and use biomarker positive and negative populations in trials. However, Drug developers have moved to only needed to be able to select the same patients reliably and therefore the analytical rigor and performance in a single arm is enough. She suggested that retrospective studies be used to understand treatment responses in biomarker negative populations because like Dr. Temple, she believed everyone would want to know if this population could benefit as well but it is not a requirement for biomarker-guided therapies. Drug developers will need to carefully consider these implications for their clinical trial decisions and it appears that despite emerging recommendations for targeted therapy development, actual decisions for clinical trial strategies may differ greatly.

## Ethical Considerations for Prevalence Thresholds

Several interviewees suggested that with a hypothesis that a biomarker negative population would not respond to a drug, it would be unethical to (1) expose those patients to drug-related toxicities who would not benefit and (2) possibly impact the timeframe and effectiveness of another treatment even if the hypothesis had not yet been validated. Interestingly in speaking with physicians, I found their viewpoint and comfort with unknown responses to a drug were considerably different. Dr. Flaherty, an academic medical center specialist oncologist stated that patients were frequently placed on therapies with an uncertain response outcome (Flaherty 2012). They also recognize the biological complexity of the biomarkers and even getting 2/3rds of the way to identifying an appropriate treatment is a benefit – it is not necessary or even feasible to be 100% positive that the patient will respond. Dr. Mansfield did provide perspective on this discordance. Both the FDA and companies are evaluating the response to a

drug at a population level where as a group, it may be unethical to include patients on a trial where it is suspected they will not benefit. Physicians, however, are evaluating this at an individual level and taking into account the multitude of factors that could be different for this one individual (Mansfield, 2012).

## Combination Therapy

The increasing biological complexity, such as with the interactions of multiple aberrations that can occur in any given pathway, resistance mechanisms or the extent of tumor heterogeneity, suggests that combination therapies may be required to achieve the desired curative or at least maintenance patient responses. If there are multiple potential targets in any one tumor, theoretically, diagnostics will still be needed to understand which targeted therapies are necessary for treatment. More than one interviewee, however, noted that a companion diagnostic may not be required in a combination setting, especially as long as there was patient response seen in such a scenario. This could be due to the fact that the number of challenges increases to an even greater extent in a combination setting. Therapy regimen design is only one aspect but yet the combination agent, doses, sequence of therapies, duration of each agent, all must be selected in clinical trials while balancing mechanisms of action and toxicities. Combining targeted agents has the potential to increase overall benefit to the patient by synergizing the anti-tumor effect without a complementary increase in toxicities, hopefully improving the therapeutic window and potentially counteracting primary and secondary resistance shown by both high and durable responses. A discussion during ASCO's annual meeting this year (and published in its education book) focused specifically on this idea; combining targeted agents in clinical trials. The above benefits were listed as opportunities as well as further agents that do not have single agent lethality, which may be the case for these targeted agents dealing with complex biological pathways. One of the key challenges was in fact how to use biomarkers to guide treatment. The slow pace of biomarker

development to support such hypotheses and the lack of standardized designs for clinical trials are key impediments to combining targeted agents (Mateo, 2012), which is what the interviewees in this study also mentioned. Further investigation will need to be performed to better understand how to best capitalize on the impact of stratified therapies in a combination setting.

## Areas for Future Research

This study provides interesting insights that actual activities surrounding biomarker guided drug development still face significant challenges despite now several successful case studies of stratified medicine (trastuzumab, imatinib, vemurafenib, crizotinib). Several opportunities for future research that could be undertaken in order to facilitate continued understanding of the factors influencing targeted drug development decisions. A similar type of qualitative study could be performed with a wider set of biologically complex drug targets and a more complete set of respondents for each program. Such a study could be augmented by a quantitative study in which respondents would be forced to choose levels of prevalence and companion diagnostic performance levels to understand what decisions would be made in a given scenario.

Modeling could also be performed to understand the interplay between the primary factors of therapeutic efficacy, prevalence and companion diagnostic performance and given a particular level, what thresholds would be required for commercial success. A retrospective study on which indications companies pursued could provide insight on while there may not be theoretical prevalence thresholds to guide decisions, there may have been certain levels needed to pursue clinical investigation. In a number of years it would also be interesting to survey the clinical trial protocols of biomarker guided

therapies and evaluate whether trends or even thresholds emerged as knowledge of complex biological pathways and drug response increased.

A secondary line of questioning could surround the burgeoning area of companion diagnostics. Given the limited knowledge of the intricacies of this industry that was found in drug development leaders, it would be interesting to understand where this information is being pulled from in order to progress a targeted drug through clinical development. These partnerships have immense challenges as discussed in the Background section. Further studies could be done to understand the operational strategies, incentive alignment and factors that contribute to overall diagnostic success. Interestingly, there is limited data on the adoption patterns of diagnostics and it may be critically important to understand what factors contribute to companion diagnostic success and what factors also affect the drug adoption and commercial value as well.

## 6. Conclusion

This qualitative pilot study found that developers of "pathway-modifying" targeted therapies are not using common thresholds of prevalence or companion diagnostic performance to guide the biomarker strategy for clinical trial decisions. Prevalence thresholds for choosing an all-comers versus biomarker-guided clinical trial strategy varied widely indicating that further investigation and modeling is needed to understand the implications of biomarker prevalence on clinical trial results. Each program investigated was planning to use a companion diagnostic, however, specific target performance levels were lacking for the majority of study respondents. Interestingly, most of the respondents felt that the drug program would either not move forward or be prioritized without the companion diagnostic, making the

knowledge gaps even more critical. Conservative biomarker cutoffs, such as complete tumor suppressor loss, may be an approach to dealing with the technological challenges facing assay validation. Essentially, as was heard from several respondents, drug activity was the primary outcome of early stage clinical trials and understanding how best to identify those patients with desired responses was a secondary objective. This illustrates the juxtaposition where although each drug program faces similar challenges, in early phase clinical trials there is not a standard trial design. An almost equal number of trials were using an all-comers or biomarker-guided strategy. Only upon phase II trials were the strategies aligned as biomarker-guided although the results were not statistically significant. Enriching a phase I trial was an approach that 2/3rds of the respondents were planning to take, suggesting a movement towards more standardized processes.

With several successes case studies of stratified medicine, especially in oncology, discussions have yielded several "best practices" that do not in fact represent the actual activities or decisions of drug developers. Recommendations for developing targeted therapies have begun to emerge and are slowly being implemented such as using phase I expansion arms to better validate a scientific hypothesis while hopefully minimizing the disadvantages of using a phase I biomarker selected only population. Processes to incorporate companion diagnostics into the drug development process have also begun to take shape in order to anticipate regulatory requirements. The key message here is to initiate a companion diagnostic as soon as possible and have a potentially commercializable assay available for pivotal trial testing. Based on this study, for biological complex targets such as "pathway-modifying" programs (CDK4/6, MDM2, PI3Kβ), the companion diagnostic is a concern but may not be reliable even once the drug enters clinical trials and even into phase II and perhaps greatly threatening the drug's success.

Although several drug programs have achieved clinical, regulatory and commercial success, significant work still needs to be done in order to better align the drug and companion diagnostic development processes since future programs will experience arguably greater challenges. The challenges surrounding scientific hypotheses, biomarker validation, clinical trial design, regulatory requirements, commercial value and ethical considerations all contribute to the significant uncertainty plaguing targeted therapies and likely leads to the findings of this study. Standards for prevalence and companion diagnostics thresholds for "pathway-modifying" targeted therapies are not yet established and the current actual clinical trial decisions made by drug developers do not yet correlate with recommendations emerging in the literature and stratified medicine community.

## Implications

Based on my research findings, I suggest the following implications for drug developers of targeted oncology therapies when making decisions on biomarker-guided clinical trial strategies:

1. Drug developers are not using common prevalence thresholds to determine whether to pursue biomarker-guided strategies in early stage clinical trials for "pathway-modifying" targeted therapies. Further evaluation is needed to understand what prevalence levels are required for efficient clinical trials and ultimately commercially successful targeted therapies.
2. Biomarker-guided clinical trial strategy decisions are not influenced by companion diagnostic performance levels as there were no common desired sensitivity or specificity levels. Drug developers have limited knowledge of the companion diagnostic processes and significant expertise will need to be gained through partnerships with diagnostic companies, further complicating the drug development process.

3. Significant uncertainty and challenges still exist for validating biomarkers due in part to increasing biological complexity. Processes will need to be developed to support biomarker validation but this can also only be accomplished through further clinical investigations. Using more phase I expansion cohorts during clinical trials to test hypotheses may be valuable.
4. Choosing conservative biomarker cutoff values may be a way for drug developers to minimize the uncertainty and challenges that surround biomarker validation. Initially conservative cutoffs can help the drug achieve approval but further investigations will be needed to understand how best to benefit applicable patients.
5. Phase I expansion cohorts are being used to further investigate and confirm scientific hypotheses for "pathway-modifying" therapies, however, some trials are pursuing biomarker-selected only patient populations in early-stage clinical trials suggesting that oncology community recommendations are not being taken into account or ethical considerations are overpowering scientific inquiry.
6. There is potentially a disconnect between drug developers and clinicians in that clinicians are willing to consider all the factors contributing to the challenge of accurate biomarker detection in determining which patients may benefit from treatment while some drug developers are more concerned with eliminating the possibility of exposing patients to toxicities without a therapeutic benefit.
7. The additional challenges of combining targeted therapies may be causing drug developers to abandon the use of biomarker-guided strategies in combination settings.
8. The assays to support companion diagnostics, regardless of singular or multi-modality approaches, are still being investigated even through phase II trials despite insistences that the companion diagnostic process should begin as early as possible and have a commercializable assay before entering pivotal/phase III trials. The considerable technical challenges of companion diagnostic development are potentially threatening the success of future stratified targeted therapies.

## Appendix: References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