The biomarker is not the end

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As drug discovery and translational scientists think about using stratification with biomarkers to improve the chances of getting medicines to patients, several areas of expertise need to be considered. These include analytical validation, clinical validation, regulatory affairs and intellectual property. Getting the right input from the right expert at the right time can make or break an effort to bring a biomarker-based companion diagnostic successfully into clinical practice.

Introduction

Congratulations. Your sequence analysis has revealed a set of biomarkers that have the potential to improve the overall clinical impact of your drug candidate through improved safety, efficacy or dosing. What do you need to know, as a drug discovery scientist, to work effectively with your partners in clinical development to translate your biomarker into an effective companion diagnostic? Specifically, what is the difference between having a biomarker that is scientifically interesting and having a test that is going to help a drug improve the practice of medicine?

Biomarkers have a long history, reaching back millennia. Texts from several cultures show, for example, that ancient physicians knew that urine from patients with diabetes is sweet to the taste [1]. Today, new classes of biomarkers based on the molecular biology revolution over the past 50 years provide an insight into disease etiology, which is unprecedented in scale and scope [2]. This explosion in the discovery of potentially clinically relevant biomarkers has fueled growth of the diagnostics market.

A report by PriceWaterhouseCoopers suggests that the overall in vitro diagnostics (IVD) market is expected to grow by 5% per annum to US$50 billion during 2012, with sales of molecular diagnostics expected to grow by 14% per annum to US$5 billion [3]. One of the key drivers for the future growth in molecular diagnostics is likely to be in companion diagnostics for targeted medicines. Reasons for this growth include: (i) a desire for ever-improved therapeutic indices of safety and efficacy; (ii) continual

increases in healthcare spending and the need to control those expenditures; (iii) less than optimal drug response rates for current drugs, ranging from 20% to 75% depending on the drug and the disease; and (iv) a move away from mass-market therapies, which can be costly, toward targeted therapies intended to treat smaller patient populations with specific disease subtypes, and who need to be identified as most likely to benefit.

Thus, the future marketing model foreseen for most specialist therapies has a companion diagnostic as a key component. This is neither a new nor unexpected development; in fact, it has emerged more slowly than expected following the completion of the Human Genome Project a decade ago. Still, pharma and biotech companies, in collaboration with a growing academic investigator community, have made progress in confronting major barriers to the growth of the biomarker-based molecular diagnostics market, establishing scientific approaches, technical platforms, and standards and levels of clinical evidence.

Several molecular diagnostic tests have had positive clinical and business impacts. These include a predictive companion diagnostic to select the patient population for trastuzumab by human epidermal growth factor receptor 2 (HER2) status; a detection and/or prognostic diagnostic test for irritable bowel disease that differentiates Crohn's disease from ulcerative colitis to identify patients with Crohn's disease for budesonide treatment; and a treatment-response test measuring *breakpoint cluster region* (*BCR*)-*Abelson murine leukemia viral oncogene homolog 1* (*ABL*) expression levels in patients treated with tyrosine kinase inhibitors. Benefits of these few, but highly successful, diagnostics include: increased total

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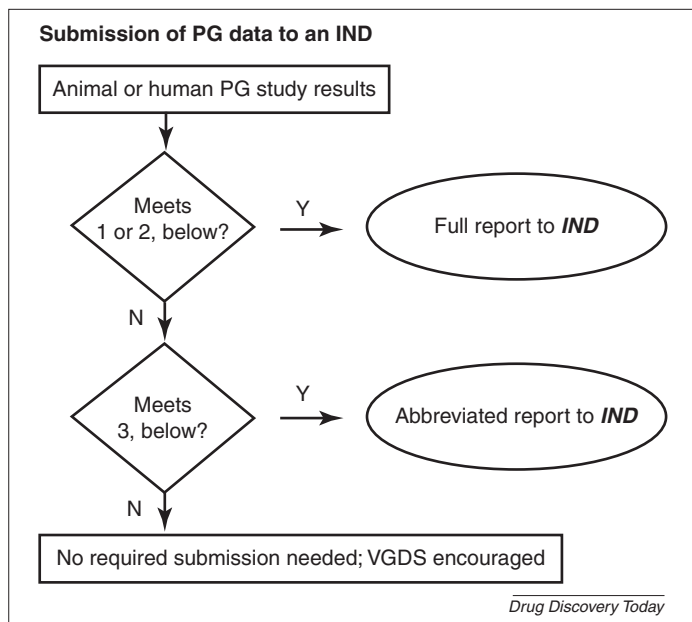
FIGURE 1

Submission of pharmacogenomic data to an investigational new drug (IND) application.

BOX 1

Pharmacogenomic data must be submitted to the IND under Section 312.23 if any of the following apply: (i) the test results are used for making decisions pertaining to a specific clinical trial, or in a animal trial used to support safety (e.g. the results will affect dose selection, entry criteria into a clinical trial safety monitoring, or subject stratification); (ii) a sponsor is using the test results to support scientific arguments pertaining to, for example, the pharmacologic mechanism of action, the selection of drug dosing or the safety and effectiveness of a drug; (iii) the test results constitute a known, or probable, valid biomarker for physiologic, pathophysiologic, pharmacologic, toxicologic, or clinical states or outcomes in humans, or is a known valid biomarker for a safety outcome in animal studies. If the information on the biomarker (e.g. human CYP2D6 status) is not being used for purposes (i) or (ii) above, the information can be submitted to the IND as an abbreviated report. Submission to an IND is not needed, but voluntary submission is encouraged (i.e. information does not meet the criteria of Section 312.23) if information: (i) is from exploratory studies or is research data, such as from general gene expression analyses in cells, animals or humans, or single nucleotide polymorphism (SNP) analysis of trial participants; or (ii) consists of results from test systems where the validity of the biomarker is not established. Abbreviations: IND, investigational new drug; PG, pharmacogenomic; VGDS, voluntary genomic data submission.

patient life years; greater impacts on sales, margins and reimbursement rates for medicines; lower overall costs of treatment and earlier access to proper care.

However, as an industry, there is still some way to go. Drug discovery and translational scientists can have a role in the establishment of best practices by considering some specific areas of expertise and incorporating them into the development effort as early as possible. These include analytical and clinical validation, regulatory affairs and intellectual property (IP). Getting the right input from the right expert at the right time can make or break an effort to bring a biomarker-based companion diagnostic into clinical practice.

I include here three figures that help to define some of the essential steps. The first is a decision tree from the US Food and Drug Administration (FDA)'s 2005 Guidance for Industry; it provides guidelines for the submission of data to an investigational new drug (IND) application (Fig. 1 and Box 1) [4].[a] The second is a detailed overview of the drug-diagnostic co-development process based on this ideal (Fig. 2) [5]. Fig. 3 represents a more pragmatic perspective of the co-development process, which accounts for some of the practical challenges and limitations of a 'real-world' view. Specifically, the development timelines for a companion diagnostic remain constant despite the stage of the development of a drug, a reality that is not fully addressed in the 'ideal' view of the process.

The necessity for crossfunctional competence is especially pronounced in the USA, where tests used to stratify patients for treatment must meet the most rigorous regulatory hurdle for devices, the premarket approval (PMA) process. The PMA carries a burden of data that demonstrates the analytical accuracy and
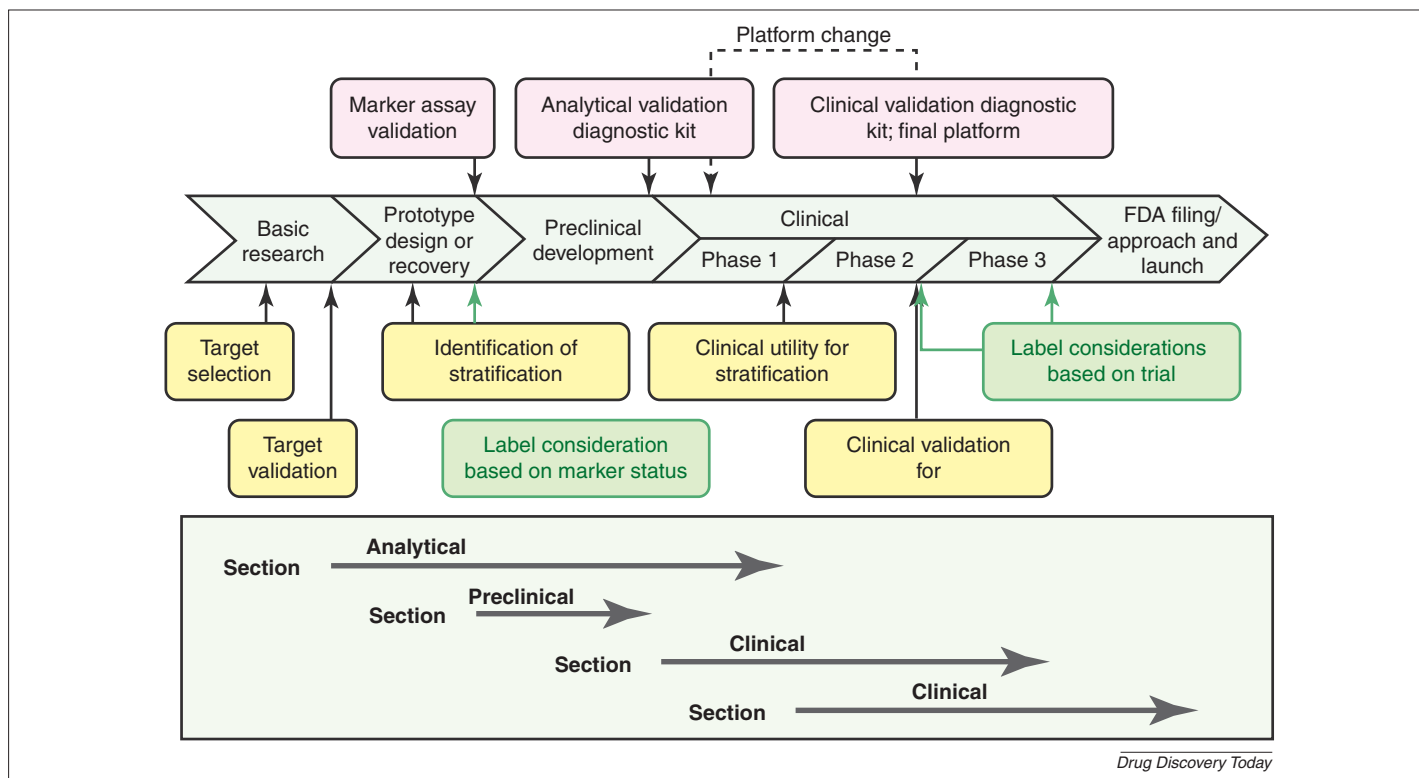
reproducibility of the diagnostic along with its clinical validity, utility and data integrity. Furthermore, if the test is used to generate pivotal data in a registration study for a companion drug, the drug itself might face delays or not be approved without a validated test. Although the European Union (EU) presently has a lower hurdle for these tests (the CE mark), there is ongoing discussion of how this will evolve over time and could follow the risk-based PMA process of the FDA. Similar discussions are also under way elsewhere in the world.

Analytical validation

Even before validating a biomarker and designing a diagnostic test system, the development researcher needs to be aware of two important points that can impact the relevance of biomarkers:

- Choosing a platform for biomarker qualification that is as close as possible to the final diagnostic test system will reduce the risk that biomarkers will not ultimately reproduce in clinical trials. For example, markers that might appear relevant in microarrays during qualification might not appear relevant in a quantitative (QT) PCR system during clinical trials. Aligning systems with the teams that will develop the diagnostics can reduce complications and frustration downstream.

- The biomarker is only as good as the data set. Serious data management issues, including cut-and-paste errors in spreadsheets, reversed sample labels, inadvertent use of duplicate samples and others, are documented examples that have distorted the signatures of biomarkers and led to irreproducible results [6]. Thus, a discussion with a good biostatistician or bioinformatician is crucial.

Once the test system design is complete, analytical validation of the technology and sample preparation that is being used is crucial to establish the robustness and stability of the analytic protocol for the diagnostic. Current genomic and proteomic technologies are

[a] At the time of writing, the FDA had only just published its new Draft Guidance for *In Vitro* Companion Diagnostic Devices [10].
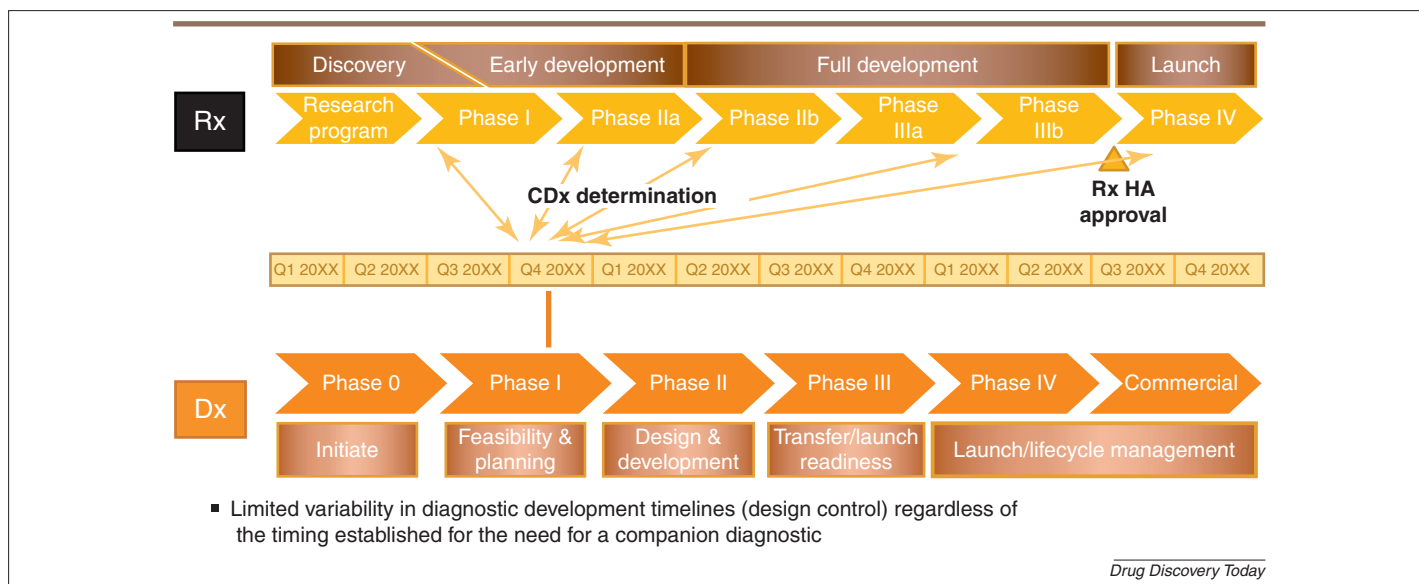
FIGURE 2

An ideal drug-diagnostic co-development process.

remarkably precise and powerful; however, issues with sample collection, sample preparation and cut-off definitions that convert quantitative biomarkers to qualitative diagnostics can generate large analytical uncertainties with data. Even areas such as sequencing or PCR, which seem fairly digital in readout, are susceptible to failures in processing protocols around labeling, handling and contamination of samples. An effort should be made to select analytical equipment that has been cleared or approved by regulatory authorities for clinical diagnostic use. This will shorten the development time and help expedite approvals.

These issues need to be addressed early in, and throughout, the process of research and translation. This can be offset, in part, by good laboratory practices and also by following the guidelines for analytical validation laid out by the FDA and in the Clinical and

FIGURE 3

Pragmatic view of drug-diagnostic co-development process. *Abbreviations*: CDx, companion diagnostic; Dx, diagnostic; HA, health authority; Rx, drug.

Laboratory Standards Institute (CLSI) standards and Clinical Laboratory Improvement Amendments/College of American Pathologists (CLIA/CAP) guidelines.

As the biomarker program moves into translational medicine and full development, it will start generating clinical samples that will be used in support of an FDA filing. Here, the need for analytical validation becomes acute. Conducting a bridging study between the test used in the pivotal clinical trial and the test intended for FDA approval can be difficult if this has not been taken into account from the beginning. Lack of adequate concordance between the two can raise questions of bias and the true treatment effect of the drug on the target population for a companion diagnostic. Timely expert guidance in diagnostics development and quality requirements can save significant time and money.

In the USA, analytical validation of the final test system for IVD products must meet FDA regulations on the minimum requirements. These can include cutoff selection, reproducibility of specimens around the cutoff, sensitivity, specificity, interfering substances, precision and sample collection, shipping and processing. If the final test system is not available until after the clinical trial has started, then a bridging strategy will be needed, using an early version or clinical trial assay (CTA) of the IVD. If patients are stratified by this CTA, then the assay should be expected to be analytically validated before the initiation of trials.

One cause of analytical discrepancies in clinical trials is the use of multiple laboratories and a variety of methods in these trials. In just one example, when ChemGenex advanced Omapro™, it designated two central laboratories for its study, each using different methods for detecting the presence of the key mutation [7]. That lack of uniformity in mutation-testing methods, including establishing an appropriate 'positive' cut-point, figured prominently in the decision of the FDA to hold up approval of the drug. It is clear that drugs that are linked to a companion diagnostic will require an adequate analytically validated test in conjunction with the regulatory filing data.

The recommended approach is to use one central laboratory with one well-controlled method and consensus protocol to minimize trial diagnostic variability. If that is not possible, then a detailed assay procedure with training and proficiency testing and regular cross-comparison of the laboratories is needed. Proficiency testing should be continued at set time periods to ensure accurate results throughout the testing of patients. In addition, sample collections and preparation should be part of a validation and proficiency testing. The clinical test laboratory(s) should be audited for uniformity of results and to ensure that a solid system is in place. These steps will help minimize variables that might impact the quality of a bridging study and concordance results.

Clinical validation

Several issues need to be considered on the clinical side to maximize chances of success. One of the most difficult issues is the ascertainment rate of samples from the trials if a prospective–retrospective bridging strategy is being used for clinical validation and qualification of the IVD [8]. Regulators naturally have concerns about trials with low ascertainment rates.

This raises questions about bias and impact on reproducing treatment effect. Thus, the clinical protocols and site management need to emphasize the importance of achieving the greatest rate of samples possible. This requires aggressive management of informed consents, good sample collection and investigator focus. Poor sample handling, labeling or storage can render samples nonevaluable. These factors need to be considered carefully in planning early clinical trials and require input from knowledgeable clinical operations experts with experience in getting sample rates and crossvalidation of sites that meet the expectations of regulatory authorities.

Another important issue is the biostatistical plan, which is crucial in two respects. First, it is important for doing prospectively designed retrospective analyses with banked samples; however, this is still a somewhat controversial area. Unlike the case with medicines, well-designed retrospective studies based on initially randomized prospective studies can sometimes be used in support of a clinical validation of the test; however, the emphasis is on 'well-designed' in either case. Extremely valuable sample sets should not be used for validation until a sound predefined biostatistical plan is in place and preferably reviewed by regulatory authorities.

One common approach is to use these sample sets for drug discovery work. The sample set must be divided before use to provide a group for discovery work or training set and another test set for validation. Failing to plan for this ahead of time can leave one with an important new biomarker that has been qualified but no easy way to validate it properly. Getting input from a biostatistician experienced in this area of diagnostic development is crucial.

The second aspect is related to the issue of designing adequately powered prospective clinical trials. Peták et al. have noted how clinical trial design can reveal or obscure the importance of biomarkers [9]. Beyond the standard issues of careful trial design, one issue particular to diagnostics, and that differentiates them from general biomarkers, is the need to address the behaviour of marker-negative populations. Trials designed to look only at outcomes in patients who are marker-positive might be cheaper and faster, and address diagnostic sensitivity, but they fail to address specificity adequately and could raise issues with regulators. More generally, they fail to get positive and negative predictive clinical values.

However, the inclusion of marker-negative populations also raises issues. First, it increases the size of the trial (or reduces the power if one does not want to increase the size). Second, ethical considerations can make it difficult to design appropriate randomizations, if there are good reasons to believe that marker-negative populations might not benefit. One key question is how much data from the marker-negative population does the regulatory authority need to see, and can it be generated outside of the pivotal study, perhaps during Phase II. Thus, strong and careful clinical design work from a biostatistics expert with experience with these issues is required.

Regulatory affairs

Receiving expert regulatory guidance from an experienced diagnostics regulatory expert is a must. Because diagnostics are regulated by the Office of In Vitro Diagnostic Device Evaluation and

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Safety (OIVD) within the Center for Devices and Radiological Health (CDRH) rather than the drug divisions, special expertise is necessary, which many drug regulatory experts do not have. However, both skill sets are necessary, as a drug–diagnostic combination will be reviewed concurrently by both centers of the FDA.

However, based on the current view, one can expect that the final document will provide a clear definition of an 'IVD companion diagnostic' and specify the review and approval requirements for both the targeted therapy and the diagnostic test. Recent FDA actions emphasizing the significance of personalized medicine, such as the establishment of the Critical Path Initiative (CPI), suggest that this guidance will lead to greater clarity in the regulation of companion diagnostics [11].

Clinical laboratory testing is overseen by the CLIA program administered by the Centers for Medicare and Medicaid. A test could undergo one of two major types of review: a 510(k) review, for a device that is considered low to medium risk; or a PMA, for high-risk IVDs, such as companion diagnostics. The clinical claims of the proposed intended use for a device in a PMA must be supported by substantial clinical data similar to those submitted in a new drug application.

One particularly valuable step is to get in front of the FDA early in the process for the drug and diagnostic. For a companion diagnostic, this is accomplished through the pre-Investigational Device Exemption (IDE) meeting interaction. Again, diagnostic regulatory expertise is necessary to make these interactions as productive as possible in defining a successful path forward for PMA of the diagnostic test system.

Emphasizing the importance of a coordinated approach, Philip et al. of the FDA listed several prominent reasons for setbacks at this stage [12]: (i) difficulty acquiring samples; (ii) lack of knowledge about proper test design or statistical methods; (iii) shortage of resources for translational research; and (iv) lack of reproducibility across laboratories and over time.

In the EU, the communication of the European Medicines Agency (EMA) on the requirement for biomarker testing is less transparent than that of the FDA, but European initiatives should not be overlooked. For example, the EMA had a role in requiring biomarker testing for Vectibix® (Amgen; http://www.amgen.co.uk/) despite the accelerated approval of the FDA without specific testing requirements. The EMA also has a larger number of drugs for which biomarker testing is required [3].

Intellectual property

There are several issues to consider when designing an IP strategy. One question is when, in the R&D process, to file for a patent protection for a biomarker. If it is filed too early, then the test might not be sufficiently described to get protection. If it is filed too late, then competitors might get to the patent office first. Filing too early for a multi-marker test can also create prior art, which could prevent patenting more refined versions of the test later. In these areas, the guidance and input of an IP lawyer skilled in IP around biomarkers and diagnostics is invaluable.

The recent activity in the courts and legislatures around the world to limit the enforcement of biomarker patents or limit the eligibility of biomarkers for patent coverage has received significant media attention. At the time of writing, the Supreme Court of the USA had agreed to take up the case of Mayo Collaborative

Services (http://www.mayomedicallaboratories.com/) versus Prometheus Laboratories, Inc. (http://www.prometheuslabs.com/), which directly challenges the patent eligibility for biomarkers (Mayo Collaborative Services v. Prometheuslaboratories, Inc; http://www.supremecourt.gov/qp/10-01150qp.pdf). The litigation challenging the patent eligibility of the breast cancer 1, early onset (BRCA1) and breast cancer 2, early onset (BRCA2) gene mutations is also winding its way through the courts. An experienced patent practitioner can establish, and more importantly, execute an effective IP strategy that includes contingencies that account for diminished patent protection for biomarker innovation. Establishing and enforcing exclusivity for biomarker tests might require a more sophisticated IP strategy as the legal landscape for biomarker patents evolves. Freedom to operate (FTO) is an even larger concern and must be determined on a case-by-case basis.

Finding the experts

All this leaves the drug discovery and translational scientists trying to get a drug–diagnostics combination to patients with a long list of diverse areas to think and act upon, and reveals the crucial need to interact early with expert colleagues to get the input to make it happen. Given the ways that pharmaceutical and medical science are evolving, everyone involved in discovering and developing drugs is going to need to know how to get access to the expertise to make a companion diagnostic possible. There are many paths one can take to get this input, but the principal players are finite: (i) biostatistician and/or bioinformatician with experience in diagnostics; (ii) diagnostics development and/or quality expert; (iii) clinical operations and/or trial design expert; (iv) diagnostics regulatory expert; and (v) patent attorney skilled in IP around biomarkers and diagnostics.

A final note about company strategy: one popular choice is to work with a diagnostic partner. However, given the plethora of regulatory pathways that exist for diagnostics, many partners have limited experience with the highest level of regulatory scrutiny, the PMA required for a new companion diagnostic.

Another approach is to develop an in-house group to provide some or all of the requisite expertise. At Novartis (http://www.novartis.co.uk/index.shtml), a discrete business unit within the pharmaceutical division, Novartis Molecular Diagnostics, is staffed with professionals with expertise in all the relevant diagnostics areas, including regulatory affairs, intellectual property, biostatistics, clinical medicine, sample management, assay design and development, reimbursement, and market access and health economics, among others. This strategy reflects an early and ongoing focus on targeted medicines, driven by a rigorous pathways approach to drug discovery spearheaded by the Novartis Institutes for Biomedical Research (NIBR), one byproduct of which is a robust stream of biomarkers to translate into diagnostics.

Acknowledgements

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