Impact of Biomarkers on Personalized Medicine

Patricia Carrigan and Thomas Krahn

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P. Carrigan • T. Krahn (✉)
Bayer Pharma AG, 42096 Wuppertal, Germany
e-mail: patricia.carrigan@bayer.com; thomas.krahn@bayer.com

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Abstract

The field of personalized medicine that involves the use of measuring biomarkers in clinical samples is an area of high interest and one that has tremendous impact on drug development. With the emergence of more sensitive and specific technologies that are now able to be run in clinical settings and the ability to accurately measure biomarkers, there is a need to understand how biomarkers are defined, how they are used in clinical trials, and most importantly how they are used in conjunction with drug treatment. Biomarker approaches have entered into early clinical trials and are increasingly being used to develop new diagnostics that help to differentiate or stratify the likely outcomes of therapeutic intervention. Tremendous efforts have been made to date to discover novel biomarkers for use in clinical practice. Still, the number of markers that make it into clinical practice is rather low. In the next following chapters, we will explain the various classifications of biomarkers, how they are applied, measured, and used in personalized medicine specifically focusing on how they are used in de-risking the 10 plus years drug development process and lastly how they are validated and transformed into companion diagnostic assays.

Keywords

Biomarker · Biomarker classification · Biomarker technologies · Clinical trial · Companion diagnostics (CDx) · Drug development · Patient stratification · Personalized medicine · Personalized treatment · Predictive biomarker · Prognostic biomarker

Abbreviations

510(k)	Premarket notification submission
ADC	Antibody drug conjugates
ALK	Anaplastic lymphoma kinase
B-RAF	Isoform B of rapidly accelerated fibrosarcoma gene
BRCA1	BReast CAncer gene 1
BRCA2	BReast CAncer gene 2
BUN	Blood urea nitrogen
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDx	Companion diagnostic
CECs	Circulating endothelial cells
CFR	Code of federal regulations

CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare & Medicaid Services
CREST	Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia
CTCs	Circulating tumor cells
DCIS	Ductal carcinoma in situ
DM1	Mertansine, a derivative of maytansine
DNA	Deoxyribonucleic acid
Dx	Diagnostic
EGFR	Epidermal growth factor receptor
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EML4-ALK	Echinoderm microtubule associated protein-like 4-anaplastic lymphoma kinase
EpCAM	Epithelial cell adhesion molecule
FDA	Food and Drug Administration
FFDCA	Federal Food Drug and Cosmetic Act
GWAS	Genome-Wide Association Studies
HbA1c	Glycosylated hemoglobin
HER2	Human epidermal growth factor receptor 2
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
IUO	Investigational use only
IVD	In vitro diagnostic
KIM-1	Kidney injury molecule-1
lncRNA	Long noncoding RNA
LC–MS	Liquid chromatography–mass spectrometry
LDT	Laboratory developed test
miRNA	MicroRNA
mRNA	Messenger RNA
MRM	Multiple reaction monitoring
MS	Mass spectrometry
ncRNA	Noncoding RNA
NGS	Next-generation sequencing
NIH	National Institutes of Health
NSCLC	Non-small cell lung cancer
PAM50	Signature of 50 genes for subtyping breast cancer
PCA3	Prostate cancer gene 3
PCR	Polymerase chain reaction
PMA	Premarket approval
PoC	Proof of concept
PoM	Proof of mechanism
PSA	Prostate-specific antigen
QMS	Quality management system
RNA	Ribonucleic acid

RNase	Ribonuclease
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
RUO	Research use only
SAGE	Serial analysis of gene expression
SCr	Serum creatinine
SNPs	Single-nucleotide polymorphisms
STRs	Short tandem repeats
TMA	Transcription-mediated amplification

1 Introduction

As far as medical literature dates back, references to "biomarkers" can be found. Most often these biomarkers were associated with something noticeably different in a biological sample from a patient that presented with symptoms. These associated differences were then used for the diagnosis, prognosis, and therapy to treat the disease. A well-known example of a biomarker of disease is the analysis of urine. Not only the color and smell but also the sweet taste of urine was used to diagnose and treat diabetic patients. The first official documentation of the term "biomarker" was published by Karpetsky et al. (1977) in a paper describing the potential use of serum ribonuclease levels as a "biomarker" that could be used to aid in diagnosis of patients with multiple myeloma (Eknoyan and Nagy 2005). The term "biomarker" since then and its usage have dramatically increased over the last three decades due to the revolution of molecular biology and medicine, in part because it is now 15 years since the human genome was sequenced but also because the scientific community is now more closely linked by the World Wide Web and large data base consortiums. Through collectively publicized programs like the Cancer Genome Atlas (Kucherlapati et al. 2012; Perou et al. 2012) and Stand Up to Cancer (2015) (www.standup2cancer.org), biomarker initiatives are actively underway identifying numerous biomarkers that may serve as targets for personalized therapy. Increasing efforts to detect and diagnose diseases at the earliest stage are a major driving force behind these biomarker initiatives. However, it is important to mention that there is a substantial amount of work that needs to take place on both the drug development and biomarker fronts to validate a biomarker that is capable of predicting drug response. A bad marker can easily translate into a bad drug, respectively, leading to a wrong treatment.

In 2001, the National Institutes of Health formed a Biomarkers Definitions Working Group to clearly define (Biomarkers Definitions Working Group 2001) what they view as a biomarker since the use of the term "biomarker" can be interpreted differently. Based on this group, a biomarker has been defined as "*a characteristic that is objectively measured and evaluated as an indicator of normal biological processes*, *pathogenic processes*, *or pharmacologic responses to a therapeutic intervention*." In addition to the NIH, the European Medicines Agency (EMA) also came to a consensus on the meaning of what a biomarker is and defined

it as "*a measurable DNA and/or RNA characteristic that is an indicator of normal biologic processes*, *pathogenic processes*, *and/or response to therapeutic or other interventions*." There are several more definitions of biomarkers found within the literature (Strimbu and Tavel 2010), but fortunately there is a general consensus on how biomarkers are being classified and used within the community.

With the emergence of new innovative technologies, the identification and validation of a biomarker is becoming one of the key elements to *personalized medicine*. For example, in cancer, personalized medicine uses specific biomarker information that has been measured from a person's tumor to help diagnose, plan treatment regimens, monitor a treatment response, or aid in determining a prognosis. The goal of personalized medicine is to provide each patient based on his individual biomarker characteristics, beyond the functional diagnosis of his disease, with the right treatment and dose at the right time. Since biomarkers are often expressed differentially in tumor tissues in comparison to healthy tissue, measuring such protein or nucleic acid markers are now proving useful in identifying alternative treatment strategies when the approved first-line therapy has failed. Technologies such as next-generation sequencing (NGS), real-time PCR, and mass spectrometry that used to be only found in academic laboratories are now being used for measuring patient samples in a real-time setting. Such technologies allow for the identification of potential new druggable targets that are specific to the patient's tumor profile. Personalized medicine is seen by some reviewers as more than just tailoring the right therapeutic strategy for the right patient but a way to identify alternative treatment options.

Personalized medicine means something different to patients, physicians, scientists, regulators, and payers. For patients, it is a refined diagnosis of the underlying disease that eliminates the "trial and error" period and expedites a new stratified approach. As not all patients respond favorably to new targeted drugs, research and development scientists heavily rely on biomarker-driven patient stratification to enrich for the population of patients that have the highest chance of a successful drug response. Regulatory bodies, however, view personalized medicine as a way to protect patients demanding that drug development companies investigate in identification of such biomarkers so patients are only given therapies that show some sort of benefit. Lastly insurance companies are demanding greater proof of positive patient outcomes to justify approval, reimbursement, and price on new targeted therapies.

Personalized medicine is here to stay with biomarkers being implemented into early drug development processes and early clinical trials. Scientists are getting better at understanding the demanding processes that are needed to validate, verify, and implement biomarker strategies for all types of drugs ranging from oncology rare diseases to more frequent cardiovascular disorders, while at the same time companion diagnostics are too becoming more of the norm rather than the exception.

2 Biomarker Classification

During the drug development processes, biomarkers are used to determine the success of development milestones. These milestones help with de-risking the drug program as well as de-risking the patient population in which the new drug is to be tested. Biomarkers are used to answer important questions such as "Does this new drug hit the planned target?" "Is this drug safe?" "Is the therapy more effective in one population?" "Does the biomarker predict survival?" "Is this biomarker unique to disease status?" "Does this biomarker guide treatment decisions?" Herein we will describe and answer the aforementioned questions in more detail explaining how biomarkers are used in monitoring the drug development process and clinical responses.

2.1 Does This New Drug Hit the Planned Target?

Prior to and after administering the new drug, biological samples will be collected and used for pharmacodynamic biomarker studies. A pharmacodynamic biomarker shows that a biological response has occurred in a patient who has received a therapeutic intervention, and for which, the magnitude of change of the respective biomarker is linked to the response. Pharmacodynamic biomarkers are largely used for dose finding and often provide decision relevant information that supports the proof of concept (PoC) and/or proof of mechanism (PoM) that is required to be met in order for the drug to continue on through the drug development process. The use of specific pharmacodynamic biomarker in the development of targeted therapies defines important data enabling early go/no-go decisions, selecting combinations of targeted agents, and optimizing schedules of drug combinations. The respective biomarker assays need to provide robust and accurate measurements.

2.2 Is This Drug Safe?

Safety or toxicity biomarkers reflecting a response to treatment are used to detect or monitor adverse effects in a patient receiving a therapeutic intervention. Prior to be administered to humans, several preclinical animal models of different species are used to understand the potential toxicological profile for the new drug. Routine biomarkers such as liver enzymes are measured and are used to determine a suggestive toxicological profile. Urinary kidney injury molecule-1 (KIM-1) is the first biomarker of kidney toxicity qualified by the FDA and EMA and is expected to significantly improve kidney safety monitoring. Traditional biomarkers of renal injury, including serum creatinine (SCr) and blood urea nitrogen (BUN), do not show the sensitivity and/or specificity to adequately detect nephrotoxicity prior to significant loss of renal function. In multiple models of kidney injury, urinary KIM-1 significantly outperformed SCr and BUN (Vaidya et al. 2010). In addition,

these markers are used to identify an adverse reaction at an early stage in a subject receiving drug.

2.3 Is the Therapy More Effective in One Population?

Stratification biomarkers predict efficacy and categorize patients by likelihood of response to a particular treatment, enabling enrichment of patients most likely to respond to therapy. The best described example is again HER2 status for treatment with trastuzumab (Herceptin®). Stratification biomarkers also predict whether a patient develops an adverse reaction to a prescribed drug, enabling enrichment of patients that can be safely treated with a specific drug. For example, P450 variants predict drug metabolism in multiple indications. Many genetic variants have been identified that are known to alter cytochrome P450 (CYP) enzymes and drug receptors, transporters, and targets (Crews et al. 2012). Known mutations in cytochrome P450 are, for example, used to select the starting dose of the anticoagulant warfarin.

2.4 Does the Biomarker Predict Survival?

Prognostic biomarkers are biomarkers that provide information on the risk or likely course of the disease in an individual and the potential overall outcome. Sometimes the terms susceptibility or risk biomarker are used for biomarkers linked to the risk of developing a disease. The BRCA1 and BRCA2 mutations are typical examples in oncology. Germline mutations in the BRCA1 or BRCA2 tumor-suppressor genes are strong predictors of breast and/or ovarian cancer development (Fackenthal and Olopade 2007). In addition, four gene signature panels, the Oncotype DX® (Genomic Health), the MammaPrint® (Agendia), the ProSigna™ (Nanostring), and the Mammostrat® (Clarient Diagnostic) panels, are commonly used to test breast cancer patients. While all four tests are somewhat similar, there are differences: The Oncotype DX® test is used to estimate a woman's risk of recurrence of early-stage, hormone-receptor-positive breast cancer, as well as how likely she is to benefit from chemotherapy after breast cancer surgery. The Oncotype DX® test also is used to estimate a woman's recurrence risk of DCIS (ductal carcinoma in situ) and/or the risk of a new invasive cancer developing in the same breast, as well as how likely she is to benefit from radiation therapy after DCIS surgery. The Oncotype DX® test analyzes the activity of 21 genes. The MammaPrint® test is used to estimate a women's recurrence risk for early-stage breast cancer. The breast cancer can be hormone-receptor positive or hormone-receptor negative. The MammaPrint® test analyzes 70 genes to see how active they are and then calculates either a high-risk or a low-risk recurrence score. MammaPrint® results can help a woman and her doctor make a more informed decision about whether to use chemotherapy to reduce recurrence risk. The Mammostrat® test is used to estimate a woman's risk of recurrence of early-stage, hormone-receptor-positive breast

cancer. The Mammostrat® test measures the levels of five genes in breast cancer cells. These measurements are used to calculate a risk index score. The higher the risk index, the more likely the cancer is to come back. The Prosigna™ test was developed based on the signature of 50 genes. The expression of these PAM50 genes is an accepted standard for subtyping breast cancer and used to classify tumors into 4 different subtypes.

2.5 Is This Biomarker Unique to Disease Status?

Diagnostic biomarkers are biomarkers that help defining a disease in a group of similar diseases. These biomarkers are used to identify a disease, a subtype of a disease, or a specific condition. Typical examples are elevated blood glucose levels and increased glycosylated hemoglobin (HbA1c) as indicators of diabetes.

2.6 Does This Biomarker Guide Treatment Decisions?

Predictive biomarkers are biomarkers that are used for patient stratification, predicting response to a specific treatment, which may increase the chance for a successful treatment. These biomarkers are used to predict whether or not a patient is likely to respond to a specific therapy or treatment regimen. The expression of the drug target is, for example, used in this respect for targeted therapies in oncology. A well-known example is the detection HER2 overexpression in patients suffering from, e.g., breast cancer predicting a likely response to trastuzumab (Herceptin®). Two additional biomarkers that are used to determine treatment decisions for patients that have been diagnosed with advanced stage non-small cell lung cancer are epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK). Whether or not these biomarkers contain a mutation or a gene rearrangement determines if patients will be treated with either the drugs erlotinib (Tarceva®) or crizotinib (Xalkori®), respectively.

3 Biomarker Technologies

3.1 Technology Trends and Applications

For the last 10 years, biomarkers (nucleic acid, mutation presence, gene panels/signatures, gene rearrangements, and protein expression patterns) have gained a great deal of attraction and application into clinical trials. This goes in parallel with the increasing number of new targeted approaches in drug discovery and a broad range of new technologies that have helped facilitate these applications. As a consequence of new technological platforms, a significant drop in costs for pharmacogenomics and pharmacogenetic testing has occurred leading to more deep sequencing of patient tumors and large uncombed data sets. In turn, the

diagnostic industry has used these advancements and newly identified gene signatures/panels of biomarkers to show that their technologies are possible for utility in clinical settings.

Emerging technologies such as RNA sequencing, SNP analyses, and multiplexing platforms such as Luminex® and Mesoscale Discovery® have significantly fuelled biomarker identification. With such technologies, routine clinical studies are now able to include biomarker tests that yield the supporting evidence needed to validate the biomarker hypothesis and its use as the surrogate marker for the diagnostic assay. Implementation of these novel technologies and assessments of the clinical relevance and the utility is a time-consuming effort required by pharmaceutical companies. However, there is such an importance and added value to the data generated by these technologies that public–private partnerships have been established, and more collaborations between pharmaceutical and diagnostics companies are happening. Since the biomarker technology landscape is in high flux, the field requires continuous monitoring and changes in regulation of standards set forth by agencies such as the FDA and EMA not only to monitor the quality of the data but also to guarantee consistency within the drug and diagnostic development/implementation processes employed within clinical trials.

The next generation of platforms that are most attentively being watched and provide the most sophisticated and cutting edge advances are the technologies that can deliver the highest specificity and sensitivity but can also detect multiple nucleic acids or proteins simultaneously from a limited amount of starting material. This focus is mainly for clinical use since reproducible biomarker data is needed to support the drug in question. In addition, technologies that have a very streamlined, standardized preanalytic upfront sample handling processes, e.g., supported by point of care devices and less invasive procedures to gain access to human specimens, are being watched. The concept of liquid biopsies is an example of a technology that is still in its infancy but one that will allow closer monitoring of patients over time and can contribute to a better understanding and clinical management of drug resistance in patients with cancer (Pantel and Alix-Panabières 2013).

3.2 Technologies Based on the Molecular Basis of Biomarker

Beside the classical tissue-based immunohistochemistry and in situ hybridization technologies, the current biomarker research field is very much focused on technologies to detect biomarkers in blood and other body fluids. There is especially in cancer patients an urgent need for these less invasive procedures and for technologies that require the least amount of material. Technologies to detect and monitor cancer formation range from the analysis of DNA including mutations, rearrangements, and methylation to mRNA and miRNA to proteins, including altered processing and other modifications. Additional sources for biomarkers are circulating rare cells, e.g., circulating tumor cells (CTCs), immune cell profiles,

exosomes/microvesicles, as well as autoantibodies, cytokines, and metabolites discharged into the different body fluids (Hanash et al. 2011).

The development and implementation of genomics and other "omics" technologies into clinical trials have significantly promoted the identification and validation of novel biomarkers. Pharmacogenomic technologies like NGS are key elements of the emergence of personalized medicine. The progress of technologies generates new knowledge on potential association of genomic biomarkers of health and disease at an unprecedented speed in science: however, translation of this information into clinical tools currently lags behind due to the difficult processing and interpretation of such large data sets. Complex algorithms are being developed and used to support biomarker gene panel signatures that are proving to be not only diagnostic but also prognostic. Also the lack of a clear rationale for the choice of genomic techniques and difficulties in obtaining sufficient samples to properly validate and address the needed clinical correlations lead to non-reproducible and clinically non-validated biomarker data, highlighting the potential of genomics but slowing down the delivery of clinically usable tools (European Medicines Agency, Concept paper on good genomics biomarker practices 2015).

3.3 DNA Biomarkers

Genetic information that is coded within DNA directs the production of proteins required for the cell structure and function of cells over a lifetime. Some authors state that DNA is stable over an individual's lifetime, and biomarkers explicitly representing this stability are termed "DNA biomarkers." Single-nucleotide polymorphisms (SNPs), short tandem repeats (STRs), deletions, insertions, or other variations on the DNA sequence level are among this group. Due to the availability of high-throughput molecular biological facilities, SNPs are the most commonly used type of DNA biomarker. Beside the established role of point mutations in protein-encoding genes as diagnostic or pharmacogenetic biomarker, genetic variation contributes to both disease susceptibility and treatment response. Genome-wide association studies (GWAS) have enabled rapid discovery of genetic variants contributing to the pathogenesis of complex genetic diseases as well as detection of many pharmacogenetic markers. Such pharmacogenetic markers have been successfully used to explain drug exposure and clinical response variability, to calculate the risk for adverse events, to guide genotype-specific dosing, and to explain mechanisms of drug action in case of polymorphic drug target. However, it is fair to say that the vast majority of risk alleles that have been found by GWAS studies since 2005 are still far away from being used for individual disease prognosis or even for drug treatment decisions. A good example is the Factor V Leiden mutation that is consistently found in GWAS studies as being strongly associated with an increased risk of venous thromboembolism, without justifying preventive treatment of mutation carriers with anticoagulants. The driving hope of these major advances in genetic epidemiology is that promotion of personalized medicine will improve medical decision-making (Ziegler et al. 2012).

3.4 RNA Biomarkers

In contrast to the very stable DNA biomarkers, the versatile transcriptome with all its different components like mRNA, microRNA, and short and long noncoding RNAs is the next level on which dynamic changes on the molecular level can occur. Some of the methods used to detect biomarkers at the RNA expression level include quantitative reverse transcription polymerase chain reaction (RT-qPCR), serial analysis of gene expression (SAGE), differential display, bead-based methods, and microfluid card and microarray analysis. Comparative analysis of RNA expression in terms of heat maps, supervised algorithms, and snapshots are eventually linked with diagnosis and prognosis.

The major attraction in transcriptomics as a starting point for biomarker identification is the ability to measure mRNA concentrations of all genes under any condition that allow studying regulation of gene expression at a genome-wide scale. The genome-wide search for mRNA biomarkers is since more than 20 years an established method in different life science fields. In pharmacogenomics mRNA expression levels were successfully applied to establish treatment prediction with specific drugs. In most studies, a number of genes whose expression was influenced by treatment could be identified. Hence the identification of a biomarker pattern consisting of various expressed genes will be more promising than finding standalone single markers. Despite the impressive number of publications aiming on the detection of mRNAs that predict disease progression or indicate the appropriate use of a specific drug treatment, the current FDA list of pharmacogenomic biomarkers in drug labeling does not even count ten different mRNA-based assays.

3.5 microRNA

Instead of the classical analysis of mRNA, the quantitative analysis of microRNA (miRNAs) is more and more used for biomarker identification and establishment. miRNAs are small noncoding RNA molecules with 20–22 nucleotides which are involved in posttranscriptional processing of mRNA. They are able to regulate physiological pathways and metabolic processes and therefore impact the entire cellular physiology, organ development, and tissue differentiation. Most miRNAs are known to be expressed in a physiological-, tissue-, and disease-specific manner. Due to their short length, they are less sensitive to RNase exposure and hence are more stable than the longer mRNA with an average length of 2 kb. It is already proven that miRNAs have the potential in the diagnosis of specific types of cancer. For example, tissue derived from gastrointestinal cancer can be differentiated from non-gastrointestinal cancer tissue by analyzing specific miRNA profiles. As also described for mRNA, the miRNA profile characterization gives insights in the progression of specific diseases or the response to a given therapeutic approach, e.g., in breast cancer, miR-210 levels correlate with sensitivity to trastuzumab (Herceptin®) and miR-125b is predictive of chemoresistance (Roosbroeck et al. 2013).

The expression of miRNAs cannot only be measured in tissue or cell culture samples; they are also present in body fluids, like urine, blood, or even milk. Some of those circulating miRNAs are already known to be specific disease markers, especially for different forms of cancer. In the field of cardiovascular research, several microRNAs in serum have been found to improve the diagnosis of acute coronary syndromes on top of the clinical gold standard high-sensitive cardiac troponin. To be used as a routine biomarker for ACS, the speed of the quantification of microRNAs must be increased. The current PCR-based quantification of miRNAs takes much too long to affect decision-making in acute life-threatening conditions such as acute coronary syndrome and is thus unlikely to replace or even complement cardiac troponin assays.

Some level of caution should be taken into consideration when assessing the usefulness of circulating RNAs as biomarkers, as recent studies report on the importance of the origin of biomarkers and their impact on biomarker specificity. For example, a significant proportion of miRNAs derived from red and white blood cells have been found to be present as contaminants in plasma preparation. In addition, inherent differences in biological samples and the methods of collecting and analyzing them can dramatically affect the detection and quantification of miRNAs and other (noncoding) ncRNAs. To identify true disease-specific circulating RNAs, the approaches used for quantification of these RNAs should be optimized and validated for accurate quantification of circulating RNAs (Roosbroeck et al. 2013).

3.6 lncRNA

Noncoding RNAs with a length of more than 200 nucleotides belong to the group of long noncoding RNAs (lncRNAs). Long noncoding RNAs have only just recently been identified to play a major role in gene regulatory pathways for a wide spectrum of human disease conditions, including multiple cancer models. Presently there are already numerous regulatory (and other) roles for which lncRNAs have been identified to be responsible for, though such roles can be classified as either positive or negative expressions of gene regulation at either one of the transcriptional or posttranscriptional levels (Ayers 2013).

In biomarker research, the group of lncRNAs is coming into focus, especially in cancer research. Due to its regulatory functions, different potential lncRNA biomarker candidates are already available. One of the first identified lncRNAs, H19, is a biomarker for tumors of the esophagus, liver, bladder, and colon and for metastases in the liver. A loss of methylation in its promoter region leads to a strong upregulation of this lncRNA, indicating the presence of tumor tissue. Similar to miRNAs, lncRNAs are also detectible in body fluids, although they are less stable than microRNAs.

One FDA-approved diagnostic assay for prostate cancer is based on the detection of the long ncRNA PCA3 in urine samples of patients at risk for prostate cancer. The Progensa™ PCA3 test from Gen-Probe Inc. uses the transcription-

mediated amplification (TMA™) technology to determine a PCA3 score from male urine. TMA is an isothermal nucleic acid-based method that can amplify RNA or DNA targets a billion-fold in less than 1 h. PCA3 is the first long ncRNA to be used in clinical diagnostic assays, but with the recent developments in the ncRNA world, many more will most likely follow soon.

3.7 Epigenetic Factors

The term "epigenetic" defines all heritable changes in gene expression and chromatin structure that are not coded in the DNA sequence itself. With minor exceptions (T- and B-cells of the immune system), all differentiation processes are triggered and maintained through epigenetic mechanisms. Epigenetic inheritance includes DNA methylation, histone modifications, and RNA-mediated silencing, all of which are essential mechanisms that allow the stable propagation of gene activity states from one generation of cells to the next. Several of these major epigenetic aberrations have been developed into biomarkers. Epigenetic biomarkers can be detected in tissue and in blood as circulating DNA (Greenberg et al. 2012). The exploration of epigenetic biomarkers in cancer for clinical use is a relatively new but rapidly developing field. Applications include screening, diagnosis, classification, surveillance, and targeted therapies. If epigenetic factors are to be effective biomarkers in clinical practice, they must be detectable by noninvasive means and outperform the current gold standard, as is true for all new emerging biomarkers. One of the most exciting cases for the use of epigenetic biomarkers outside oncology was the recent finding that DNA methylation status can predict response to therapy with either methotrexate or blockers of tumor necrosis factor alpha in patients with rheumatoid arthritis (Plant et al. 2014).

3.8 Protein Biomarker

While early work has been strongly focused on nucleic acid-based biomarkers (DNA, SNPs and mRNA expression profiles), recent experience suggests that the utility of these markers as clinically applicable decision tools may generally be limited. Protein biomarkers, which offer a significantly greater degree of differentiated information content, are likely to close this gap. Two types of protein assay platforms are currently applied to discover protein biomarkers and to measure them quantitatively and qualitatively (i.e., to determine the isoform state of a protein such as phosphorylation). It is instructive to point out here that an antibody use is a "fit-for-purpose" approach. For example, the requirement for an ELISA is substantially different from that of immunohistochemistry (IHC) or diagnostic assay versus laboratory assay (Qoronfleh and Lindpaintner (2010), www.ddw-online.com).

3.9 Immunoassays: Direct Use of Antibodies

Immunoaffinity-based assays are the mainstay of testing for proteins. They use antibodies directed against the protein or isoform of interest. Detection of the antibody–antigen (protein) complex provides the quantitative measurement of the amount of antigen present in the sample. A variety of methods are used that vary both by how the antibody and antigen come into proximity of each other to form a complex (based on what the antibody or antigen is fixed to) and by the detection method used to monitor the amount of complex. Western blots are the simplest and most widely used immunoassay method in biomedical research. ELISA is the method most often used in clinical settings (e.g., PSA test). A number of platform technologies offer methods for multiplexed and miniaturized immunoaffinity assays (e.g., Luminex®, Meso Scale Discovery®, and PerkinElmer®). Development of antibody-based assays is a time-consuming, resource-intensive effort and frequently hampered by cross-reactivity to other antigens. Moreover, results from immunoassays often do not discriminate among closely related forms of specific proteins. Currently, commercial tests are available for several hundreds of different proteins (using various methodologies); custom-immunoaffinity assay services for others are provided by a number of specialty providers.

3.10 Mass Spectrometry Assays

One of the major challenges facing the emerging field of protein biomarkers is the fact that many biomedical relevant biomarkers are present at very low abundance in human samples. The immunoaffinity LC–MS/MS approach has been specifically devised to address the analytical challenge imposed by the tremendous dynamic range of protein biomarkers, especially in biofluids. For instance, serum or plasma analytes of interest are first enriched in the sample using immuno-based approaches, followed by mass spectrometry-based further characterization. An example of this approach is the design and validation of an immunoaffinity LC–MS/MS assay for the quantification of a collagen type II neoepitope peptide in human urine as a biomarker of osteoarthritis (Nemirovskiy et al. 2010).

While mass spectrometry has been widely used over the years for hypothesis-free detection of protein biomarkers, its application has been impeded by lack of sensitivity and the nonquantitative nature of the tests. More recently, a variant of the technology commonly referred to as peptide MRM (multiple reaction monitoring) is gaining importance as a more quantitative variant for protein biomarker measurements of this platform. Peptide MRM can combine the high selectivity and specificity of mass spectrometry for the protein of interest with impressive quantitative accuracy and dynamic range. Quantitation obtained by this method is based on the peak area for the mass spectra data of the analyte relative to a known quantity of an isotope-labeled standard. The peak area can be used to provide relative quantitation (similar to most immunoaffinity assays) or absolute quantitation (protein concentration). Proteins at low abundance levels in samples will

require a method to enrich for the protein(s) of interest. One such enrichment method is immuno-enrichment with antibodies to the protein or the peptides, another one is the immune-adsorption-based depletion of the sample of abundant protein species. Thus, even mass spectrometry is highly dependent on antibody technology. Indeed, the marriage of the two approaches is fast emerging as one of the most powerful approaches in biomarker research.

3.11 Autoantibodies

An autoantibody is defined as an antibody that an organism produces against the individual's own proteins originating from cells or cell components. Autoantibodies can be used as highly sensitive and specific biomarkers, and their occurrence in blood offers easy access and cost-effective assays. Autoantibodies appear often in quite early disease states and can therefore be used for a respective early diagnosis. The use of immunofluorescence techniques, in which tissue culture cells were used as antigen substrate for detecting autoantibodies, led to the detection of autoantibodies in various autoimmune diseases, including systemic lupus erythematous, scleroderma, dermatomyositis, and mixed connective tissue disease. Certain autoantibodies produce distinct patterns of staining because they react with specific organelles in the nucleoplasm, nuclear membrane, nucleolus, or cytoplasm. An outstanding example is an autoantibody in the CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) subset of scleroderma, in which immunostaining revealed a limited number of dots in the nucleoplasm of interphase cells but a total redistribution of these dots to the centromeric regions of condensed chromosomes of cells in mitosis. It became clear that there were multiple autoantibodies of different specificities in any individual autoimmune disease, a few autoantibodies were disease specific, and different autoantibody profiles were associated with different diseases. Such profiles of autoantibodies now serve as diagnostic biomarkers in autoimmune diseases. Their clinical application has, however, been hindered by low sensitivity, specificity, and low predictive value scores. These scores have been shown to improve when panels of multiple diagnostic autoantibody biomarkers are used. A five-marker biomarker panel has been shown to increase the sensitivity of prostate cancer diagnosis to 95% as compared with 12.2% for prostate-specific antigen alone (Zaenker and Ziman 2013).

3.12 Exosomes, Microvesicles

Exosomes and micro-vesicles can be used as biomarkers in translational and personalized medicine. Exosomes are small micro-vesicles which are secreted into different body fluids, e.g., blood, urine, or cerebrospinal fluid by a variety of cells. The expression of proteins in the membrane of these microvesicles and the cytoplasmic RNA and protein content facilitate the allocation to the cell of origin in

healthy volunteers as well as patients. Isolation and molecular profiling of exosomes can provide evidence for the existence of a therapeutic target and therefore be used for patient stratification. Exosomes are on the other hand a valuable source for miRNAs.

3.13 Rare Cells, Immune Cells

A large variety of technologies are under development to capture rare cells of different origin from the blood stream. Molecular characterization of circulating rare cells allows a prediction with respect to the prognosis of the course of a disease as well as the effectiveness of therapeutic interventions. Capturing fetal cells in the bloodstream of pregnant women enables the diagnosis of genetic diseases of the unborn avoiding a risky amniocentesis. Circulating endothelial cells (CECs) can be separated into endothelial progenitor cells and detached mature cells, the latter being indicative for a damage of the endothelial lining. Endothelial progenitor cells reflect a sustained vascularization capability and are suspected to be a marker of angiogenesis and tumor growth in cancer patients.

The increasing number of treatment options under development for patients with metastatic cancer creates an accompanying need for biomarkers to determine whether the tumor will be responsive to the intended therapy, to monitor early response to treatment, and to anticipate emerging drug resistance. Ideally, these biomarkers would be obtained by minimally invasive means to allow serial sampling and to enable quantitative real-time molecular analyses of tumor heterogeneity and evolution as well as drug responsiveness. Molecular characterization of CTCs captured from the blood stream or other body fluids may fulfill this need. The well-known heterogeneity of the primary tumor and metastasis as well as the respective CTCs shedded into the bloodstream determines the need for analysis of multiple CTCs. The current standard for CTC detection is the FDA-approved CellSearch® system by Veridex LLC. This system captures CTCs out of a single blood draw of 7.5 mL based on membrane expression of EpCAM and intracellular cytokeratins. The number of tumor cells circulating in the bloodstream of metastatic cancer patients has been shown to correlate with overall survival (Cristofanilli et al. 2004). As metastasis is the main cause of death in cancer patients, CTCs are seen as a prerequisite for distant metastasis.

4 De-risking Drug Development

Biomarkers are now used in drug discovery and development processes and are proving to be both useful and beneficial in translational and early experimental studies. Biomarkers are incorporated as soon as possible starting with preclinical animal studies to determine and measure pharmacodynamic and safety/toxicity biomarkers. These biomarker assays are then transferred if possible to first-in-man phase I clinical studies. Moreover, depending on the human material that is

collected or archived at the time of diagnosis, the drug developers apply as many high-value information-generating technologies to gleam as much data as possible. Next-generation sequencing (NGS) is now being applied in a retrospective manner to allow the drug development companies more options for the discovery of additional biomarker/gene signature panels that can be used to further determine response, resistance, or patient populations that will most likely benefit. Using prospective or retrospective analyses of pharmacodynamics markers in phase I trials are giving drug developers the window to access the proof of mechanism/concept in limited number of patients. These data interpretations are just one example of how a drug program can be de-risked very early. Predictive markers as well as stratification markers also are being tested in early phase I studies. These studies confirm the biomarker hypothesis. Collectively any biomarker data that can be generated in early phase I/II clinical studies is the ideal way a drug program can be de-risked or refined for greater success.

New classes of drugs that are accelerating personalized medicine are antibody drug conjugates (ADC) that are composed of an antibody that is linked to a drug toxophore. These ADCs are routinely tested in phase I studies in combination with a biomarker assay as the biomarker that is to be measured to determine the patient population to be used in is also the drug target. Archived tissue that has been collected at the time of diagnosis or prospectively collected tissue will be tested by an IHC assay to confirm the biomarker expression level and also determine the prevalence of the biomarker in the test population. An example of a newly approved ADC is a combination of trastuzumab® and a chemotherapy drug called DM1. Trastuzumab® finds the cancer cells and delivers the DM1 upon internalization within the cell. In principle this is how all ADCs work. ADCs are the best example of how biomarkers can speed up the drug development process and how personalized medicine can be applied. The processes in which biomarkers are applied to the drug development are outlined in Fig. 1. Briefly, the use of biomarkers starts with the exploration studies in preclinical models and continues all the way through the pivotal phase II/III trials. These data are continually used to monitor and validate the hypothesis that is needed to support the proof of mechanism/proof of concept in the early development phases and allow for go/no-go decisions to be made.

In parallel with the drug development process, the biomarker also has to undergo a series of development and validation steps. In the event that the biomarker is predictive or a diagnostic marker, the biomarker assay that is used to measure the marker as well as the technology that is used has to be verified and validated just like the development of the drug. Ideally, the biomarker assay and drug should be codeveloped and tested in all clinical trial phases as outlined in Fig. 1. Most often, the biomarker assay lags the drug development phases and will require an expedited development of an assay and a partnership with a diagnostic manufacturer.

Biomarkers/drug combinations are being tested in over 13,000 clinical trials (www.clinicaltrials.gov) with the number of trials increasingly each year (National Institutes of Health 2015). However, it is important to point out that both the drug and the biomarker strategies have to be vetted before the drug can be approved

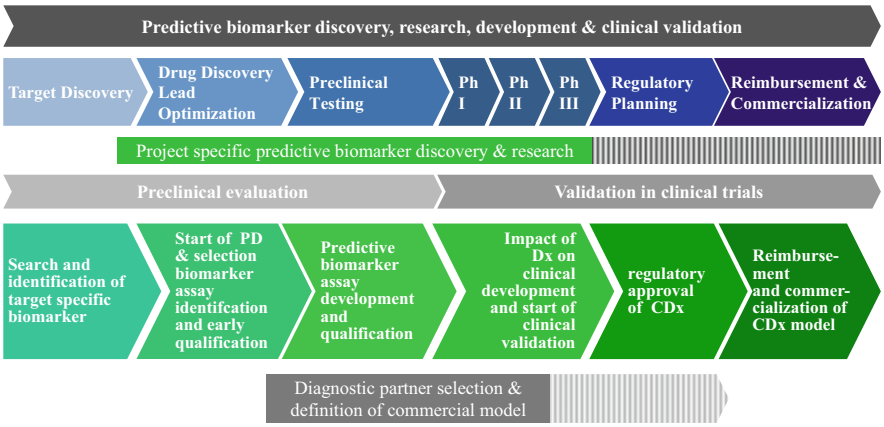

Fig. 1 Overview of the tasks and needs to identify, develop, and validate predictive biomarker along the value chain of drug development. Whereas the target-specific biomarker hypothesis starts to be generated in parallel to the early target validation, the discovery and development of biomarkers can take place along the complete value chain of drug development. Project-specific biomarker assay development starts with the target-specific lead optimization. The development of a diagnostic assay (Dx) which might be used as a companion diagnostic (CDx) in later stages of development should start as soon as the drug candidate and the respective patient population has been selected. Early clinical trials might be used to generate the required data sets for the validation of a diagnostic test to achieve regulatory approval and be used as a CDx

which often is not the case. Recent examples of biomarker/drug programs that did not lead to drug approvals are hENT1 (CP-4126) for treatment of pancreatic cancer and onartuzumab (MetMAb®) for treatment of NSCLC. Even though the biomarker hypothesis seemed to be valid and vetted in the early phase trials, when tested in a randomized phase III pivotal trial, the hypotheses did not yield statistical results with increased survival and overall benefit to BM-positive patients. These trials are devastating to the drug development community and challenge the biomarker/drug development processes. It is still not clear why these programs failed, but programs like these raise the importance of collecting additional biomarker data as early as possible and even after the trials have failed to further de-risk the drug development process or prevent similar failures in other ongoing trials.

5 Biomarkers and Companion Diagnostics

In 2011 the US Food and Drug Administration (FDA) released a guidance document (www.fda.gov, 2015a) that emphasized that all drug development programs should include biomarker measurements. The push to include such biomarkers studies was seen as a way to expedite the drug discovery process, a way to de-risk the treatment population in which the drug is to be administered, and lastly a way to further support the drug mechanism of action. This guidance document in a nut shell upturned the way pharmaceutical companies had been developing new

therapeutic compounds. In turn, the drug development process had to be revamped to include the implementation of validated biomarker assays and in some cases required the pharmaceutical company to start working collaboratively with diagnostic partners. This minor change of including a biomarker assay into a clinical trial has revolutionized and redefined approaches on how to develop therapeutics.

As a result of these new changes, the FDA established a Biomarker Qualification Program to assist with this new guidance and to support the Center for Drug Evaluation and Research's (CDER) work with external scientists and clinicians in developing biomarker assays. The Biomarker Qualification Program provides a framework for the scientific development and regulatory acceptance of biomarker data that are to be used during drug development. Moreover, this program has been integral in facilitating the identification and implementation of new and emerging biomarkers that are to be evaluated and utilized in conjunction with drug programs regulatory decisions/approvals.

Considering that the United States is not the only drug market, the European Union realized the need to standardize drug development across markets. Like the FDA and the Biomarker Qualification Program, Europe and other countries realized the need to rationalize and harmonize the regulations. This was impelled by concerns over rising costs of healthcare, escalation of the cost of R&D, and the need to meet the public expectation that there should be a minimum of delay in making safe and efficacious new treatments available to patients in need. Therefore, in 1990, a meeting was held with representatives of the regulatory agencies and industry associations from Europe, Japan, and the United States to establish the "rules" that the International Conference on Harmonization (ICH) would support. The ICH since then has outlined the required standards for the technical requirements for the registration of pharmaceutical products that are to include biomarkers. The ICH guidance's have evolved over the last three decades to keep current with the technologies and standards surrounding biomarkers. An example of such guidance is ICH 16 which describes the recommendations regarding context, structure, and format of regulatory submissions for qualification of genomic biomarkers (as defined in ICH E15). Briefly, the qualification of a biomarker is a conclusion that, within the stated context of use, the results of the assessment with a biomarker can be relied upon to adequately reflect a biological process, response, or event and supports the use of the biomarker during drug or biotechnology product development, ranging from discovery through post-approval. Through these various agencies in conjunction with the various guidance documents, strict rules have been defined which must be taken into consideration when incorporating a biomarker within a drug development program independent of the country in which these activities are taking place. Special consideration must be taken early within your biomarker and drug development programs to assure that there will be no delay when trying to register your drug/diagnostics within the EU and especially for registration within the United States.

5.1 Biomarkers: Laboratory Developed Test or Companion Diagnostic

It is important to note that not every biomarker that can be measured in an academic or research laboratory can be translated into a diagnostic assay and used in combination with a new therapeutic drug. This is due to the required levels of assay development, verification, and validation data that needs to be generated before the test can be used in a clinical setting to diagnose, provided prognostic value, or determine treatment strategies. Based on where the biomarker assay will be run determines the level of validation and designates the assay as either a laboratory-developed test (LDT) or a companion diagnostic (CDx). An LDT is an in vitro diagnostic test that is manufactured by and used within a single laboratory. LDTs are also sometimes called in-house developed tests or "home brew" tests. Similar to other in vitro diagnostic tests, LDTs are considered "devices," as defined by the Federal Food Drug and Cosmetic Act (FFDCA), and are therefore subject to regulatory oversight. LDTs are regulated by the Centers for Medicare & Medicaid Services (CMS) (2015) through the Clinical Laboratory Improvement Amendments (CLIA). In total, CLIA covers approximately 244,000 laboratories (Centers for Medicare & Medicaid Services, www.cms.gov). Any tests performed on humans are to be performed following CLIA guidelines to ensure accurate and reliable test results. When a laboratory develops a test system such as an LDT in house without receiving FDA clearance or approval, CLIA prohibits the release of any test results prior to the laboratory establishing certain performance characteristics relating to analytical validity for the use of that test system in the laboratory's own environment. The majority of pharmaceutical companies outside of the United States follow these rules when conducting their pivotal trials since the FDA will not allow drugs to be marketed or registered if the CLIA guidelines are not followed and data are not aligned or compliant.

A companion diagnostic is a medical device/in vitro diagnostic (IVD) that provides information that is essential for the safety and effectiveness use of a corresponding therapeutic product. CDx differs from LDTs in that these tests are run in multiple global laboratories. IVD devices range from needles to pacemakers to immunohistochemical (IHC) or polymerase chain reaction (PCR) assays. The term companion diagnostic denotes that a drug is used in conjunction with the readout of the in vitro diagnostic device. Moreover, CDx is used to identify patients who are most likely to benefit from the therapeutic product, identifies the patients likely to be at increased risk for serious adverse reactions as a result of treatment with the therapeutic product, is used to monitor responses to treatment with the therapeutic product for the purpose of adjusting treatment (e.g., schedule, dose, discontinuation) to achieve improved safety or effectiveness, and identifies patients in the population for whom the therapeutic product has been adequately studied to be found safe and effective, i.e., there is insufficient information about the safety and effectiveness of the therapeutic product in any other population. Depending on how the device data will be used dictates the level of design control (inclusion of special controls and documentation of the whole development process) and

validation the device must undergo before being approved by the FDA. As an example, if the data from an IHC assay will be used to decide what treatment a patient will receive, the device must undergo the highest level of testing, validation, and documentation. This device would be considered a class III medical device since it contains the highest risk to a patient. The FDA uses a risk-based approach on deciding the regulatory pathway for IVD companion diagnostic devices. This means that the regulatory pathway will depend on the level of risk to patients, based on the intended use of the IVD companion diagnostic device and the controls necessary to provide a reasonable assurance of safety and effectiveness. Thus, the level of risk together with available controls to mitigate risk will establish whether an IVD companion diagnostic device requires a premarket approval application (PMA, Class III) or a premarket notification submission (510(k), Class II). FDA recommends that sponsors consult early with FDA on the likely regulatory pathway for the IVD companion diagnostic device. Premarket review by FDA will determine whether the IVD companion diagnostic device has adequate performance characteristics for its intended use. As shown in Fig. 2, the three classes of medical devices are listed. The specifics regarding the other level of devices and further information surrounding design control will not be discussed in this chapter, but the relevant information can be found on the FDA website (www.fda.gov, 2015b).

5.2 Laboratory Developed Test and Companion Diagnostic Requirements

The requirements for laboratory-developed tests and companion diagnostics have been very well outlined by CLIA and the FDA, respectively. In general, because LDTs have not been evaluated by the FDA, they must undergo a more lengthy and rigorous validation process by the individual laboratory wishing to implement the new method. The minimum analytical validation standards that need to be measured for an LDT are accuracy, precision, the test sensitivity, and specificity. Additional metrics may be required if the laboratory is accredited by the College of American Pathologist or the Joint Commission. Often the standards set by these different accrediting groups exceed those set by CLIA. In brief, accuracy is the ability of the test to most closely measure the "true" value of a biomarker. The precision of the assay is the reproducibility and repeatability of the test commonly evaluated by performing replication experiments. The results should show a mean value with a statistical significance that does not exceed 15% of the coefficient of variation. The sensitivity of a biomarker assay defines the proportion of actual positive values which are correctly identified as such (e.g., the patients who are precisely identified as being affected by a specific disease). Assays with a very low incidence of false negatives are considered highly sensitive. The specificity of a biomarker assay defines the proportion of negatives which are correctly identified as such (e.g., patients or healthy volunteers who are precisely identified as not being affected by the disease, the assay has been designed for). Assays with a very low incidence of false-positive results are highly specific.

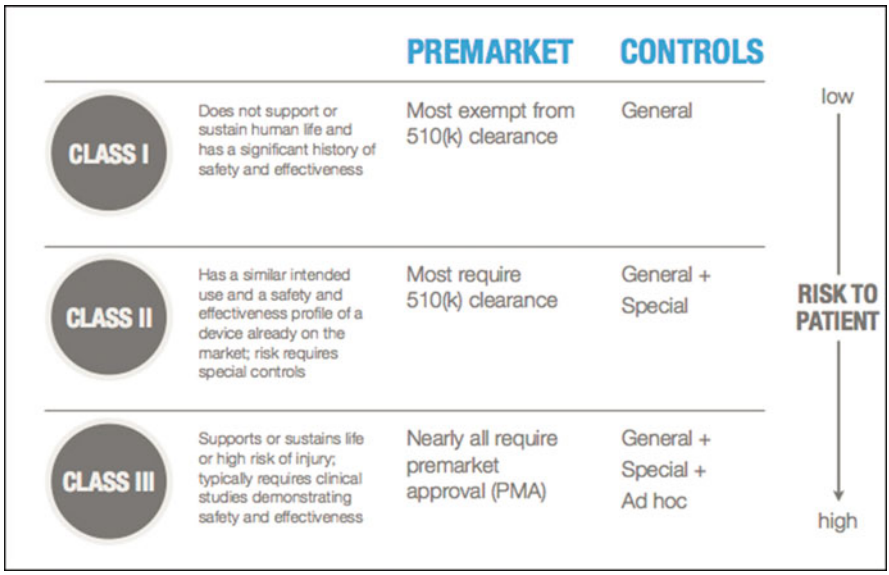

Fig. 2 The FDA has categorized the medical device classes based on risk. Properly classifying a potential medical device will determine the regulatory pathway to be followed to gain FDA approval. Devices are classified as 1, 2, or 3. The time to commercialization can range from 0 days (Class I) to a 90-day FDA review (Class II). In some cases, a multiyear FDA review (Class III) is to be expected. Step-by-step guidelines for classifying a medical device can be found on the FDA CDRH website. All Class II and III medical devices marketed in the United States must be manufactured under the quality systems described in 21 CFR (Code of Federal Regulations) 820. However, it is important to note that the designer of a medical device is considered to be a manufacturer, so the design process must fall within the quality systems. Design controls are critical, especially as the final design is being developed. This is to ensure that medical devices are designed to perform their desired function in a repeatable manner, which is consistent from unit to unit and lot to lot and that changes to the medical device during the design phase are planned, verified, and validated

The requirements for a companion diagnostic include the same analytical validation standards as mentioned above for an LDT but also include that a clinical validation of the biomarker be shown in a clinical trial setting. In some cases, the diagnostic manufacturers have in-house CLIA laboratories where LDTs are developed and use for early phase I/II clinical trial where the assay is used to support the biomarker hypothesis. If the data are compelling enough and the pharmaceutical company decides that the test must be administered globally and in conjunction with the newly developed therapeutic, the diagnostic manufacturer will then use the LDT assay as feasibility data and transfer the assay into a design control mode. Briefly design control consists of the interrelated set of practices and procedures that are incorporated into the design and development process of the assay, i.e., a system with checks and balances. This guarantees quality assurance practices are used and that they are consistent with quality system requirements worldwide. For companion diagnostics that are developed in the United States, the diagnostic

manufacturer must comply with the Code of Federal Regulations, specifically 21 CFR Part 820 which mandates design control and use of a quality management system (QMS) for tracking. Of importance, the time it takes to develop a companion diagnostic can vary depending on the biomarker assay and technology being used. Typically, development times range from 12 to 15 months so planning is quite important when incorporating a CDx into a pivotal clinical trial.

There are various stages of the development of a CDx. As mentioned previously, the LDT or prototype assay can be used early on to support drug trials. This type of assay will be labeled as a research use only (RUO) assay preventing any results to be used in treatment decisions. An investigational use only (IUO) assay is the next phase of the CDx development and builds upon the RUO assay: Using the RUO assay, this assay will be further tested, the reagents will be checked for stability issues, and the conditions to achieve the maximum sensitivity and specificity will be identified. Once all of the various components and conditions of the assay have been tested and identified, the assay is considered locked and at this point the IUO assay cannot be further changed or optimized. The additional testing of the RUO to the IUO is all documented in the QMS and a design history file is created. Over the course of time, if one reagent changes, this change will be documented in the design history file and shows why and when this change occurs. The IUO is the assay that will be used in most pivotal phase III trials.

5.3 Regulatory Authorities

In addition to the design control element of the CDx, there is also a regulatory component of the assay. In order to use the RUO/IUO assay to test human samples that are prospectively collected specifically for testing purpose, an Investigational Device Exemption application must be submitted to the Center for Device and Radiological Health (CDRH) at the FDA. This application consists of manufacturing information, the analytical study design and data (validation), and the clinical trial plan including statistical analysis and cutoff values. Once received at CDRH, there is a 30-day waiting period, and if CDRH has no comments or concerns and the 30 days has passed, the test can be used. If there are concerns surrounding the test or the clinical program, a presubmission packet can be assembled collaboratively between the diagnostic and pharmaceutical companies and sent to CDRH for comments as well requesting a meeting. This packet allows for the diagnostic and pharmaceutical companies to align on expectations and assure that the assay and clinical approach/suggestive cutoff is acceptable to the FDA. The last two additional regulatory elements that are needed are the completion of the premarket approval application which contains all of the diagnostic data collected during the pivotal trial. This data should be filed to CDRH at the same time the drug data is submitted to CDER/CBER which is a very crucial part of the process. The last remaining regulatory element is the postmarket surveillance that occurs after the CDx and drug have been granted approval. This monitoring accesses the safety, effectiveness, and performance of the diagnostic and drug.

There are currently about 20 approved and in-use companion diagnostics. However, the majority of these 20 tests are for the detection of the HER2/new oncogene for treatment with Herceptin. The approval of Herceptin in conjunction with the first approved companion diagnostic IHC test in 1998 has paved the path forward for the search for more novel biomarkers that resemble the same paradigm. A recent success is BRAFV600E companion diagnostic for melanoma. The drug vemurafenib (Zelboraf®) was co-developed with the companion diagnostic to identify the patients that had the mutations BRAFV600E(K,D). This drug received accelerated approvals since the response and survival rates were significantly better than the current standard of care first-line available therapies. The search continues for combination of biomarkers and therapies that can replace the current first-line treatments where often the percentages of patients that respond and have complete remission are relatively low. Personalized medicine is now being applied most often in oncology practices, but in time other diseases such as cardiovascular, infectious disease, rare diseases, etc., too will hopefully have additional companion diagnostic test that help speed up drug development times, identify the correct patient populations, and most importantly provide overall benefit to the patient. In summary, with the increase in emerging technologies and the better understanding of disease heterogeneity, there is a high likelihood for better treatment strategies in the near future.

6 Conclusions

6.1 What Is the Current Impact of Biomarkers on Personalized Medicine?

The expectations regarding the potential of biomarker to transform clinical development and personalized medicine are incredibly high. A lot of biomarker approaches have entered early clinical trials within the last decade. Biomarkers are increasingly being used to develop diagnostics that could help to differentiate, or stratify, the likely outcomes of therapeutic intervention. However, the pace of the uptake of personalized medicine is widely perceived to have been slower than hoped (Milne et al. 2014). Beside a few success stories on companion diagnostics, the impact on personalized treatments and personalized medicine is still limited. The field is still in its early days, but the pharmaceutical as well as the diagnostics industry expects a rapid growth based on the cornerstones that have been laid. Advances in genomics and proteomics help reveal more about the molecular basis of diseases, especially the understanding of disease heterogeneity and drug response.

Tremendous efforts have been made over the past decade to discover novel cancer biomarkers for use in clinical practice as well as developing the required technologies to compliment the clinical setting. Still, a striking discrepancy exists between the effort directed toward biomarker discovery and the number of markers with proven clinical utility. This is not because effort has not been put forth. The list

of Food and Drug Administration (FDA)-approved protein tumor biomarkers in current clinical use is comprised of only 23 proteins (Füzéry et al. 2013) with the majority being to the HER2 oncogene. Extending the approval to include DNA and RNA biomarkers, in the table of pharmacogenomic biomarkers in drug labeling available on the FDA homepage (www.fda.gov, 2015c), 160 entries are found with the vast majority of them applying to the identification of fast or poor drug metabolizers. When broken down to clinical meaningful biomarker, this list ends up with only 25 entries (Majewski and Bernards 2011). It is the case that most biomarkers which are clinically useful were identified through retrospective analysis of clinical trial data. Supporting additional biomarkers should be explored clinically in parallel with the drug development process. In theory this sounds plausible but in reality it is a challenging task. It is quite difficult to develop and validate numerous biomarker assays and technologies and implement them without delaying clinical trials or development timelines. Even with the most sophisticated molecular technologies, there still is a lag with approval of biomarker–drug combinations due to the large amount of data that is generated and the fact that there is a lack of a meaningful clear-cut conclusion. More importantly, the amount of material that is available for testing during clinical trials is often either nonexistent or not compatible with the technology that will be applied for testing. This is not to say that things will always be as they are. With each drug program failure, the community learns lessons and proactively applies new clinical trial strategies to the ongoing trials.

It is fair to say that technology drives discovery and with that leads to better personalized treatment. As mentioned previously, the cost of whole-genome sequencing is no longer cost prohibitive allowing now for patient tumors and genomes to be completely sequenced. Initiatives to sequence 100,000 genomes are being launched by companies and countries, for example, England (2015) (www.genomicsengland.co.uk). The 3-year project, launched by the prime minister of England late 2014, will transform diagnosis and treatment for patients with cancer and rare diseases. The initiative involves collecting and decoding 100,000 human genomes. Projects like these have the potential to transform the future of healthcare. They can improve the prediction and prevention of disease, enable new and more precise diagnostic tests, and allow personalization of drugs and other treatments to specific genetic variants. By participating in initiatives like these, patients will benefit because a conclusive diagnosis can be reached for rare and inherited diseases more quickly. The ultimate benefit will be in the improvement of our knowledge of the influence of genetics on disease and how it is expressed in an individual. Most importantly once this information is available, drug developers can quickly apply this information to their clinical programs and work with diagnostic manufacturers to develop the right tests to detect the biomarkers that are the most meaningful.

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