Cancer biomarker discovery and validation

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Abstract

With the emergence of genomic profiling technologies and selective molecular targeted therapies, biomarkers play an increasingly important role in the clinical management of cancer patients. Single gene/protein or multi-gene "signature"-based assays have been introduced to measure specific molecular pathway deregulations that guide therapeutic decision-making as predictive biomarkers. Genome-based prognostic biomarkers are also available for several cancer types for potential incorporation into clinical prognostic staging systems or practice guidelines. However, there is still a large gap between initial biomarker discovery studies and their clinical translation due to the challenges in the process of cancer biomarker development. In this review we summarize the steps of biomarker development, highlight key issues in successful validation and implementation, and overview representative examples in the oncology field. We also discuss regulatory issues and future perspectives in the era of big data analysis and precision medicine.

Keywords

Cancer; biomarker; drug response biomarker; prognosis; companion biomarker

Introduction

The Precision Medicine Initiative unveiled in January 2015, included an investment of $70 million to the National Cancer Institute (NCI), to "scale up efforts to identify genomic drivers in cancer and apply that knowledge in the development of more effective approaches to cancer treatment" (1). In the field of cancer research and care, the concept of precision medicine – prevention and treatment strategies that take individual variability into account – hinges on the development of valid biomarkers interrogating key aberrant pathways potentially targetable with molecular targeted or immunologic therapies (1). Although biomarkers such as prostate-specific antigen (PSA), have been known and used for decades to attempt to guide prognostic and therapeutic decisions, the recent revolution in molecular

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biology, with the rise of high-throughput sequencing and increased molecular characterization of tumor tissue has led to an exponential increase in attempts to measure and target aberrant pathways at the molecular level. Nevertheless, there has been a large gap between multiple initial reports of biomarkers, often with diagnostic performance that cannot be reproduced in later studies, and full clinical implementation and validation of the biomarkers due to issues in study design, assay platforms, and availability of specimens for biomarker development (2, 3).

Nevertheless, with the recent emergence of highly selective molecular targeted agents and high-throughput genomic characterization technologies, robust and well-validated cancer biomarkers are increasingly needed. For instance, more than 90% of oncological drugs that enter clinical development will not reach market approval due to failure of clinical trials to demonstrate therapeutic benefit, contributing to costly and slow cancer drug development (4). As acknowledged by the US Food and Drug Administrations (FDA), the judicious use of biomarkers is expected to play an important role in minimizing risk of clinical trial failure by enriching the trial populations with specific molecular subtypes responding better to tested therapies. In this review, we overview the recent trend in the cancer biomarker development and discuss the issues in clinical translation of cancer biomarkers.

Biomarkers in cancer care

A biomarker is an objectively measured characteristic that describes a normal or abnormal biological state in an organism by analyzing biomolecules such as DNA, RNA, protein, peptide, and biomolecule chemical modifications (5). However, it must be acknowledged that the definition of biomarkers has been evolving over the past decade, with one especially broad definition by the World Health Organization suggesting that "A biomarker is any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease." (6, 7) More specifically in terms of clinical utility, a cancer biomarker may measure the risk of developing cancer in a specific tissue or, alternatively, may measure risk of cancer progression or potential response to therapy. Besides providing useful information in guiding clinical decision making, cancer biomarkers are increasingly linked to specific molecular pathway deregulations and/or cancer pathogenesis to justify application of certain therapeutic/ interventional strategies. The conceptual framework of cancer biomarker development has also been evolving with the rapid expansion of our omics analysis capability of clinical biospecimens based on the traditional path of biomarker deployment (5).

Cancer biomarkers can be classified into the following categories based on their usage. *Predictive biomarkers* predict response to specific therapeutic interventions such as positivity/activation of *HER2* that predicts response to trastuzumab in breast cancer (8-10). Similarly, *KRAS*-activating mutations predict resistance to EGFR inhibitors such as cetuximab in colorectal cancer (11). *Prognostic biomarker*, on the other hand, may not be directly linked to or trigger specific therapeutic decisions, but aim to inform physicians regarding the risk of clinical outcomes such as cancer recurrence or disease progression in the future. An example of a prognostic cancer biomarker is the 21-gene recurrence score which was predictive of breast cancer recurrence and overall survival in node-negative,

tamoxifen-treated breast cancer (12). Another class of biomarker, the *diagnostic biomarker*, is used to identify whether a patient has a specific disease condition. Diagnostic biomarkers have recently been implemented for colorectal cancer surveillance by testing for stool cancer DNA (13).

Processes of biomarker development

Biomarker development involves multiple processes, linking initial discovery in basic studies, validation, and clinical implementation (Figure 1) (5, 14-21). The ultimate goal of the processes is to establish clinically accessible biomarker tests with clinical utility, informing clinical decision-making to improve patient outcomes (21, 22). However, there are many hurdles as evidenced by the low estimated rate (0.1%) of successful clinical translation of biomarkers (23). Here we elaborate each of the processes, which should be designed/planned prior to the conduct of the study to ascertain validity of cancer biomarkers.

Biomarker discovery

At the start of any biomarker development, biomarkers should be "discovered" and are typically validated within the same initial report. Validation based on predefined prediction rule in an independent patient series is ideal, but it is often substituted by cross-validation-based methods when independent patient sets are not available. The research question and plan , including the fundamental use of the biomarker, should traditionally be clearly defined prior to the analysis, although this can be challenging at the very early stages of biomarker development. In this era of ever-evolving high-throughput omics technologies where thousands of individual molecules can be easily interrogated without *a priori* assumptions, research hypotheses are often generated in a *post hoc* manner, following often serendipitous discovery from unbiased mining of the genome-wide measurements (data-driven hypothesis generation) (20). Another relevant issue to be addressed early in biomarker development is the target population to be tested in specific clinical contexts, which will guide subsequent clinical evaluation and implementation. In general, broader target populations could lead to increased costs and risks of failure during the development stage.

Study design/setting, from which analyzed biospecimens are derived, is the major source of bias that hampers subsequent biomarker development. Ideally, the specimens should be prospectively collected based on well-defined inclusion and exclusion criteria together with accompanying clinical annotations pre-specified in the study protocol. A cohort or case-control study design is typically employed. In a cohort study, clinical characteristics of enrolled individuals as well as information of intervention and follow-up are critical in identifying molecular correlates associated with clinical outcomes of interest. In a case-control study, potential confounding factors should be properly matched between cases and controls to minimize false discovery. In practice, biomarker discovery is often based on "samples of convenience", which were incidentally available to the investigator at the time of research and collected without prior intention of specific biomarker discovery (24). This could introduce unrecognized confounding factors, which may contribute to the false positive associations of the biomarkers. The study design quality may be semi-quantitatively evaluated by using scores such as level of evidence scale proposed by Simon *et al*. (16). In general, evidence derived from large-scale well-predefined prospective trials is regarded as

most reliable. Retrospective, observational studies may be affected by multiple sources of bias, which can be better identified if reporting guidelines such as REMARK for prognostic studies (25), STROBE for observational studies (26) and STARD for diagnostic studies (27) are used to determine reliability and quality of biomarkers in the initial reports.

A common cause of failure in developing robust predictive and especially prognostic biomarkers is to define them based on clinically invalid surrogate endpoints such as objective response in oncology trials as well as short-term outcomes from retrospective studies. Biomarkers trained for poorly-defined endpoints are more likely to fail in subsequent prospective evaluation. A prognostic gene-expression signature trained on long-term outcome using archived specimens has been successfully validated in a series of independent clinical and experimental studies (28-31). While the most optimal setting is prospective sample collection and follow-up based on a fully predefined protocol, this requires costly and lengthy biomarker assessment, which hampers timely deployment of cancer biomarkers. As an alternative, retrospective analysis of samples archived as part of previously completed prospective trials (prospective-retrospective design) is proposed to shorten the time frame while ascertaining quality of study design (16). Another solution is to develop a biobank in which biospecimens and complete clinical annotations are prospectively accumulated based on well-defined protocols. However, in part due to the complex and heterogeneous nature of cancer, it has become increasingly recognized, that there is a need for larger integrated biobanks (32, 33) which require careful development and adherence to published biobanking guidelines (34). The practical challenges of biobanking in cancer patients has been underlined by a recent US survey of NCI-funded cancer researchers who conduct tissue-based research showing that 39-47% reported difficulty obtaining biospecimens of adequate numbers and quality and low-quality biospecimens resulted in 60% questioning their findings and 81% limiting the scope of their work (35). Quality of clinical annotations is another key factor in utilizing the resources to identify reliable biomarkers and validate their clinical utility. A recent NCI joint workshop recommended improved sharing of existing specimens and data and creation of NCI-wide inventory of prediagnostic specimens and cancer diagnosis data, ongoing engagement of the clinical, translational and basic research communities, and encouraging the development of pilot projects (18).

Robustness of sample processing and data analysis procedures is another factor that influences reproducibility of biomarker studies. For example, a high diagnostic accuracy of a peptide signature for ovarian cancer was not confirmed in subsequent independent reanalysis of the original dataset possibly due to variation in sample processing (36, 37). One report of proteomic biomarker discovery noted that common statistical algorithms run on data with low sample sizes can overfit and yield misleading misclassification rates and that prefiltering variables exacerbated this problem (38). Similarly, a critical review of prognostic microarray studies in cancer revealed that half of the reported prognostic gene signatures were not reproducible due to critical flaws in the data analysis methods (39). These reports highlight the importance of careful assessment of technical soundness and methodological validity and disclosure of information to the research community to enable fair evaluation of reported biomarkers and identification of candidates for further development. In addition, ensuring reproducibility of bioinformatics analysis is a critical

determinant of successful clinical translation of genome-based biomarkers. There have been several efforts to develop informatics infrastructure to address this issue, including public repository of datasets with relevant annotations on biological, clinical, and experimental parameters, analysis software repository, and systems to record whole process of data analysis itself to allow anyone to rerun or modify the analysis to verify robustness of reported findings (40, 41).

Biomarker assay development and analytical validation

Following the discovery phase that typically includes internal validation, candidate biomarkers are adapted to clinically applicable assay platforms, and subjected to two types of validation, namely *analytical validation*, i.e., how accurately and reliably does the test measure the analyte(s) of interest in the patient specimen and *clinical validation*, i.e., how robustly and reliably is the test result correlated with the clinical phenotype or the outcome of interest. Analytical validation is typically performed by assaying the same set of samples by both the assay used in the initial discovery and the clinical deployment platform to determine robustness and reproducibility of the measurements. Frequently used assay technologies generally used for analysis of single gene/protein anomalies include real-time polymerase chain reaction (RT-PCR) to assess gene expression or DNA mutations (e.g., *BRAF* V600E mutation in melanoma), fluorescent in situ hybridization (FISH) to assess DNA copy number or genetic translocation (e.g., *HER2* amplification, *BCR–ABL* translocation), and immunohistochemistry (IHC) to assess protein expression and subcellular localization (e.g., estrogen receptor expression in breast cancer).

More recently, several multi-gene assays classified as *in vitro* diagnostic multivariate index assays (IVDMIA) have been introduced into clinic (13, 42, 43). The implementation of gene expression-based multi-gene assays has been a challenging task due to poorer reproducibility of the measurements (44). Currently available tests, such as MammaPrint (45) and Oncotype Dx (12), are performed in centralized laboratories to minimize technical variability. Emerging technology such as direct digital counting of transcripts without target amplification could enable more robust gene expression measurements reproducible across individual laboratories (46, 47). Resequencing of a targeted panel of genes (disease-specific, exome, etc.) has been tested as another option (48), identifying somatic DNA mutations potentially driving cancer in nearly two-thirds of patients with lung adenocarcinomas and linking to molecular targeted therapy in 28% of patients (49). Clinical sequencing is a promising approach, but the interpretation and reporting of incidental findings from non-targeted sequencing is still being debated (50). In addition, high demand on data analysis, referred as the "$1000 genomic test [but] $100,000 genomic analysis", is another layer of challenge in sequencing-based approaches (51). Capability to analyze formalin-fixed, paraffin-embedded (FFPE) tissue samples greatly enhances general applicability of biomarker assays (52-54). Emergence of highly sensitive assays, e.g., single cell profiling, are expected to enable analysis of body fluid-derived specimens such as whole blood, plasma, serum, ascites, and urine to assess circulating microRNA, circulating DNA, and circulating tumor cells (CTCs)-derived biomolecules (55, 56). These technologies are expected to achieve less-invasive assessment of molecular biomarkers (liquid biopsy) (55). Circulating tumor DNA was highly accurate in assessing mutation status of *BRAF* V600E

mutation (100% specificity and sensitivity reported) and *KRAS* point mutations (>90% sensitivity and specificity) in subjects with metastatic colorectal cancer in one blinded prospective trial (57). Another report, assessing the role of CTCs, defined as 5 or more per 7.5mL of whole blood in this study, in metastatic breast cancer, did not find an improvement in outcomes after changing therapy in case of persistently elevated CTCs but confirmed that CTCs were strongly prognostic for overall outcome (58). In addition to their role in diagnosis, circulating cell-free microRNAs are also being currently assessed as a predictive cancer biomarker with some encouraging preliminary reports (59, 60).

Validation of clinical utility

After analytical validity is confirmed, the biomarker assay in the clinical deployment platform must be evaluated to confirm its performance in predicting or diagnosing the clinical phenotype or outcome of interest as demonstrated in the discovery and initial validation phase (5, 21, 61). Ideally, the biomarker should be evaluated in statistically well-powered prospective trials as performed in the TransATAC study for breast cancer recurrence prediction (62). However, it is realistically infeasible to test all candidate biomarkers in this manner due to financial constraints and/or limited availability of patient cohorts. Therefore, similar to the setting of biomarker discovery, the use of prospective-retrospective design and/or biobank/biorepository samples could be a potential alternative to overcome these obstacles. Clinical utility assessment could also include analysis of clinically meaningful outcome benefit, comparative effectiveness, cost-effectiveness of biomarker-guided clinical care, and assessment of alternatives and availability of the biomarker based on real-world clinical data or mathematical modeling (21, 63).

Clinical implementation

An analytically and clinically validated biomarker assay is now ready for implementation in clinical care. This phase includes the following four key elements, which vary widely across regions: regulatory approval, commercialization, coverage by health insurance companies, and incorporation in clinical practice guidelines. In the US, there are two paths for regulatory approval: *in vitro* diagnostic device (IVD) as commercial medical device with 510(k) clearance overseen by the FDA, and laboratory developed tests (LDT), home-grown assay developed and optimized at a diagnostic lab performing the test, which will likely be regulated by the FDA although current oversight is more limited (64). Clinical biomarker tests must be conducted in diagnostic laboratories certified for Clinical Laboratory Improvement Amendments (CLIA) and in accordance with state-specific regulations. Coverage by health insurance is critical for physicians to order the tests. Assignment of current procedural terminology (CPT) codes as well as incorporation into clinical practice guideline/recommendation supports payer's decision. Centers for Medicare & Medicaid Services (CMS) classifies the tests into tier 1 (CPT code-assigned, commonly performed tests) and tier 2 (less commonly performed tests grouped by complexity). CMS defers pricing for new CPT codes to the local Medicare administrative contractors in a procedure known as "gapfill", which causes delayed reimbursement for many biomarker tests (65). Post-marketing clinical utility validation will further support the use of biomarker tests, and may result in indication for additional diseases and/or clinical scenarios. Resources such as

the National Comprehensive Cancer Network Biomarkers Compendium (66) are available to access the current recommendation for biomarkers in clinical guidelines, (67).

Cancer biomarkers currently available in clinic

An example of a molecular biomarker in clinic is overexpression/amplification of HER2 (*ERBB2*), a member of the epidermal growth factor receptor (EGFR) family, predictive of response to monoclonal antibodies such as trastuzumab and pertuzumab in breast cancer (8-10). It has been shown in pivotal phase III trials in breast cancer that subjects with HER2 overexpression (approximately 20% of patients) treated with anti-HER2 therapy have improved disease-free and overall survival (8-10). American Society of Clinical Oncology and College of American Pathologists recommend primarily IHC and *in situ* hybridization for assessment of HER2 status (68). Currently, the FDA has approved 10 HER2 assays as companion diagnostic devices (50% of all approved companion diagnostic devices) and 3 other HER2 assays as nucleic acid based tests cleared by the Center for Devices and Radiological Health (FDA website accessed on March 20th 2015 (69)). HER2 overexpression is similarly predictive of response to trastuzumab in esophago-gastric adenocarcinoma (70). OmniSeq Target assay analyzes clinically actionable somatic DNA alterations in 23 known cancer-related genes, which acquired the New York state approval as LDT. Other major predictive biomarkers, including *BCR-ABL* in chronic myeloid leukemia and *KRAS* mutations in colorectal cancer and multiple mutations in non-small cell lung cancer (NSCLC), are listed in Table 1.

Despite the numerous prognostic biomarkers reported in the literature, only seven biomarkers have been approved by the FDA Center for Devices and Radiological Health (Table 2) (48). One of the major reasons is that prognostic prediction itself often does not directly change clinical decision making unless coupled to specific therapeutic options. Despite this, many other prognostic biomarkers are available through the LDT pathway. Mammaprint is one of the first gene expression signature-based assays based on the measurement of 70 genes to predict breast cancer recurrence after chemotherapy, which was recently adapted for use in FFPE tissue (45). Another gene expression-based assay, Oncotype Dx Breast Cancer Assay measures 21 genes predicting breast cancer recurrence in women with node negative or node positive, ER-positive, HER2-negative invasive breast cancer (12, 71). Similar tests are also available for colon and prostate cancer, all of which analyze gene expression in tumor tissue (72, 73). A 186-gene expression signature in non-tumor stromal liver tissue has been validated to predict hepatocellular carcinoma development and recurrence as well as liver cirrhosis progression, and was recently implemented in an FDA-approved diagnostic device (28-30). .

Diagnostic biomarkers are one of the most diverse classes of biomarkers ranging from assays developed for cancer screening to diagnostic tests assessing progression of a known cancer (see Table 2 for a list of FDA-approved diagnostic genetic tests). One recent example of a diagnostic biomarker is Cologuard, a multigene DNA (*KRAS* mutations, aberrant *NDRG4* and *BMP3* methylation) stool test combined with fecal immunochemistry designed to screen for colorectal cancer in individuals at average risk of colorectal cancer. In a recent clinical trial of nearly 10,000 participants, sensitivity of the test for detecting colorectal

cancer was higher than fecal immunochemical test alone (92.3% and 73.8% respectively) although the test also had a higher rate of false positives (specificity 86.6% and 96.4% for Cologuard and fecal immunochemical test respectively) (13). These encouraging results led to the approval of this test by the FDA in August 2014. Recently, there has also been increased interest in developing minimally invasive diagnostic tumor biomarkers, using the measurement of circulating DNA or microRNA. For instance, a new technology termed cancer personalized profiling by deep sequencing (CAPP-Seq) has been tested on circulating tumor DNA in patients with non-small-cell lung cancer. Levels of circulating DNA correlated with tumor volume and provided earlier response assessment than radiography in this preliminary trial while potentially allowing the non-invasive detection of actionable mutations (74). Another report, focusing on circulating microRNA serum profiles identified a microRNA profile thought to distinguish subjects with pancreatic cancer from healthy controls, even at early stages of the disease (75). This result requires further validation but may suggest a direction towards which the field of diagnostic biomarkers is moving. However, even when FDA-approved, commercialization may still be a challenge due to the high cost required for assay development.

Cancer biomarkers under evaluation in clinical trials

Multiple predictive biomarkers, mostly based on single gene/protein, are currently in phase II or III evaluation along with their companion therapeutic agents (Table 3). From this snapshot, the increasing importance of predictive biomarkers is apparent as is a trend to develop minimally invasive cancer biomarkers. Biomarkers validated in a certain type of cancer are undergoing discovery and validation in other cancers (for instance BRAF mutations or HER2 overexpression) underlying certain shared oncogenic drivers and less prevalent cancers are also benefitting from the rapid developments in the field. The 70-gene breast cancer signature is currently being evaluated for its recurrence-predictive capability in comparison to clinico-pathological assessment in a large prospective trial enrolling more than 6,600 subjects in 9 countries (MINDACT study) with early results suggesting that the 70-gene signature added information to usual assessment (76).

Future perspectives and conclusions

In this review, we aimed to overview the current landscape of cancer biomarker development. The speed of technological development has highlighted the challenges facing regulatory oversight and legislation in their attempts to keep up with the rapid pace of scientific changes while allowing proper consideration to how the new biomarkers could shape the future of medicine (77, 78). One of the major challenges is to manage the tradeoff between safety and speed of clinical translation. For example, regulation of LDT by the FDA will improve assay quality and safety and increase overall medical utility of the tests, while it could hamper timely deployment of the tests and benefit only large commercial laboratories with capabilities to accommodate the high requirements. The large amount of data generated by the assays have posed supplementary challenges in the analysis of "big data", which requires massive computational resources for data storage, processing, and interpretation (79). Informatics resources such as ClinGen (80) are being developed to support the process. Also, systems to integrate genomic information with electronic medical

records (EMRs) are actively developed, where protection of patient privacy is a central issue such as the Electronic Medical Records and Genomics Network (eMERGE), a NIH-funded consortium aiming to develop and disseminate approaches combining DNA biorepositories with EMRs (81). However, the integration of EMRs with genomic datasets remains in its infancy, due to a number of challenges including defining optimal storage standards of genomic data, integration of rich phenotype information, interpretation of complex data in a format easily accessible to clinicians and of course ethical, legal and social issues (82). Defining unified standard for the systems and data formats is particularly challenging due to the big financial/commercial interests.

Another crucial aspect of biomarker development, especially genomic biomarkers, is the issue of intellectual property. In the US, a recent high profile Supreme Court decision, The Association for Molecular Pathology versus Myriad, determined that isolated but otherwise unmodified genes were products of nature and therefore not patent eligible subject matter (83). This decision was a response to an ongoing lawsuit between Myriad Genetics, who owned the exclusive rights to analyze the BRCA1 and BRCA2 gene mutations, and a coalition of groups who challenged the constitutionality and validity of the BRCA1 and BRCA2 gene patents. In this context, the US Patent and Trademark Office has recently issued new guidelines which enforce more stringent criteria to patent natural products such as antibiotics, or even nucleic acids, peptides and proteins. These new guidelines have generated considerable concern in the biotechnology world due to their far-reaching consequences that are still being considered (84). Of note, genetic sequences are currently still patent-eligible in the European Union and in Australia if certain conditions are fulfilled (85, 86). It is expected to take more time to reach a solution acceptable to all relevant parties.

Despite unclear future prospects and regulatory and legislative minefields, several examples of successful clinical translation summarized above have emphasized the challenges but also the opportunities at each step of cancer biomarker development. Acknowledging these challenges and implementing them in the design of biomarker development will help streamline the whole process, and eventually transform cancer patient care by fulfilling the vision of Precision Medicine.

Acknowledgments

Abbreviations

CAPP-Seq	cancer personalized profiling by deep sequencing
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare & Medicaid Services
CPT	current procedural terminology

CTC	circulating tumor cells
EGFR	epidermal growth factor receptor
EMR	electronic medical records
FDA	US Food and Drug Administrations
FFPE	formalin-fixed, paraffin-embedded
FISH	fluorescent in situ hybridization
IHC	immunohistochemistry
IVD	*in vitro* diagnostic devices
IVDMIA	*in vitro* diagnostic multivariate index assays
LDT	laboratory-developed tests
NCI	National Cancer Institute
PSA	prostate-specific antigen, REMARK, Reporting Recommendations for Tumor Marker Prognostic Studies
RT-PCR	real-time polymerase chain reaction
STARD	Standards for Reporting of Diagnostic Accuracy
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology

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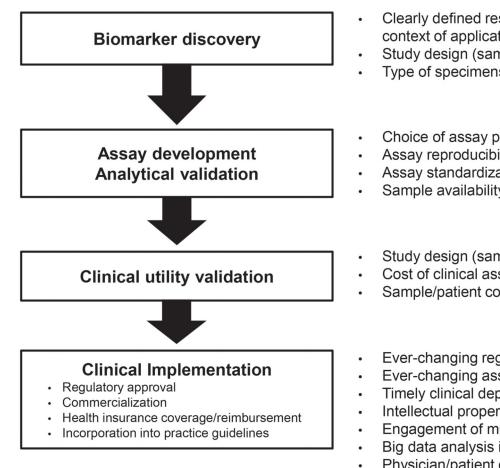
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Key issues/challenges

Biomarker discovery

- Clearly defined research question, clinical context of application
- Study design (sample size, clinical follow-up)
- Type of specimens (source, storage,...)

**Assay development
Analytical validation**

- Choice of assay platform
- Assay reproducibility
- Assay standardization
- Sample availability

Clinical utility validation

- Study design (sample size, clinical follow-up)
- Cost of clinical assessment/trial
- Sample/patient cohort availability

Clinical Implementation
- Regulatory approval
- Commercialization
- Health insurance coverage/reimbursement
- Incorporation into practice guidelines

- Ever-changing regulation/legislation
- Ever-changing assay technology
- Timely clinical deployment
- Intellectual property
- Engagement of multidisciplinary groups
- Big data analysis infrastructure
- Physician/patient education

Figure 1.
Schematic overview of the processes of cancer biomarker development.

Table 1

Predictive biomarkers in clinical use

Organ	Cancer	Biomarker and mechanism	Assay for measurement	Associated Target and Drug	Approximate proportion of positive tests	Stage of clinical validation	References
Breast	Breast Cancer	HER2: oncogene overexpression	ISH, IHC	HER2: Trastuzumab, Pertuzumab, Ado-trastuzumab emtansine	18-20%	In clinical use	(8-10)
		ER/PR: suggests sensitivity to endocrine therapy	IHC, (LBA)	ER: Endocrine therapy (tamoxifen, aromatase inhibitors)	75%	In clinical use	(87)
Gastro-intestinal	Colorectal Cancer	KRAS: mutations activate RAS-RAF-MEK pathway and resistance to EGFR therapy	PCR	EGFR: Cetuximab, panitumumab	40% mutated	In clinical use	(11, 88)
	GIST	KIT: mutation leads to consitutional activation	IHC	BCR-ABL: Imatinib	95%	In clinical use	(89)
	Esophago-gastric adenocarcinoma	HER2: oncogene overexpression	ISH, IHC	HER2: Trastuzumab	7-22%	In clinical use	(70)
Hematological	Chronic myeloid leukemia	BCR-ABL: Balanced t(9;22) leading to the formation of a constitutively active tyrosine kinase	Cytogenetics, FISH, RT-PCR	BCR-ABL: Imatinib, dasatinib, nilotinib	>90%	In clinical use	(90)
	Acute promyelocytic leukemia	PML–RARa: balanced t(15;17) leading to aberrant retinoid receptor.	Cytogenetics, FISH, RT-PCR	PML–RARa: All-trans retinoic acid	>90%	In clinical use	(91)
Lung	NSCLC	EGFR (HER1): mutations in tyrosine kinase domain	Sequencing, ISH	EGFR: Erlotinib, gefitinib, afatinib	15% adenocarcinomas in US (higher in Asians, women and nonsmokers)	In clinical use	(92)
		ALK: Inversion in chromosome 2 leads to EML4-ALK fusion oncogene	FISH (IHC)	ALK: Crizotinib, ceritinib (alectinib under development)	4% (mostly adenocarcinoma)	In clinical use	(93)
	Lung adenocarcinoma	Multiple genes:					
		BRAF (V600E and non-V600E)	Multiplex sequencing	BRAF: AZD6244	2%	Continued validation	(49)

Organ	Cancer	Biomarker and mechanism	Assay for measurement	Associated Target and Drug	Approximate proportion of positive tests	Stage of clinical validation	References
		EGFR (HER1): mutations in tyrosine kinase domain		EGFR: Erlotinib, gefitinib, afatinib, Cetuximab	17%		
		HER2: oncogene overexpression		HER2: Decinutubub, Neratinib, Lapatinib, Trastuzumab	3%		
		KRAS: mutations activate RAS-RAF-MEK pathway and resistance to EGFR therapy		KRAS: Erlotinib, Tivantinib, Everolimus, Ridaforalimus, AZD6244	25%		
		ALK: Inversion in chromosome 2 leads to EML4-ALK fusion oncogene		ALK: Crizotinib, ceritinib	8%		
		MET		MET: Cizotinib	<1%		
Skin	Melanoma	BRAF V600: 80-90% V600E mutation, a downstream mediator of RAS, leads to downstream activation of MEK and ERK	Sequencing	BRAF: Venurafenib, dabrafenib	40-60%	In clinical use	(94)

ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; EML4, echinoderm microtubule-associated protein-like 4; ER, Estrogen receptor; ERK, extracellular-signal-regulated kinases; GIST, Gastrointestinal stromal tumor; HER2, Epidermal growth factor receptor 2; (F)ISH, (Fluorescence) in situ hybridization; IHC, immunohistochemical staining; LBA, ligand binding assay; MEK, Mitogen-activated protein kinase; MLH, mutL homolog; MSH, mutS homolog; MSI, microsatellite instability; NSCLC, non-small cell lung cancer; PML, Promyelocytic leukemia gene; PR, progesterone receptor; RARa, retinoic acid receptor-alpha; (RT-)PCR, (reverse transcription) polymerase chain reaction

Table 2

Prognostic and diagnostic nucleic-acid based tumor biomarkers approved by the Center for Devices and Radiological Health (FDA).

Organ	Cancer	Biomarker target	Biomarker name	Tissue sampled	Method
DIAGNOSIS					
Bladder	Bladder Cancer	Aneuploidy for chromosomes 3, 7, 17 loss of 9p21	Vysis UroVysion Bladder Cancer Recurrence Kit	Urine	FISH
Breast	Breast cancer	MG, CK19	BLN assay	Sentinel lymph node	RT-PCR
Gastro-intestinal	Colorectal cancer	Multi-target DNA (aberrantly methylated BMP3 and NDRG4 promoter, mutant KRAS, and β-actin). Hemoglobin assay.	Cologuard	Stool	PCR Immunochemical assay for hemoglobin.
Hemato-logical	B-cell chronic lymphocytic leukemia	alpha satellite (centromeric) region, 12p11.1-q11	CEP 12 SpectrumOrange Direct Labeled Fluorescent DNA Probe Kit	Peripheral blood	FISH
Ovary	Ovarian Cancer	BRCA1 and 2 gene mutation	BRACAnalysis CDx	Blood	PCR
Prostate	Prostate Cancer	PCA3	PROGENSA PCA3 Assay	Prostate biopsy, urine	PCR
PROGNOSIS					
Breast	Breast cancer	58 gene RNA expression profile	Prosigna Breast Cancer Prognostic Gene Signature Assay	Tumor	nCounter System
		70-gene expression profile	Amsterdam 70-gene profile (MammaPrint)	Tumor	Agendia BV
		HER2	INFORM HER2 Dual ISH DNA Probe Cocktail HER2 CISH pharmDx™ Kit DakoCytomation Her2 FISH pharmDx™ Kit	Tumor	ISH
		TOP2A	Dako TOP2A FISH PharmDx Kit	Tumor	FISH
Prostate	Prostate Cancer	tPSA	NADiA ProsVue	Blood	PCR
Hemato-logical	Acute Myeloid Leukemia	EGR1	Vysis D7S486/CEP 7 FISH Probe Kit	Bone marrow peripheral blood	FISH
	B-cell chronic lymphocytic leukemia	TP53, ATM, D13S319 deletion D12Z3 gain	Vysis CLL FISH Probe Kit	Peripheral blood	FISH

The list was obtained from http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm (accessed March 20th 2015).

ATM, serine/threonine kinase; BLN, breast lymph node; ER, Estrogen receptor; HER2, Epidermal growth factor receptor 2; (F)ISH, (Fluorescence) in situ hybridization; MG, mammaglobin; PSA, prostate specific antigen; (RT-) PCR, (reverse transcription) polymerase chain reaction; TOP2A, topoisomerase II alpha

Table 3

Predictive biomarkers currently under clinical evaluation and registered in clinicaltrials.gov.

Organ	Cancer	Biomarker	Associated Drug	Phase	Clinicaltrials.gov identifier
Breast	Breast Cancer	BRCA1/2	Olaparib	III	NCT02000622
		Circulating tumor cells positive for HER2	Trastuzumab - Emtansine	II	NCT01975142
		TOP2A (in subjects with HER2 overexpression)	Anthracycline-based neoadjuvant chemotherapy	II	NCT02339532
		HER2 (negative in tumor but positive in circulating tumor cells)	Lapatinib	III	NCT01619111
Gastrointestinal	Colorectal	New biomarkers (unspecified)	Cetuximab	II	NCT01276379
		RAS (mutation- type)	FOLFOXIRI and bevacizumab	II	NCT02350530
		BRAF	LGX818, BYL719	II	NCT01719380
	Esophago-gastric	HER2	Afatinib and trastuzumab	II	NCT01522768
Head and Neck	Squamous cell carcinoma	HER and KRAS	HM781-36B	II	NCT02216916
Hematological	Cutaneous and Peripheral T-cell Lymphomas	GATA-3	MLN9708	II	NCT02158975
Lung	NSCLC	ROS1	Crizotinib	II	NCT02183870
		BRAF V600E	Dabrafenib, trametinib	II	NCT01336634
Skin	Melanoma	BRAF V600E/K	Trametinib, binimetinib	II	NCT02196181

The list was obtained from https://clinicaltrials.gov/ (accessed March 20th 2015).