Published in final edited form as:

Urol Oncol. 2015 June ; 33(6): 295–301. doi:10.1016/j.urolonc.2015.01.007.

Biomarker Development in the Context of Urologic Cancers

Gary J. Kelloff, MD,

National Institutes of Health, National Cancer Institute, Division of Cancer Treatment and Diagnosis, Rockville, MD 20850

Caroline C. Sigman, PhD, and

CCS Associates, Mountain View, CA 94043

Howard I. Scher, MD

Genitourinary Oncology Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, Department of Medicine, Weill Cornell Medical College, New York, NY 10065

For Urologic Oncology: Seminars and Original Investigations

Abstract

The FDA has called for the use of analytically validated biomarkers that have strong evidence of being fit for purpose to identify patients likely to respond and to evaluate the patient response to a therapy, potential toxicity, and drug resistance. This article discusses development and application of these biomarkers in the context of urologic cancers—specifically in cancers of the prostate and urinary bladder. The FDA has defined four specific categories for contexts of biomarker use: prognostic, predictive, response-indicator, and efficacy-response (surrogate endpoints). Prognostic and predictive biomarkers include pretreatment characteristics of the patient and the tumor. Response-indicator and efficacy response biomarkers occur after treatment and show the effects of treatment on biomarkers. Efficacy response biomarkers show changes associated with clinical benefit and can be surrogates for clinical endpoints leading to drug approvals. Well-structured development plans are required to satisfy rigorous criteria that must be met to qualify biomarkers for specific contexts of use in drug development and patient management. A description of the extensive effort applied to the validation and qualification of circulating tumor cells in castration resistant prostate cancer is described as an example of the potential utility of biomarkers in urological cancers.

Keywords

biomarker; analytical validation; biomarker qualification; prostate cancer biomarkers; bladder cancer biomarkers; clinical utility; circulating tumor cells (CTCs)

Corresponding Author: Caroline C. Sigman, PhD, President, CCS Associates, 1923 Landings Drive, Mountain View, CA 94043, Phone: +16506914400, FAX: +16502404013, csigman@ccsainc.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1.0 Biomarkers for Faster and Better Cures: Drug Development and Patient Management

Since 2000, remarkable headway has been made in our understanding of the biology that underlies development and progression of cancer (1–4). Particularly important advances have been made in the application of genomics and proteomics to identify and characterize cancer associated molecular and genetic alterations [*e.g.*, microarray analysis, next-generation sequencing (NGS)] and in technologies that may be used to measure these characteristics as well as to measure cellular and tissue changes (*e.g.*, quantitative and high definition imaging, single-cell analysis, microfluidics). Also important is progress in the design of instruments to measure patient symptoms [*e.g.*, Brief Bone Pain Inventory (5)], other quality of life factors (*e.g.*, patient reported outcome questionnaires), and to provide systematic assessment of clinical observations and measurements (*e.g.*, bone scan assay, quantitative imaging parameters). Despite these advances, many promising new drugs are failing late in development because they are tested in ill-defined patient cohorts and/or the gold standard endpoint of longer overall survival (OS) or other efficacy endpoints are uninterpretable because of confounding factors such as additional therapies during prolonged follow-up. Late failures can also arise because of unexpected safety issues from long term exposure. Drug resistance from preexisting and evolving clones, recognized clonal heterogeneity and influence of disparate factors outside of the tumor per se are universal challenges (6, 7). All this suggests a high likelihood that the development and clinical application of effective cancer treatments need to address patient-specific, continuously changing molecular defects in the tumor itself and the tumor microenvironment. To overcome these challenges, the FDA has called for the use of analytically and clinically validated biomarkers that have strong evidence of being fit for the purpose (contexts of use) of identifying patients likely to respond to therapy (prediction) and to evaluate patient response to therapy (response or sensitivity to the treatment), potential toxicity (safety), and understanding mechanisms associated with drug resistance either prior to or while on treatment (8–11). In the following sections of this article, we discuss these specific applications of biomarkers for urologic cancers—specifically in cancers of the prostate and urinary bladder.

2.0 Types of biomarkers/uses in urologic disease

Biomarkers are characteristics that can be objectively measured and evaluated as indicators of normal processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers can be clinical parameters (such as age, performance status), laboratory measures (such as PSA), imaging-based measures, or genetic and molecular determinants (12). The FDA has defined four specific categories for contexts of biomarker use: prognostic, predictive, response-indicator, and efficacy-response (surrogate endpoints) (9–11). Prognostic and predictive biomarkers include pretreatment characteristics of the patient and the tumor (9, 13). **Prognostic** biomarkers are highly correlated with clinical outcomes (*e.g.* survival time) but may not be associated with specific mechanisms of cancer development and progression, the latter representing the drivers of tumor growth. Some of these biomarkers indicate prognosis in general *e.g.*, measurements of prostate specific

antigen (PSA) (10, 14); enumeration of circulating tumor cells (CTCs) in patients with metastatic prostate cancer (10, 15); and gene expression patterns such as the OncotypeDx Genomic Prostate Score (GPS) that is used as an aid to distinguish between indolent and potentially aggressive prostate cancers in men with very low risk to low intermediate risk tumors based on standard clinical and pathological measures (14, 16, 17). Urinary levels of the protein product of the fusion of transmembrane protease serine 2 and the v-ets erythroblastosis virus E26 homolog (avian) (ERG) genes (TMPRSS2-ERG) has been studied extensively and is used to aid in the diagnosis of prostate cancer, but has not been established as a prognostic biomarker (14, 18). Other biomarkers in bladder cancer are not as well developed as those in prostate cancer but several indicate the likelihood of response to therapy in general, *e.g.*, in patients with muscle invasive bladder cancer (MIBC), high meiotic recombination (MRE11) expression may be indicative of potential response to radical radiotherapy (19, 20), low excision repair cross-complementation group 1 (ERCC1) expression suggests potential benefit from chemotherapy and chemoradiation (19, 21–23), and low multidrug resistance gene 1 (MDR1) expression is associated with benefit from chemotherapy (19, 22, 24).

Predictive biomarkers (which are often early genetic events in cancer) used to determine sensitivity to a specific form of therapy, often reflect specific mechanisms of cancer progression, correlate with clinical outcomes (there are some exceptions to this, *e.g.*, a cell surface protein used as the target to enhance delivery of a therapeutic is not necessarily prognostic or associated with cancer progression), and predict tumor response to specific drug interventions (9, 10). Many current drug development strategies use predictive biomarkers. For example, studies have been designed that evaluate new drugs targeting specific genes or mutations by identifying patient populations carrying these mutations (and/or genes) or gene expression patterns. Although not yet validated, potential predictive biomarkers in castration resistant prostate cancer (CRPC) include changes in the androgen receptor (AR) and AR signaling axis (*e.g.*, AR overexpression, increased androgen biosynthesis, splice variants and mutations, altered PTEN signaling, and translocations that allow the ETS transcription factor to be under the control of androgen) (10, 14, 18). Similarly, unvalidated examples of potential predictive biomarkers in advanced bladder cancers are those measuring epidermal growth factor receptor (EGFR), HER2, and vascular endothelial growth factor receptor (VEGFR) pathways, which identify patients who are likely to respond to drugs interfering with these specific targets (19). The prognostic biomarkers listed above, which are useful for managing patients with bladder cancer, are also being increasingly used in drug development trials to define patient populations, both for selecting patients who are likely to respond and for excluding patients who are not (9, 25). Such biomarkers may be identified retrospectively after a trial has been completed and used as part of the eligibility criteria for future trials (9, 10).

As is evident, biomarkers that predict the response of the tumor to specific drugs may also be prognostic for these patients. A biomarker that informs the choice of a specific therapy on an investigational protocol or in practice is called an integral biomarker (26). If the biomarker is shown to be predictive, an approved companion diagnostic assay may be required before the drug can be approved for clinical use (26, 27). Under the revised FDA

guidance on companion diagnostics (27), if the results of a pretreatment biomarker assay are used to guide the choice of one treatment versus another in a clinical trial, in many cases the assay and the device used to measure it are subject to regulatory review for an Investigational Device Exemption (IDE) and must be formally approved for investigational use by the FDA. Once clinical development starts, the assay itself cannot be changed. More recently, the requirements for an IDE have been amended; if the FDA judges that the use of the assay poses only non-significant risk to the patient, then an IDE may not be required.

Response-indicator and **efficacy response** biomarkers occur during and after treatment. Response-indicator biomarkers show that there has been a change following treatment but do not necessarily demonstrate that clinical benefit has been derived from the treatment (10, 11). Such biomarkers include pharmacodynamic measurements to assess the on-target effects of a drug, changes in tumor size, and changes measured against other response criteria (*e.g.*, patient symptoms). Response-indicator biomarkers in CRPC include the control and/or relief of symptoms (28); post-therapy changes in PSA, which are now reported using waterfall plots (10); changes in bone scans (reported as improved, unchanged or stable, or progression); and changes in soft-tissue disease reported using RECIST 1.1 [but because tumor size does not adequately account for changes in PSA and bone metastases RECIST alone is a limited measure of response in CRPC (10)].

In non-MIBC (muscle invasive bladder cancer), measurement of clinical outcome from treatment with Bacillus-Calmette Guérin (BCG) is monitored by follow-up with local cystoscopy wherein serial cytologies and alternative molecular assays of choice are used to do upper tract evaluations. Examples of these molecular assays include measurement of urinary interleukin 2 (IL-2) level; measurements of urinary IL-8, IL-18, and tumor necrosis factor apoptosis-inducing ligand (TRAIL) levels may also be useful (29).

Efficacy response biomarkers also show changes following treatment, and further are associated with clinical benefit. In drug development, efficacy response biomarkers indicate clinical outcomes and depending on the level of evidence may ultimately be qualified as surrogates for a clinical endpoints and lead to drug approvals.

Post therapy PSA declines are an indication that a treatment effect has been achieved, but are not universally evidence of efficacy, nor are they surrogates of clinical benefit that can be used for regulatory approvals. In contrast, the control and relief of pain in patients with CRPC is a clinical benefit (10, 28, 30, 31). Biomarkers to assess presence of pain at baseline and change in pain post-treatment are derived from measurements on quality-of-life instruments—such as the Brief Pain Inventory (5, 32) or QLC-30 (5, 32, 33)— which undergo a detailed validation process similar to the process performed for laboratory assays (see below). Of the measurements reported, maximal pain intensity in the previous 24 hours has been used as an efficacy-response indicator of clinical benefit for drug approval (5). Dramatic improvements in the intensity, area and number of lesions with tracer uptake on a bone scan following treatment with cabozantinib is an example of a response indicator that in some cases was associated with significant pain relief but no effect on PSA (34). One of the two phase 3 trials evaluating cabozantinib was designed to show an effect on survival relative to prednisone, and the other was designed to superiority in the relief of pain in men

with a maximal pain intensity despite narcotic analgesics relative to mitoxantrone and prednisone which has a formal approval in this indication. Neither trial was successful and development in prostate cancer has been halted (35).

One of the authors (Dr. Scher) has been heavily engaged in the development and use of biomarkers for prognosis and assessing treatment effects in prostate cancer. In 2008, he and the Prostate Cancer Working Group (PCWG2) presented a framework for clinical trial conduct in CRPC in response to a challenge by the FDA (13). PCWG2 recommended focusing on signs that treatment was failing, rather than on evidence of treatment activity, since treatment failure was closely tied to the need for change of therapy. Applying this approach requires a prospectively defined primary end point that can be measured with minimal bias and that is a clinical benefit or can serve as a surrogate for that benefit. Delay or prevention of death from the disease and reduction or delay in the development of skeletal-related events are key endpoints. The indications for drug approvals in CRPC are now consistent with this model.

3.0 Requirements for clinical validation/clinical utility and qualification

The authors note the continued high interest in the academic and pharmaceutical research communities and by the FDA in identifying relevant biomarkers for characterizing cancers. However, the well-structured development plan that is required and rigorous criteria that must be met to qualify biomarkers for specific contexts of use in drug development and patient management are not often fully appreciated (Table 1) (9, 10, 13, 36, 37). As suggested in the discussion above regarding urological cancers, the most progress in developing biomarkers has been made for evaluation of CRPC. In the section following, the FDA's general requirements for validating and qualifying a biomarker are described along with the work carried out and progress made in developing biomarkers for use in drug development and patient management in CRPC, particularly some of the most promising biomarkers in this setting—enumeration and characterization of CTCs.

Many assays for analysis of CTCs have been proposed recently with the developers working to increase sensitivity and specificity for detecting these rare cells and to avoid artifacts from extensive processing (36, 38). Enrichment for CTCs can involve density gradient centrifugation, immunomagnetic isolation, microfluidics, or some combination of techniques. CTC enumeration platforms are generally image-based approaches using immunocytochemistry or laser scanning cytometry, and CTC characterization techniques are typically nucleic acid or protein-based molecular assays, imaging, or a combination of molecular and imaging methods.

Biomarker development consists of three components: analytical validation, clinical validation, and qualification for specific contexts of use (9, 10, 36, 37, 39–42).

To establish the **analytical validation** of a biomarker, data are generated to describe performance characteristics of the biomarker measurement itself; these include the device, imaging modality, and/or molecular assay, and the range of conditions under which the measurement gives reproducible and accurate results (including in clinical samples). This includes the pre-analytical assessment of specimen and/or image acquisition, processing and

storage; determining the consistency and reproducibility of the analysis each time it is performed at the same or independent laboratories, and quality control measures; the post-analytical data reduction, including how the results will be recorded, reported, and the specific measures that will be associated with clinical outcome (Tables 2 and 3) (36).

For CTCs, many parameters need to be considered to document pre-analytical processing. For example, collection tubes for various assays can differ: some have fixatives to preserve the cells, while those for RNAs, short-term culture, xenograft development, or functional characterization generally do not. Some methods require sample processing within hours, while others provide consistent results up to several days after the blood draw. Sensitivity and specificity are issues for all of the techniques owing to the heterogeneity of tumor cells with respect to size, density, and marker expression. Epithelial cellular adhesion molecule (EpCAM) is the most widely used capture antibody, and cytokeratins (CK8 or CK18, CK19) are the most widely used biomarkers for the detection of CTCs from epithelial prostate tumors (36, 43). However, a proportion of the CTCs in the blood have undergone an epithelial mesenchymal transition (EMT) or acquired stem like properties and no longer express EpCAM and other cytokeratins, and are not being captured or detected using positive-selection techniques based on these antibodies, leading to false-negatives (36, 43, 44).

Currently, CellSearch® (Veridex LLC, Warren, NJ, USA) is the only CTC assay that has been analytically validated and FDA-approved for use as a prognostic biomarker in clinical settings in breast, colon, prostate, and lung cancer (37, 45). This assay captures CTCs using an EpCAM conjugated ferrofluid, and defines them after immunofluorescent staining as morphologically intact cells with a 4,6-diamidino-2-phenylindole (DAPI)-positive nuclei surrounded by cytoplasm that express the cytokeratins CK8 and CK18 or CK19, but do not express CD-45 (46). The assay reports results as the number of cells in 7.5 ml of blood that meet the defined criteria; notably, this repeated value represents a small proportion of the EpCAM-expressing cells captured.

CellSearch® was analytically validated using cell-line-spiking experiments, with over 450 duplicate breast cancer patient samples showing consistent results over a wide range of cell numbers analyzed in the same laboratory by different technicians, and in a reference laboratory compared to a local laboratory (45). In prostate cancer, it was shown that the captured cells expressed PSA and alpha-methylacyl-CoA racemase (AMACR), and that prostate cancer samples had unique copy number alterations and androgen receptor (AR) amplification by fluorescent *in situ* hybridization (FISH) (46).

After analytical validity has been established, clinical studies are initiated to establish **clinical validity**: the demonstration that the biomarker is fit for purpose for a specific context of clinical use—that is, that the results will inform a medical decision (47). Establishing clinical validity requires trials designed to address the biomarker question. For our example in prostate, this has included evaluation of treatment effects on CTCs alone or in combination with other parameters compared with clinical benefit (PFS, OS) in trials of abiraterone (48), enzalutamide (49), and docetaxel plus prednisone with or without lenalidomide. Notable here is that in a trial of abiraterone plus prednisone vs. placebo plus

prednisone, biomarkers containing CTC and LDH measured at 12 weeks met the four Prentice criteria for surrogacy (Scher *et al.*, J Clin Oncol, in press). Further validation of this finding is ongoing.

By definition, an assay that demonstrates **clinical utility** informs a medical decision that leads to improved patient outcomes when applied for a specific context of use. The improvement in patient risk/reward ratio for a clinical outcome is analogous to the clinical benefit of a drug for needed for an approval for a specific indication. The proven utility can include diagnostic accuracy for information for the patient population, improved patient management, safety, and/or reduced costs (37), both of which affect reimbursement. Although the FDA does not formally request clinical utility in the biomarker development process, it is a vital consideration that impacts on how widely the marker will be used and ultimately reimbursed by private and public payers. For example, clinical utility would be a deciding factor when comparing the value of a costly molecular analysis of a tumor compared to inexpensive clinical parameters routinely available in practice to assess prognosis.

Clinical qualification is an additional step in biomarker development that has regulatory implications. A clinically qualified biomarker is one for which sufficient evidence has been generated for FDA acceptance for use in regulatory submissions, without a re-review of the data supporting the use of the biomarker. A biomarker that qualifies for a clinical benefit end point (an improvement in survival time or patient function) could potentially lead to accelerated drug approval (8, 11, 50). Generating the level of evidence needed for clinical qualification can be a long process involving the prospective testing of the biomarker in multiple phase 3 trials (11). Because CTCs can be obtained non-invasively and they contain information about the molecular and phenotypic characteristics of the tumors from which they originate, CTCs have attracted interest as biomarkers for prognostic, predictive, and response uses. As noted, enumeration of CTCs, as measured by the CellSearch[®] assay, has been cleared by the FDA as a correlate for poor prognosis in patients with breast, prostate, colon and lung cancers, and efforts are ongoing to qualify the measurement of CTCs for determining patient response and drug efficacy in CRPC (36, 44). Although results are promising, issues related to sensitivity and enumeration without functional characterization of the cells may limit the generalizability of the assay. Newer technologies — such as fiberoptic scanning, filtration and electrophoretic separation methods, single cell analysis, and microfluidic techniques [reviewed in (36, 43, 44)]— MAY enable more precise definitions of the characteristics of CTCs (for example, by identifying cells that are undergoing or have undergone EMT and by allowing the molecular characterization of the cells through genomic profiling (10, 36).

4.0 Looking to the future

The development of biomarkers to meet FDA requirements for validation and qualification to inform drug development and patient management requires a systematic and stepwise approach, beginning with an assessment of the performance characteristics and reproducibility of the measurement itself, followed by a series of prospectively designed studies with adequate statistical support to demonstrate a clinical effect within a well-

defined context of use. To date, many potential biomarkers have been identified in prostate and urinary bladder cancers, but few have sufficient demonstration of analytical and clinical validity to meet FDA standards for use in clinical settings. However, CTC assays are particularly promising candidates for informative new biomarkers to measure disease before and after treatment. New technologies are providing opportunities for high definition, more informative analysis. Nonetheless, with each technology enhancement comes the burden of establishing that the new method meets the analytical validation criteria for performance, clinical qualification for the context of use, and the qualification process/criteria. Analytical validation includes the performance of the device, the reagents, the measurements, the software, as well as the data elements and outputs. The statistical and computational methodologies to describe assay results are also rapidly evolving. These advances will lead to better diagnosis, earlier indications of treatment response and failure, and better definition of patient cohorts that will respond to a specific treatment.

References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–74. [PubMed: 21376230]
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000; 100:57–70. [PubMed: 10647931]
3. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell*. 2009; 136:823–37. [PubMed: 19269363]
4. Aukema TS, Kappers I, Olmos RA, Codrington HE, van Tinteren H, van Pel R, et al. Is 18F-FDG PET/CT useful for the early prediction of histopathologic response to neoadjuvant erlotinib in patients with non-small cell lung cancer? *J Nucl Med*. 2010; 51:1344–8. [PubMed: 20720059]
5. Atkinson TM, Mendoza TR, Sit L, Passik S, Scher HI, Cleeland C, et al. The Brief Pain Inventory and its “pain at its worst in the last 24 hours” item: clinical trial endpoint considerations. *Pain Med*. 2010; 11:337–46. [PubMed: 20030743]
6. Polyak K, Shipitsin M, Campbell-Marrotta LL, Bloushtain-Qimron N, Park SY. Breast tumor heterogeneity: causes and consequences. *Cancer Res*. 2009; 11:18.
7. Polyak K, Kalluri R. The role of the microenvironment in mammary gland development and cancer. *Cold Spring Harb Perspect Biol*. 2010; 2:a003244. [PubMed: 20591988]
8. Woodcock J. Paving the critical path of drug development: the CDER perspective. *Nat Rev Drug Discov*. 2014; 13:783–4. [PubMed: 25359359]
9. Kelloff GJ, Sigman CC. Cancer biomarkers: selecting the right drug for the right patient. *Nat Rev Drug Discov*. 2012; 11:201–14. [PubMed: 22322254]
10. Scher HI, Morris MJ, Larson S, Heller G. Validation and clinical utility of prostate cancer biomarkers. *Nat Rev Clin Oncol*. 2013; 10:225–34. [PubMed: 23459624]
11. FDA. Guidance for Industry and FDA Staff: Qualification Process for Drug Development Tools. 2014. [cited; January 2014; Available from: <http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm230597.pdf>]
12. Wagner JA, Williams SA, Webster CJ. Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. *Clin Pharmacol Ther*. 2007; 81:104–7. [PubMed: 17186007]
13. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. *J Clin Oncol*. 2008; 26:1148–59. [PubMed: 18309951]
14. Crawford ED, Ventii K, Shore ND. New biomarkers in prostate cancer. *Oncology (Williston Park)*. 2014; 28:135–42. [PubMed: 24701701]
15. Danila DC, Fleisher M, Scher HI. Circulating tumor cells as biomarkers in prostate cancer. *Clin Cancer Res*. 2011; 17:3903–12. [PubMed: 21680546]

16. Klein, E.; Maddala, C.; Millward, C., et al. Development of a needle biopsy-based genomic test to improve discrimination of clinically aggressive from indolent prostate cancer. Poster presentation at the American Society of Clinical Oncology annual meeting; Chicago, IL. 2012;
17. Cooperberg, M.; Simko, J.; Falzarano, S., et al. Development and validation of the biopsy-based genomic prostate score (GPS) as a predictor of high grade or extracapsular prostate cancer to improve patient selection for active surveillance. Presented at the American Urological Association annual meeting; San Diego, CA. 2013;
18. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science*. 2005; 310:644–8. [PubMed: 16254181]
19. Ru Y, Dancik GM, Theodorescu D. Biomarkers for prognosis and treatment selection in advanced bladder cancer patients. *Curr Opin Urol*. 2011; 21:420–7. [PubMed: 21814055]
20. Choudhury A, Nelson LD, Teo MT, Chilka S, Bhattarai S, Johnston CF, et al. MRE11 expression is predictive of cause-specific survival following radical radiotherapy for muscle-invasive bladder cancer. *Cancer Res*. 2010; 70:7017–26. [PubMed: 20843819]
21. Kawashima A, Nakayama M, Kakuta Y, Abe T, Hatano K, Mukai M, et al. Excision repair cross-complementing group 1 may predict the efficacy of chemoradiation therapy for muscle-invasive bladder cancer. *Clin Cancer Res*. 2011; 17:2561–9. [PubMed: 21177407]
22. Hoffmann AC, Wild P, Leicht C, Bertz S, Danenberg KD, Danenberg PV, et al. MDR1 and ERCC1 expression predict outcome of patients with locally advanced bladder cancer receiving adjuvant chemotherapy. *Neoplasia*. 2010; 12:628–36. [PubMed: 20689757]
23. Bellmunt J, Paz-Ares L, Cuello M, Cecere FL, Albiol S, Guillem V, et al. Gene expression of ERCC1 as a novel prognostic marker in advanced bladder cancer patients receiving cisplatin-based chemotherapy. *Ann Oncol*. 2007; 18:522–8. [PubMed: 17229776]
24. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011; 364:2507–16. [PubMed: 21639808]
25. Tang G, Shak S, Paik S, Anderson SJ, Costantino JP, Geyer CE Jr, et al. Comparison of the prognostic and predictive utilities of the 21-gene Recurrence Score assay and Adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. *Breast Cancer Res Treat*. 2011; 127:133–42. [PubMed: 21221771]
26. Schilsky RL, Doroshow JH, Leblanc M, Conley BA. Development and use of integral assays in clinical trials. *Clin Cancer Res*. 2012; 18:1540–6. [PubMed: 22422406]
27. FDA. In Vitro Companion Diagnostic Devices: Guidance for Industry and Food and Drug Administration Staff. 2014. [cited; August 2014; Available from: <http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf>]
28. Tannock IF, Osoba D, Stockler MR, Ernst DS, Neville AJ, Moore MJ, et al. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. *J Clin Oncol*. 1996; 14:1756–64. [PubMed: 8656243]
29. Mertens LS, Neuzillet Y, Horenblas S, van Rhijn BW. Biomarkers for assessing therapeutic response in bladder cancer. *Arch Esp Urol*. 2013; 66:495–504. [PubMed: 23793767]
30. Sartor O, Reid RH, Hoskin PJ, Quick DP, Ell PJ, Coleman RE, et al. Samarium-153-Lexidronam complex for treatment of painful bone metastases in hormone-refractory prostate cancer. *Urology*. 2004; 63:940–5. [PubMed: 15134985]
31. Berthold DR, Pond GR, Roessner M, de Wit R, Eisenberger M, Tannock AI. Treatment of hormone-refractory prostate cancer with docetaxel or mitoxantrone: relationships between prostate-specific antigen, pain, and quality of life response and survival in the TAX-327 study. *Clin Cancer Res*. 2008; 14:2763–7. [PubMed: 18451243]
32. Osoba D, Tannock IF, Ernst DS, Neville AJ. Health-related quality of life in men with metastatic prostate cancer treated with prednisone alone or mitoxantrone and prednisone. *J Clin Oncol*. 1999; 17:1654–63. [PubMed: 10561201]
33. Sternberg CN, Petrylak DP, Sartor O, Witjes JA, Demkow T, Ferrero JM, et al. Multinational, double-blind, phase III study of prednisone and either satraplatin or placebo in patients with

- castrate-refractory prostate cancer progressing after prior chemotherapy: the SPARC trial. *J Clin Oncol*. 2009; 27:5431–8. [PubMed: 19805692]
34. Smith DC, Smith MR, Sweeney C, Elfiky AA, Logothetis C, Corn PG, et al. Cabozantinib in patients with advanced prostate cancer: results of a phase II randomized discontinuation trial. *J Clin Oncol*. 2013; 31:412–9. [PubMed: 23169517]
 35. ClinicalTrials.gov. 2013. [online][cited December 16, 2014; Available from: <http://clinicaltrials.gov/show/NCT01522443>
 36. Parkinson DR, Dracopoli N, Petty BG, Compton C, Cristofanilli M, Deisseroth A, et al. Considerations in the development of circulating tumor cell technology for clinical use. *J Transl Med*. 2012; 10:138. [PubMed: 22747748]
 37. Parkinson DR, McCormack RT, Keating SM, Gutman SI, Hamilton SR, Mansfield EA, et al. Evidence of clinical utility: an unmet need in molecular diagnostics for patients with cancer. *Clin Cancer Res*. 2014; 20:1428–44. [PubMed: 24634466]
 38. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. *Cancer Discov*. 2014; 4:650–61. [PubMed: 24801577]
 39. Goodsaid FM, Mendrick DL. Translational medicine and the value of biomarker qualification. *Sci Transl Med*. 2010; 2:47ps4.
 40. Khleif SN, Doroshow JH, Hait WN. AACR-FDA-NCI Cancer Biomarkers Collaborative consensus report: advancing the use of biomarkers in cancer drug development. *Clin Cancer Res*. 2010; 16:3299–318. [PubMed: 20501613]
 41. Park JW, Kerbel RS, Kelloff GJ, Barrett JC, Chabner BA, Parkinson DR, et al. Rationale for biomarkers and surrogate end points in mechanism-driven oncology drug development. *Clin Cancer Res*. 2004; 10:3885–96. [PubMed: 15173098]
 42. Gutman S, Kessler LG. The US Food and Drug Administration perspective on cancer biomarker development. *Nat Rev Cancer*. 2006; 6:565–71. [PubMed: 16794639]
 43. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer*. 2008; 8:329–40. [PubMed: 18404148]
 44. Danila DC, Pantel K, Fleisher M, Scher HI. Circulating tumors cells as biomarkers: progress toward biomarker qualification. *Cancer J*. 2011; 17:438–50. [PubMed: 22157288]
 45. FDA. FDA clearance document for Veridex LLC. CellSearch(TM) circulating tumor cell kit. Premarket notification - expanded indications for use - metastatic prostate cancer. 2008 Feb 26. [cited Feb 1, 2013]; Available from: http://accessdata.fda.gov/cdrh_docs/pdf7/K073338.pdf
 46. Shaffer DR, Leversha MA, Danila DC, Lin O, Gonzalez-Espinoza R, Gu B, et al. Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. *Clin Cancer Res*. 2007; 13:2023–9. [PubMed: 17404082]
 47. Cummings J, Raynaud F, Jones L, Sugar R, Dive C. Fit-for-purpose biomarker method validation for application in clinical trials of anticancer drugs. *Br J Cancer*. 2010; 103:1313–7. [PubMed: 20924371]
 48. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. *N Engl J Med*. 2013; 368:138–48. [PubMed: 23228172]
 49. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012; 367:1187–97. [PubMed: 22894553]
 50. Buyse M, Sargent DJ, Grothey A, Matheson A, de Gramont A. Biomarkers and surrogate end points--the challenge of statistical validation. *Nat Rev Clin Oncol*. 2010; 7:309–17. [PubMed: 20368727]

Table 1

Validation and Qualification of Biomarkers¹

The The FDA Center for Drug Evaluation and Researchhas provided a guidance document on the qualification of biomarkers for use as drug development tools (8, 9, 11). Some of the features of this process are as follows:	
<ul style="list-style-type: none">• Biomarkers are measured using specific devices (for example, assays or instruments), and if they are marketed for managing patients in clinical practice, the devices are reviewed by FDA's Center for Devices and Radiological Health (CDRH)for the ability to analytically measure the biomarker.• Biomarkers that are being considered for qualification are independent of the specific device that performs the measurement, and devices that measure the biomarker are expected to yield equivalent results.• Biomarker qualification cannot be achieved without analytical and clinical validation of at least one device to measure the biomarker. Analytical validity may be defined as the ability of an assay to accurately and reliably measure the analyte of interest in the laboratory and in specimens that are representative of the population of interest. Clinical validation requires the detection or prediction of the associated disorder of interest in specimens from targeted patient groups.• Clearance of a measurement device by CDRH does not imply that the biomarker has been demonstrated to have a qualified use in drug development; additional data are needed from studies designed to establish qualification.• Qualification data include consensus methods and evidence that correlates biomarker measurements with sufficient clinical outcomes. The lack of standardization of protocols for biomarker specimens is a limiting factor in acquiring these data.• Biomarker qualification is intended to provide some degree of generalizability for the use of the biomarker as a drug development tool across multiple clinical disorders, drugs or drug classes, and for benefiting patients. Historically, acceptance of biomarker measurements has usually been specific to a sponsor and a drug.• Once qualified, a biomarker can be used by drug developers for its qualified 'context of use' in regulatory submissions without the need for further review by CDER.• The term 'context of use' used by the FDA describes the setting (or settings) in which the biomarker is qualified, and boundaries within which the available data justify its use. The context of use may expand as new data are obtained.	
Biomarker qualification also enables collaboration among stakeholders, reduces costs for individual stakeholders and provides biomarkers that are useful to multiple parties	

Adapted from (9)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2**CTC Assay Analytical Validation Considerations¹**

Pre-analytic Variables Controlled	
<ul style="list-style-type: none"> • Materials at study site, <i>e.g.</i>, sample collection tubes, shipping containers • Materials at analytic laboratory, <i>e.g.</i>, assay-specific kit (with instructions for use, reagents, buffers, controls, <i>etc.</i>), additional equipment (centrifuge, test tubes, micro-pipettes, vortex mixer, <i>etc.</i>) • Reagent storage conditions (temperature, time, <i>etc.</i>) • Specimen collection, <i>e.g.</i>, timing of specimen collection relative to study treatments and procedures, phlebotomy procedure (patient positioning, needle size, blood draw site, sample size and number of samples, collection tube, tube inversion for clot prevention) • Specimen handling, <i>e.g.</i>, storage time and temperature, shipping time, <i>etc.</i> 	
Analytic Variables Controlled	
<ul style="list-style-type: none"> • Type of analysis (enrichment/enumeration/ molecular characterization of target cells) • What is the range of specimens tested? • Sensitivity of assay (enrichment/enumeration/molecular characterization of all cells) • Specificity of assay (enrichment/enumeration/molecular characterization of only target cells) • How often does the assay give a useable result? • How similar are the results with repeated measurements? • What are the analytical range, reproducibility, and clinical applicability of the test? • How similar are the results obtained within the same or in multiple laboratories using the same or different technology? • Assay-specific controls should be run each day of analysis or when a new assay lot is used to check overall performance, including instrument, reagents, and operator technique • Assay-specific controls should be run regularly (monthly or quarterly) to show performance at the lower limit of analysis • Assay-specific samples should be run semi-annually to document the lower limit of quantitation • Calibration/calibration verification (at least semi-annually) 	
Post-analytic Variables	
<ul style="list-style-type: none"> • All participating laboratories in the same study should use common controls to document continuously across laboratory performance. • Splitting samples across laboratories prior to the study is recommended to document assay performance across laboratories. 	

¹. Adapted from (36)

Table 3

CTC Assay Clinical Validation Considerations

Assay Characteristics	
<ul style="list-style-type: none">• Clinical sensitivity and specificity of assay• Methods to resolve clinical false positive assay results in a timely manner	
Disease Characteristics	
<ul style="list-style-type: none">• Prevalence (how often target cells are detectable in the study population)• Relationship between detectable target cells and the study disease• Positive predictive value (the probability that a positive assay result means that the subject has the target disease)• Genetic, environmental, or other modifiers affecting detection of the target cells	

1. Adapted from (36)