There has been significant activity in the development of biomarkers for the diagnosis and prognosis of diseases such as cancer. The development of diagnostic agents will allow the integration of this technology with highly specific therapies with the likelihood of improved outcomes for the patient.

Keynote review:

Recent advances in biomarkers for cancer diagnosis and treatment

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With the availability of new technologies and the increased interest of medical practitioners to use molecular biomarkers in early detection and diagnosis, and in the prediction of therapeutic treatment efficacy and clinical outcomes, the academic and research institutions, as well as the pharmaceutical industry, have increased their efforts to develop novel molecular biomarkers for several human diseases, including cancer. The identification of molecular biomarkers also enables the development of a new generation of diagnostic products and to integrate diagnostics and therapeutics. This integrated approach will aid in 'individualizing' the medical practice. Here, we address issues related to the development of biomarkers, novel technological platforms used for drug development, future technologies and strategies for validating biomarkers for their clinical utility.

During the past two decades, fewer than 12 biomarkers have been approved by the US Food and Drug Administration (FDA) for monitoring response, surveillance or recurrence of cancer [1]. This is surprising because hundreds of thousands of biomarkers have been discovered or declared to be potential biomarkers for cancer diagnosis and detection. However, to date, none has proven to be effective. To be useful, biomarkers must distinguish between people with cancer and those without. Many biomarkers do not progress beyond this point because the investigators are either unable to develop robust, accurate assay methods, or validation studies show that the biomarker lacks sufficient sensitivity and/or specificity [2]. In this regard, a recent review by Stoss and Henkel also addressed some of the issues relevant to cancer biomarker development and the difficulties of moving biomarkers from basic research into the clinic [3].

In the context of cancer biomarker testing, the sensitivity of a biomarker refers to the proportion of case subjects (individuals with

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BOX 1

Laboratory measures for surrogate endpoints

Reliability
Repeatability, a high correlation between two measurements
Precision
The total error is zero
Accuracy
Measure the true level, devoid of bias
Validity
Measure the true change or effect of intervention on outcome
Bias
Systematic error introduced into sampling or testing by selecting or encouraging one outcome or answer over others

confirmed disease) who test positive for the biomarker, and specificity refers to the proportion of control subjects (individuals without disease) who test negative for the biomarker. An ideal biomarker test would have 100% sensitivity and specificity; in other words, everyone with cancer would have a positive test and everyone without cancer would have a negative test. The lower the sensitivity, the more often that individuals with cancer will not be detected, and the lower the specificity, the more often someone without cancer will test positive [4].

None of the currently available biomarkers achieve 100% sensitivity and specificity. For example, prostate specific antigen (PSA), currently the best overall serum biomarker for prostrate cancer, has high sensitivity (greater than 90%) but low specificity (~25%), which results in many men having biopsies when they do not have detectable prostrate cancer [5–8]. The serum tumor biomarker for breast cancer CA15.3 has only 23% sensitivity and 69% specificity and is only useful in monitoring therapy for advanced breast cancer or recurrence. Other frequently used terms are positive predictive value (PPV), the chance that a person with a positive test has cancer, and negative predictive value (NPV), the chance that a person with a negative test does not have cancer. PPV and, to a lesser degree, NPV, are affected by the prevalence of disease in the screened population. For a given sensitivity and specificity, the higher the prevalence, the higher the PPV.

Definitions and types of biomarkers

The definitions and types of biomarkers are illustrated here by looking at cancer as a disease process. A tumor marker is also referred to as a 'cancer biomarker'. It is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention. This characteristic/biomarker is either produced by the tumor or by the body in response to cancer. A characteristic could be measured by genetics, proteomics, cellular or molecular substances found in higher than normal amounts in the blood, urine or body tissues of a cancer patient. The biomarker is a measurable and reliable indicator or marker used to assess the disease process, or an outcome or to estimate whether a drug used in the treatment was effective or not [9].

Based on their utility, biomarkers can be divided into the following categories:

1) Early detection – if used for screening patients to find cancer early;
2) Diagnostic – if used to assess the presence or absence of cancer;
3) Prognostic – if used to assess the survival probabilities of patients or to detect the aggressive phenotype and determine how the cancer will behave;
4) Predictive – if used to predict whether the drug and other therapies will be effective, or to monitor the effectiveness of treatment; and
5) Target – if used to identify the molecular targets of novel therapies and which molecular markers' expression were affected by therapy.

Surrogate endpoints (see Box 1) are biomarkers used to substitute for a clinical endpoint and could be used to measure clinical benefit, harm or lack of benefit or harm. Surrogates could replace traditional endpoints, such as incidence of disease, mortality due to disease or the recurrence or relapse of disease.

The advantages of biomarkers are well recognized by the research, medical and pharmaceutical communities. Biomarkers can reduce time factors and costs for Phase I and II clinical trials by replacing clinical endpoints. Biomarkers can also be helpful in redefining the diseases and their therapies by shifting the emphasis of traditional practices of depending on symptoms and morphology to a more rational objective molecular basis.

Issues affecting molecular detection, screening and treatment

Development of biomarkers for cancer screening, detection and treatment involves both biological and economic challenges. Most detection methods in use to date identify fully developed cancer, not the pre-malignant or early lesions (intermediate stages) amenable to resection and cure. Although a screening test might detect cancer at the preclinical stage, it could fail to detect micrometastasis and thereby limit the benefit of early detection and treatment [10]. Another problem is that in many organs, for example, prostate or colon, preneoplastic lesions are much more common than aggressive cancers and only 10% or less develop into a malignant tumor [11]. However, there is evidence that a subset of prostatic intraepithelial neoplasia develop into particularly aggressive phenotypes. This raises the question of whether any screening method should just focus on early lesions, or whether it should also analyze the behavior of the tumor. To our minds, the discovery of serum, genetic or other tumor biomarkers should enable detection of the subset of cancer that is

likely to lead to clinically important cancer, such as in prostate cancer.

Cancer is a heterogeneous disease, meaning that the disease itself is composed of many biologically different phenotypes with varied responses to intervention, including screening and treatment. Heterogeneity is found between cells of a single macroscopic cancer. For example, a varying number of estrogen-receptor positive malignant cells are found within a breast adenocarcinoma. Tumors can contain several types of clonal populations, each with different sets of mutations and phenotypic changes. This heterogeneity complicates the development of biomarkers because a biomarker initially tested using one cell type might not be predictive of cancer risk or treatment effect of a tumor with several different cell types. As such, the development of biomarker by genomic and proteomic means must carefully address the heterogeneity issues. There is a misconception that microdissected specimen minimize heterogeneity within the neoplastic cell population. Although microdissection might provide pure cell populations for experimental purposes, it does not change heterogeneity in the tumor as a whole [12].

Bias in biomarker discovery

The rules of evidence are not as well established for evaluating diagnostic and prognostic markers as for clinical agents in therapy. Cancer therapy is commonly evaluated through a well controlled randomized trial that addresses issues related to bias, heterogeneity and other confounding factors, such as age, sex, hormonal status, and so on. By contrast, research studies on biomarkers are usually conducted using observational epidemiology or clinical epidemiology rules that are less well defined. However, the rules of clinical epidemiology of diagnostics and prognosis can improve the evaluation of molecular markers, especially for handling heterogeneity, complexity and bias. For example, Manne *et al.* [13] suggested that p53 abnormal expression, detected by immunohistochemistry, can be a poor prognostic indicator for non-Hispanic Caucasians with proximal colonic adenocarcinomas but not for distal or rectal adenocarcinomas, and p53 might not be a prognostic molecular marker for African-Americans with colorectal adenocarcinomas. These studies suggest that the heterogeneity or admixture of patient population could bias the outcome studies. Recently, however, several sophisticated genomic and proteomic analyses of tumor cells have provided useful information on molecular signatures for discriminating cancer cells from non-cancerous cells. However, it would be an insurmountable task to conduct a clinical trial for each promising biomarker, a task that would be prohibitively expensive and time-consuming [14].

One of the major problems with high-dimensional data derived from high throughput genomic and proteomic technologies is overfitting of the data when there are large numbers of potential predictors among a small number of outcome events. For example, a recent study of RNA micoarray analysis showed how easy it was to overfit data with a small number of samples. Simon and colleagues clearly demonstrated that expression data on 6000 genes from imaginary individuals, 10 normal and 10 cases, could be used to discover discriminatory patterns, using one common method, with 98% accuracy [15].

Many of the so-called 'omics' derived data are subjected to a similar over-fitting if the training and validation sets for analyses are small and not randomized. Most commonly used approaches to analyze 'omics' data are artificial neural networks, boosted decision tree analyses, various types of genetic algorithms and support vector machine learning algorithms [16]. Each approach has the potential to overfit the data. Overfitting has led to strong conclusions that are likely to be erroneous. The first step, therefore, would be to determine whether the results are reproducible and portable. For this purpose, information on samples should be blinded and samples be sent to several laboratories for running the sample sets under a fixed protocol. The data from each laboratory should be analyzed by an independent data manager to learn if each laboratory reproduced a similar result. Splitting the samples randomly between 'training sets and validation sets' should minimize the overfitting. The validation set should not contain samples used in training sets. Further issues related to bias, overfitting and reproducibility can be found in the article by Ransohoff [14].

How to develop and evaluate biomarkers

Because of tumor heterogeneity and other biases that might be inherently imbedded with biomarker discovery and evaluation processes, it is important that the discovery of biomarkers should proceed in a systematic manner. Unlike a clinical trial design in which there are three phases (Phase I, Phase II and Phase III), research on biomarkers has largely been guided by intuition and experience. In 2002, the National Cancer Institute's Early Detection Research Network developed a five-phase approach to systematic discovery and evaluation of biomarkers. In general, biomarker development should follow an orderly process wherein one proceeds to the next phase only after meeting pre-specified criteria for the current phase. A brief summary of the criteria [17] (Figure 1) follows:

Phase 1 refers to preclinical exploratory studies. Biomarkers are discovered through knowledge-based gene selection, gene expression profiling or protein profiling to distinguish cancer and normal samples. Identified markers are prioritized based on their relevance to the diagnostic/prognostic/therapeutic (predictive) value and with promising leads that suggest their evolution into routine clinical use. The analysis of this phase is usually characterized by ranking and selection, or finding suitable ways to combine biomarkers. The phase could be repeated several times to ensure that the initial evidence is

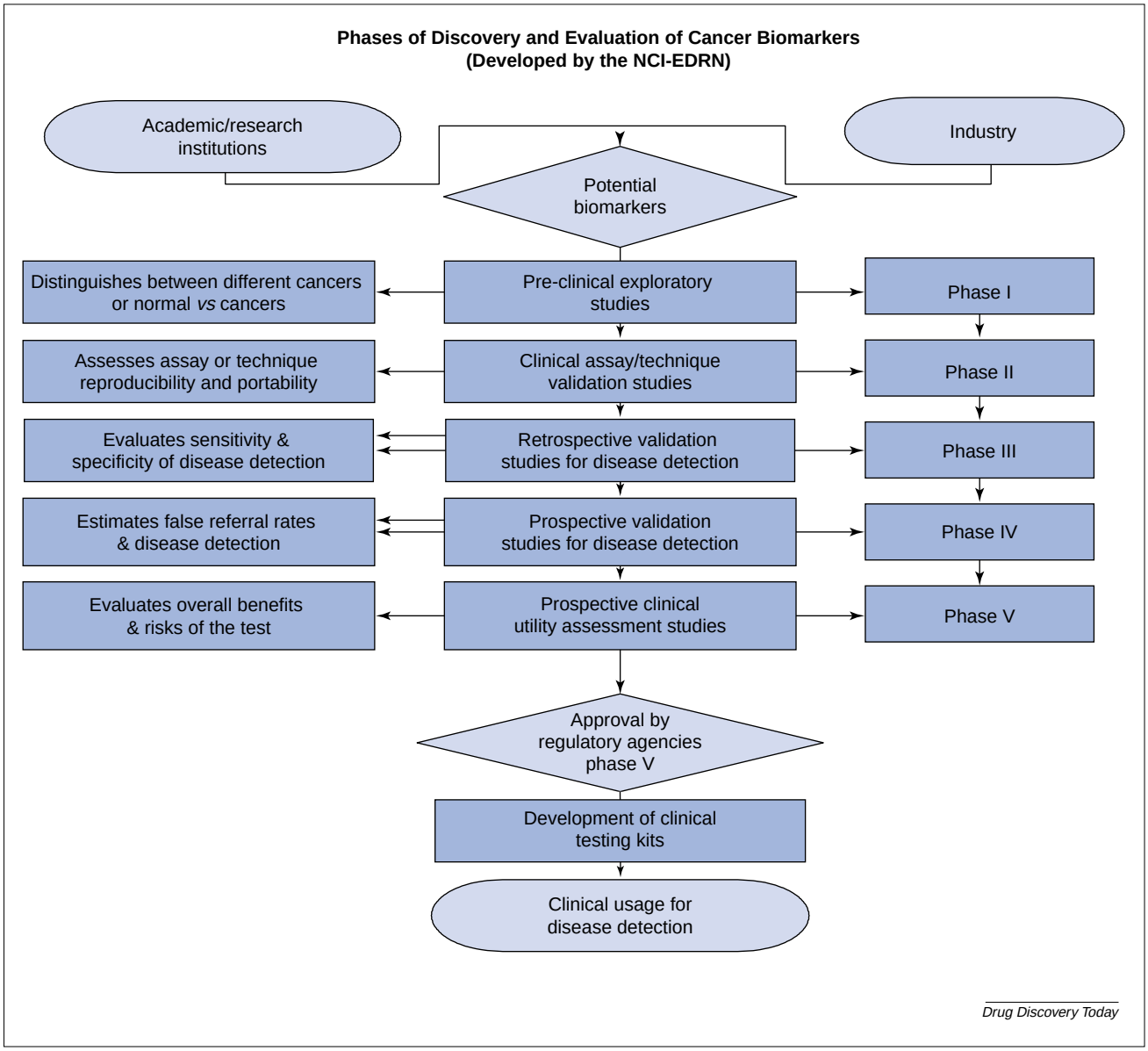

FIGURE 1

Phases of cancer biomarker development and validation. Approval by regulatory agencies will vary by the country-specific regulations and may commence after the completion of Phase III trials for limited use.

unequivocal; is based on a small number of samples; or whether there is concern about possible confounding or other sources of bias. Although it is not required, it is preferred that the specimen for this phase of discovery come from well-characterized cohorts, tissue banks or from a trial with active follow-ups.

Phase II has two important components. Upon successful completion of the Phase I requirements, an assay is established with a clear intended clinical use. The clinical assay could be a protein-, RNA-, DNA- or a cell-based technique, including ELISA, protein profiles from MS, phenotypic expression profiles, genearrrays, antibody arrays or quantitative PCR. To document clinical usefulness, such assays need to be validated for reproducibility and shown to be portable among different laboratories. Second, the assay is evaluated for its clinical performance in terms of 'sensitivity' and 'specificity' with thresholds determined by the intended clinical use.

During Phase III, an investigator evaluates the sensitivity and specificity of the test for the detection of diseases that have yet to be detected clinically. The specimens analyzed in this evaluation phase are taken from study subjects before the onset of clinical symptoms, with active follow-up to ascertain disease occurrence. It is usually time-consuming and expensive to collect these samples with high quality, therefore, Phase III should piggyback on existing large cohort studies or intervention trials whenever possible. This is probably where most biomarker validation studies will end and the biomarker will be ready for clinical use.

Phase IV evaluates the sensitivity and specificity of the test on a prospective cohort. The major difference to

Phase III is that in Phase IV a positive test triggers a definitive diagnostic procedure, often invasive and could lead to increased economic healthcare burden. Therefore, in a Phase IV study an investigator can estimate the false referral rate based on tested biomarkers and describe the extent and characteristic of disease detected (e.g. the stage of tumor at the time of detection). For rare diseases, Phase IV requires a large cohort with long-term follow-up and might often be too expensive as a stand-alone activity. These studies are difficult to perform specifically for rare diseases.

Phase V evaluates the overall benefits and risks of the new diagnostic test on the screened population. The cost per life saved is one example of an endpoint for such study. This again requires a large-scale study over a long time period and also could also be prohibitively expensive.

Phases IV and V are necessary to evaluate benefits and risks of the use of a biomarker in screening and detection. PSA is an example of a test that did not go through Phases IV and V before its widespread clinical use for prostate cancer screening and its clinical benefits are still under debate [18–20]. It is fair to say that PSA as an early detection marker was not even under a rigorous Phase II/III validation with a clear intended clinical use in mind. If hundreds of biomarker-based diagnostic tests were proposed for clinical use without thorough, orderly evaluations, the healthcare cost burden and the potential harm to the affected public could be enormous.

Biomarker discovery using high-throughput technology platforms

Historically, some screening tools (e.g. Pap smears, colonoscopy and PSA testing) have successfully reduced mortality through early detection. Despite these successes, the field of early detection has been plagued by problems of overdiagnosis (e.g. PSA), inadequate specificity of individual markers (e.g. CA125, CEA and AFP), low compliance (colonoscopy) and a lack of analytical tools for discovering new diagnostic markers. Several derivatives of PSA tests, such as the ratio of bound versus free PSA, PSA velocity and now PSA doubling-time have been brought forward as potential alternate surrogate tests for risk and treatment response (for references, see [21]). AFP is the only serum marker currently available for the diagnosis of hepatocellular cancer but its sensitivity (39–64%) and specificity (76–91%) are too low for use in early detection [22].

The limited number of useful markers has propelled investigators to use high throughput platforms, such as DNA, RNA, protein array and antibody arrays, and other approaches to identify large numbers of candidate biomarkers. The literature is full of candidate markers; however, because of concerns about the reproducibility of arrays, these results require great scrutiny. In a recent publication, investigators questioned the use of microarray in diagnostics due to inconsistency, lack of robustness and lack of concordance among microarray data [23].

The reason for using high throughput technologies is that they provide a large number of correlative data on gene or protein expression in relation to disease. Such data are then analyzed for their association to the disease. The assumption is that multiple variables will be able to provide information on associations more accurately than a single variable (marker). Such strong associations provide major impetus for the molecular profiling approaches to find patterns or profiles for a clinical test based on high dimensional gene or protein expression panels. High throughput platforms being used in the biomarker discovery phase are briefly summarized in the subsequent section in view of their potential for cancer detection and treatment (see review [24]).

Proteomics techniques are being applied to finding a biomarker that can be detected from plasma or serum. It is reasonable to expect that the serum/plasma proteome might contain diagnostic signatures: tumors 'leak' DNA and, hence, presumably also proteins into the circulation; tumors induce dramatic alterations of surrounding stroma (angiogenesis); and tumors release proteases that digest normal tissue and plasma proteins. Individual plasma proteins (i.e. PSA, CA125, CEA and AFP) originally identified using immunological approaches are already in clinical use as markers of response to therapy or tumor recurrence [25].

Comparative genomic analyses have yielded a large number of genomic expression data in relation to disease. Recent studies by Peru and colleagues on breast cancer [26], and Alizadeh and colleagues on lymphoma [27], have clearly demonstrated the potential use of gene expression patterns in stratifying patients between responders and non-responders to certain drugs. The patterns of gene expressions that were observed represent novel signatures for the respective tumor types and can be used to both develop new clinical tests based upon gene expression patterns, and identify candidate markers for diagnosis and prognosis. Similar high throughput platforms have been developed to screen genome-wide methylation and single nucleotide polymorphism patterns (haplotypes) in tumor tissues and body fluids. Aberrant DNA methylation of CpG dinucleotides is a common epigenetic alteration that contributes to colon cancer formation [28–32]. Aberrant CpG island methylation results in transcriptional silencing of genes and is a mechanism for inactivating tumor suppressor genes in colon cancer. The methylated tumor DNA can be detected using methylation-specific PCR (MSP) and thus has the potential to be used as a molecular marker for cancer. In colon cancer, the tumor suppressor genes *CDKN2A*, *MGMT* and *MLH1*, as well as other genes (e.g. *TIMP-3*, *p14^{ARF}*, *APC*, *MINT31*, *MINT2* and *THBS-1*), are commonly methylated and are thus candidate molecular markers for colon cancer. The methylation of *CDKN2A*, *MGMT*,

TABLE 1

Required characteristics of surrogate biomarkers

Characteristics	Evidence in support
Biological plausibility	Extensive and consistent epidemiological, correlative evidence on biomarkers with disease; well understood disease pathogenesis, drug mechanism of action well understood, surrogate relatively late on biological path; animal model that shows drug response.
Clinical trials for prediction	Effect on surrogate has predicted outcome with other drug of same pharmacological class; effect on surrogate has predicted outcome in several classes.
Public health considerations	There exists serious, life-threatening illness for which there is no alternative therapy available; extensive information on drug safety available, difficulty of studying clinical endpoint (rare, delayed); short-term use acceptable.

MLH1, MINT31, MINT2 and other genes occurs early in the adenoma-carcinoma sequence suggesting that these alterations could be used for the early detection of colon cancer.

Haplotypes, also known as single nucleotide polymorphisms, have been used as genetic markers of risk, treatment response, and gene and environment interactions in both rare and common cancers. Haplotypes within the *p53* gene have been associated with increased lung cancer risk, and haplotypes within *BRCA* genes, as well as in the surrounding regions, are associated with breast and ovarian cancer risk. The human leukemia antigen (HLA) genetic regions are highly variable and their haplotypes often include several genes. The HLA haplotypes are found to correlate with the outcome of cytokine therapy for renal cell carcinoma. Haplotypes might also be useful for predicting outcome of 'chemoprevention' (i.e. the use of one or several natural or synthetic substances to reduce the risk of developing cancer, or to reduce the chance of cancer recurrence), but few studies have been published that directly look for an association between haplotypes and cancer chemoprevention or response to chemopreventative agents [33–36].

Similarly, comparative analysis of serum and plasma samples by MS-based techniques, such as surface enhanced laser desorption ionization (SELDI)–MS (Ciphergen system; http://www.ciphergen.com/) has shown patterns of protein/peptide features indicative of a range of diseases, particularly cancer. Significant controversy surrounds this approach because of the reported difficulty in reproducibly detecting and using the same mass features in different laboratories, the inability to identify markers (specific profiles) and the difficulty in determining the specificity of the biomarker to the disease state. These problems are mainly attributed to manufacturing variation in the required single-use absorptive surfaces (Ciphergen ProteinChip™), the lack of a standardized data reduction and analysis protocols, and also the low resolution of the MS instruments originally employed [4,12,24].

The best platform for reproducible feature identification and quantification is debatable. Many believe that the high throughput matrix assisted laser desorption ionization (MALDI)-based instruments are well suited to clinical diagnostics, and they envision the mass spectrometer being part of the clinical laboratory. By contrast, others believe the mass spectrometer is merely a discovery tool and not a diagnostic instrument, and hence favor electrospray ionization (ESI)-based instruments, such as LC–MS–MS or LC–ESI–TOF and the ability to perform online separations. ESI is a technique used in MS to ionize small amounts of large and/or labile molecules, such as peptides and proteins, to produce a series of ions. The multiple charged ions are separated based on their charge to mass ratio. Meanwhile, schemes for fractionating plasma are being piloted in different laboratories, often on different samples, on different MS platforms and using different data analysis tools and criteria. The number of variables is too great to compare results from any one laboratory to those from another, therefore, it is nearly impossible to conclude which MS platforms, fractionation schemes or combination of analysis platforms will provide the most reliable data. As a result, many laboratories are now profiling patient samples on platforms that have not been validated or shown to be reproducible.

Biomarkers in cancer detection, diagnosis and prognosis

No single biomarker is likely to have 100% positive and negative predictive values for a specific neoplasm. Instead, panels of biomarkers seem to be a promising alternative for the use in clinical laboratories. Some examples of putative biomarkers are presented in Table 1. These biomarkers must be validated for the intended clinical use: diagnosis, prognosis and prediction for patients with cancer, as well as the selection of individuals at high risk for cancer with the aim of cancer prevention interventions. However, although many potential biomarkers are being identified using high throughput technologies, their clinical use remains to be established. Several investigators have used SELDI–TOF to evaluate a serum protein pattern as a biomarker for ovarian [37], breast [38] and prostate cancer [39] and lung [40]. Thus, a panel of biomarkers could provide more useful information and improve the statistical performance of individual biomarkers. An example of the usefulness of combining three serum biomarkers along with CA125 for increased sensitivity and specificity (the best-known biomarker for ovarian cancer and approved for ovarian cancer monitoring) was demonstrated in a five center case-control study, Chan and colleagues [41] analyzed the serum proteome of 153 patients with invasive epithelial ovarian cancer, 42 patients with other ovarian cancers, 166 with benign pelvic masses and 142 healthy women. The authors identified three early detection biomarkers: apolipoprotein, a truncated form of transthyretin and a cleavage fragment of inter-α-trypsin inhibitor. When these biomarkers were combined with CA125, the sensitivity was 74% for early

stage ovarian cancer, which is significantly higher than CA125 alone (65%) at a matched specificity of 97%. Another example is a recent study that combined CA125 with the α-chain of haptoglobin (Hp-α). Hp-α was recently identified as a serum biomarker for ovarian cancer using mass spectrometric protein profiling. In that study, CA125 was reported to have a sensitivity of 87% and a specificity of 90%, and HP-α a sensitivity of 64% and a specificity of 90%. When used in combination, the sensitivity was 91% and the specificity was 95% [42].These data demonstrate that the profiling coupled with identification of unique MS peak patterns could lead to improved detection of early stage ovarian cancer.

With no single biomarker showing 100% sensitivity and specificity, there is a need for more than one biomarker to enable molecular screening to reduce false positive and negative cases. One promise of new high throughput genomic and proteomic technologies is that they will enable the discovery and measurement of new biomarkers or more likely panels of biomarkers that will more accurately detect the presence of cancer, resulting in fewer false positive and false negative tests.

One factor that should be considered when designing a panel of biomarkers is to choose biomarkers that reflect changes in independent pathways. If two biomarkers come from the same or associated pathways, factors contributing to their increase or decrease in cancer are likely to be the same and, thus, their combined usage is unlikely to be more informative than when they are used individually. For example, prostate cancer is caused by androgen hormone specific stimulation of cell growth and its ablation is one of the therapeutic approaches. However, the more aggressive form of prostate cancer is usually due to androgen-independent pathways [43–47]. PSA is regulated by androgen and its levels are elevated in prostate cancer. However, it might not be a good biomarker to predict either the androgen independent prostate cancer or when androgen levels are depleted by chemotherapy or castration, thus making it an unattractive marker to follow the course of prostate cancer. The use of prostate biomarkers that monitor these different pathways would not only improve the present low sensitivity of PSA but also improve the measures used to follow the course of androgen independent, aggressive prostate cancer. In summary, a panel of biomarkers detected through the use of multiple, high throughput platforms allow multivariate and simultaneous analyses, enabling better and more efficient screening methods. Table 2 gives some examples of cancer biomarkers in use.

Biomarkers in drug development

The major challenges in cancer drug development are discriminate responses, efficacy and toxic side effects. The pharmaceutical industry, drug policy makers and administrators are constantly looking for novel pharmacogenomics and/or pharmocoproteomic studies that might identify potential molecular biomarkers to help solve these problems [48].

Most therapeutic agents were developed without the knowledge of their molecular target. This has led to expensive development and production of cancer drugs because of a lack of information on targets, which can be used to test the efficacy of therapeutics. Costs have been estimated at up to US$750 million to develop a single drug [49]. This does not include expenses on failed agents or drugs (http://csdd.tufts.edu/InfoServices/OutlookPDFs/Outlook2004.pdf) because early in drug discovery, hundreds of agents are evaluated in a series of experiments and only a few lead to useful discovery. To increase the efficiency and quality of drug discovery, biomarkers could be used. Biomarkers can be useful for *in vitro* evaluations of hundreds of candidates that are typically screened during the drug development process. Biomarkers can also be used in measuring drug toxicity and pharmacokinetics in Phase II clinical trials. Most Phase IIb and Phase III trials are conducted using reduction in mortality or disease-free survival as the endpoint, and studies are usually large (several thousands patients) and long (more than 10 years).

It is likely that future studies will employ a panel of biomarkers as surrogate endpoints for each stage of drug discovery. At least three elements are necessary to use biomarkers as surrogate outcomes: (1) the proper definition of the risk factor and the means to detect it; (2) the definition of definitive, clinical outcomes and how to assess them; and (3) a close association between the surrogate outcome and clinical outcome. The following criteria must be met before the potential biomarkers can serve as surrogate endpoints. First, the marker should be differentially expressed between the normal and the disease state. Second, the marker should be present throughout the progression of a tumor. Third, the assay for the biomarker should be reproducible with a high level of performance characteristics, for example, sensitivity and specificity. Fourth, the marker should preferably be measurable in samples collected non-invasively or by less invasive means. For surrogate biomarkers to serve as intermediate endpoints, they should satisfy additional criteria. First, the biomarker expression should be modulatable by the therapeutic or chemopreventative agents. Second, the change in biomarker expression should correlate with a change in disease rate [48].

Before embarking on the use of biomarkers as surrogate endpoints, the biomarker of interest must be fully validated for biological plausibility, success in clinical trials and risk-benefit and public health considerations (Table 2) [50]. The lesson from a recently concluded trial on hormone replacement therapy (HRT) for cardiovascular indications reminds us that a biomarker selected on the basis of intuition or circumstantial evidence might lead to a completely unexpected outcome. In this trial, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were used as surrogates for measuring the reduction

TABLE 2

Some putative biomarkers for cancer detection, diagnosis and prognosis

Biomarker	Cancer types	Remarks	Recent citations
CA 125 (MUC 16)	Ovarian (serum)	Sensitivity by stage: Stage I = 27 - 66%, Stage II= 65 - 100%; Stage III = 87 - 90% Stage IV = 94% Sensitivity by histology: Serous: 68 - 94.1% Mucinous: 52 - 68% Endometrioid: 92%, Clear cell: 61%;	[70]
Kallikrein 6 (Protease M)	Ovarian (serum)	Sensitivity by stage: Stage I: 16% Stage II: 27% Stage III: 75% Stage IV: 63% Sensitivity by histology: Serous: 68% Endometrioid 33% Mucinous: 9%; In combination with CA 125: sensitivity=69%, specificity = 95%	[70]
Osteopontin	Ovarian (serum)	Sensitivity by stage: Stage I/II: 80%, Stage III/IV: 85% Mean level (ng/mL) and 95% CI: In healthy: 147 (8 - 641) In benign: 254.4 (3 - 641) In ovarian cancer: 487 (315 - 751)	[70]
Prostate-specific antigen (PSA)	Prostate (serum)	Sensitivity ~70% Specificity 59–97%	[5–8]
Alpha methyl CoA-racemase	Prostate (tissue)	Sensitivity ~97% and specificity ~100%	[71–72]
Methylation of promoter regions of genes	Prostate, colon, lung, gastric	Varied sensitivity and specificity; GST	[28–32]
Protein Profiling	Prostate, colon and lung (serum)	Sensitivity 90–98% Specificity 95–99%	[37–40]
APC	Colon	Frequently mutated in colon cancer	[73]
BRCA-1 and BRCA-2 mutations	Breast (tissue)	Risk marker and prognosis	[74]
Glutathione S-transferase –1 (GSTP-1)	Breast, prostate (tissue)	Risk assessment, prognosis; detection of GSTP-1 methylation in prostate with 75% sensitivity and 100% specificty	[75]
EGFR	Lung	Prognosis and treatment	[68–69]
Haptoglobin	Lung, colon, and breast (serum)	Diagnosis and treatment response	[74]
$CDKN_2$ A	Colon	Sensitivity 16–54%; Specificity 63–94%	[76]
Microsatellite Alterations	Bladder (urine sample)	Sensitivity 83–90; Specificity 100%	[77]
Des-gamma-carboxyl- prothrombin (PCP)	Hepatocellular carcinoma (serum)	Sensitivity 54–68%; specificity 87–98%	[78]
AFP	Hepatocellular carcinoma (serum)	Sensitivity 39–64%; specificity 76–91%	[22]

in risk for cardiovascular diseases. Although the treatment lowered the LDL level and increased HDL levels, the risk of coronary disease remained unaffected. This finding suggests that a factor (e.g. LDL and HDL) and the risk of a disease (e.g. coronary disease) do not guarantee that drug-induced factors will produce a corresponding change in the risk. Therefore, a good marker of risk might not be a good surrogate for measuring drug efficacy [51].

In colon cancer, colonic polyps might be the closest surrogates that have been used as an endpoint for several clinical trials of calcium supplementation, aspirin and celecoxib [52]. More specifically, nonsteroidal anti-inflammatory drugs (NSAIDs) have reduced the incidence of and mortality from sporadic adenoma and colon cancer in several epidemiologic studies. Recently, a 2–3-times greater risk of heart attacks, strokes and/or deaths resulting from heart or blood vessel disease has been reported among people taking celecoxib than the risk of patients who did not take celecoxib (http://www.nih.gov/news/pr/dec2004/od-17.htm). This has led the National Cancer Institute (NCI) to suspend the use of celecoxib within the Adenoma Prevention with Celecoxib Trial based on a recommendation from the Data Safety and Monitoring Committee. Accordingly, drug development efforts will have to occur increasingly at molecular levels to identify genetic abnormalities that are commonly occurring in a disease process, and to assess their implications on other cellular activities by studying gene and/or protein expression profiles before identifying them as actual drug targets. This approach of identifying or monitoring 'target' effects early in the discovery and development process avoids intricacies in assessing the effects of drug treatment at systemic levels during later stages. These approaches also provide higher quality information about how compounds act, particularly how they affect other molecules and restore the internal environment of the cell to 'near normal' conditions. Such population-based molecular epidemiological studies are crucial in the drug development process.

Recent advances in drug development based on molecular biomarkers

In the treatment of cancer, there is a shift from the traditional clinical practices to novel approaches. Traditionally, cancer patients were treated with drugs of low toxicity or of high tolerance regardless of their efficacy in a given patient if the benefits of that drug are proven in both experimental and clinical conditions. However, recent advances in basic and clinical research have provided opportunities to develop 'personalized' treatment strategies. These novel approaches are intended to identify individualized patient benefits of therapies, minimize the risk of toxicity and reduce the cost of treatment.

The biggest challenge for researchers and clinicians today is which type of biomarker to use across the wide spectrum of disease processes. In cancer, genomic studies are valuable because every cancer cell shows some degree of genetic damage, which might not be present in normal cells of the body. Contrary to genomic DNA markers, phenotypic expression markers (RNA/protein) will vary among cell types and change over time and show different posttranscriptional or posttranslational modifications. However, proteins, peptides or metabolites are abundant, easily accessible in body fluids, such as blood, urine, cerebrospinal fluid and secretions, and show promise for measuring outcomes and studying changes in disease state.

Another challenge in characterizing biomarkers is the complexity of the expression profile of potential markers in benign conditions close to the disease phenotypes. The evolving trend is the usage of patterns of markers instead of a single marker. This approach could, to some extent, reduce the error rate in predicting the outcome or severity of side effects during the targeted therapies. Further challenges in biomarker development are in finding the relevant markers that have the right degree of specificity and sensitivity and a reliable test to measure the outcome.

Targeted therapies

Some of the recently developed targeted therapies for cancer are Herceptin (Genentech; http://www.gene.com/gene/products/information/oncology/herceptin/index.jsp), Gleevac (Novartis; http://www.pharma.ca.novartis.com/e/products/gleevec.shtml), Iressa (AstraZeneca Pharmaceuticals; http://www.astrazeneca-us.com/default.asp), and Velcade (Millennium; http://www.mlnm.com/products/velcade/index.asp). The selection of patients and their efficacy will be based on molecular profiling or phenotypic expression of their target molecules in malignant tissues. These targeted drugs shut down their specific pathway or sets of pathways. The predictability of the response to targeted drugs rules out their use in all patients, which helps to avoid unnecessary drug-associated side effects. Furthermore, the biomarker expression profiles will provide early hints of success to pharmaceutical companies about whether to proceed with the development of drugs specific for those target molecules, thus, helping to reduce development costs.

Surrogate endpoints and surrogate endpoint biomarkers

Several definitions and interpretations of surrogate end points and surrogate end point biomarkers (SEPB) in cancer exist. Surrogate end points are generally intermediate endpoints that should be related to the main endpoint and could represent a preliminary index of the final outcome. Based on the distinction between activity and effectiveness of a treatment, the outcomes can be cancer outcomes (e.g. response rate) and patient-outcomes (e.g. survival or quality of life) [53]. Surrogate endpoints can also represent survival and the disease-free interval in the adjuvant setting, or the number of complete regressions of the disease during chemotherapy clinical trials [54]. A recent review by Johnson *et al.* [55] summarized the endpoints used by the FDA during the past decade to approve new cancer drug proposals.

The protein/peptide, genetic, phenotypic, cytologic, histologic or morphologic markers can be used as SEPB to measure specific outcomes (end points) of the studies. One of the advantages of SEPB is that they can be used to evaluate the outcomes of clinical trials much earlier than

the clinical end points, which are usually measured at the end of the trials. The use of SEPBs curtails the time required for the trial and therefore can save millions of dollars. The FDA encourages the use of SEPB for drug trials to expedite the process of drug approval. However, it is doubtful that interim analysis of outcomes (surrogate end points) in clinical trials will always reflect or indicate the ultimate clinical endpoint. Under those conditions in which interim analyses are not possible, the SEPB should not be used and the trials should be continued for their full duration to assess the clinical endpoints. Several recent reviews discussed the effectiveness and controversies related to SEPBs in drug development for cancer [56–62]. Although the advantages of SEPBs were clearly demonstrated, the studies based on SEPB are inherently less reliable compared to studies in which true endpoints were measured. It is important to know when the use of SEPBs is appropriate and when it is not (Table 2).

The majority of recently approved drugs already have associated biomarkers. For example, the breast cancer drug Herceptin is recommended only to patients whose tumors exhibit *Her2/neu* gene amplification. In actuality, it is used in ~25% of patients with metastatic disease [63]. The benefit of Herceptin has been shown in a Phase III clinical trial in metastatic breast cancer. Treatment alone or in combination with chemotherapy resulted in a delay of the disease progression, higher response rates and longer overall patient survival [64]. Based on these trials, Herceptin was approved in 1998 by the FDA. Similar successes apply to the recently approved anti-epidermal growth factor receptor (EGFR) chimeric monoclonal antibody (cetuximab) for metastatic colorectal cancer [65–67], and anti-EGFR-tyrosine kinase (EGFR-TK) small molecules Gefitinib (ZD1839 or Iressa) for non-small cell lung cancer (NSCLC) [68,69]. The use of these targeted therapies requires biomarker-based patient selection to enroll into the clinical trials and interim evaluations of treatment efficacy by measuring phenotypic expression of these markers as SEPB.

Future directions

A large concerted effort is required to advance the field of biomarker discovery. Recent advances clearly indicate the lack of useful biomarkers for cancer detection, screening and treatment. Most biomarkers do not satisfy the required sensitivity and specificity for cancer detection and for entry into the clinical study needed to quantify drug effect. Validation of biomarkers is necessary for measuring efficiency in drug development. Biomarkers such as blood cholesterol, for example, which can identify subjects for entering into clinical trials and quantify drug efficacy, are urgently required for accelerating the drug development, measuring the adverse effect for a 'go' or a 'no go' decision, and confirming the benefit of the drug.

In its first five years, the NCI's Early Detection Research Network (EDRN) [67] has established both crucial and necessary groundwork for the validation of potential biomarkers. The Network has created and published a five-phase model of early detection research for their researchers to follow; pioneered development of common data elements to speed consistency in the way data are described across institutions; implemented informatics solutions to data sharing; and created a web-based system to automate flow of study procedures at laboratories in EDRN studies.

EDRN investigators applied SELDI–TOF–MS technology to discover signature proteins that distinguish prostate cancer from benign prostate disorders and healthy prostate tissues. Protein profiles from prostate cells in different states of disease have been shown to have discriminating differences from each other and from healthy cells. The investigators were able to distinguish early stage cancer with high sensitivity (95%) and specificity (95%). Research continues to develop global gene expression patterns as biomarkers of cancer detection and risk assessment.

Crucial validation-level studies for early detection tests are underway for several disease sites via the EDRN, including a trial to determine the sensitivity and specificity of microsatellite analysis in diagnosing bladder cancer; validation of α-fetoprotein and des-gamma carboxyprothrombin for differentiating hepatocellular cancer from nonmalignant liver disease; and validation of the protein markers annexin I and II, PGP9.5 and autoantibodies to these proteins as biomarkers for early detection of lung cancer.

References

1 Anderson, N.L. and Anderson, N.G. (2002) The human plasma proteome: history, character, and diagnostic prospects. *Mol. Cell. Proteomics* 1, 845–867

2 Srivastava, S. *et al.* (2001) Biomarkers for early detection of colon cancer. *Clin. Cancer Res.* 7, 1118–1126

3 Stoss, O. and Henkel, T. (2004) Biomedical marker molecules for cancer-current status and perpectives. *Drug Discovery Today: Targets* 3, 228–237

4 Wagner, P.D. *et al.* (2004) Molecular diagnostics: a new frontier in cancer prevention. *Expert Rev. Mol. Diagn.* 4, 503–511

5 Bangma, C.H. *et al.* (1995) Volume adjustment for intermediate prostate-specific antigen values in a screening population. *Eur. J. Cancer* 31A, 12–14

6 Gann, P.H. *et al.* (1995) A prospective evaluation of plasma prostate-specific antigen for detection of prostatic cancer. *JAMA* 273, 289–294

7 Gillatt, D. and Reynard, J.M. (1995) What is the 'normal range' for prostate-specific antigen? Use of a receiver operating characteristic curve to evaluate a serum marker. *Br. J. Urol.* 75, 341–346

8 Lepor, H. *et al.* (1994) Detection of prostate cancer in males with prostatism. *Prostate* 25, 132–140

9 Atkinson, A.J. *et al.* (2001) NCI-FDA Biomarkers Definitions Working Group; Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* 69, 89–95

10 Pollak, M.N. and Foulkes, W.D. (2003) Challenges to cancer control by screening. *Nat. Rev. Cancer* 3, 297–303

11 Neuget, A.I. *et al.* (1997) Prevalence and incidence of colorectal adenomas and cancer in asymptomatic persons. *Gastrointest. Endosc. Clin. N. Am.* 7, 387–399

12 Wagner, P.D. *et al.* (2004) Challenges for biomarkers in cancer detection. *Ann. N. Y. Acad. Sci.* 1022, 9–16

13 Manne, U. *et al.* (1998) Nuclear accumulation of p53 in colorectal adenocarcinoma: prognostic

importance differs with race and location of the tumor. *Cancer* 83, 2456–2467
14 Ransohoff, D.F. (2004) Rules of evidence for cancer molecular-marker discovery and validation. *Nat. Rev. Cancer* 4, 309–314
15 Feng, Z. *et al.* (2004) Research issues and strategies for genomic and proteomic biomarker discovery and validation: a statistical perspective. *Pharmacogenomics* 5, 709–719
16 Srivastava, S. (Ed.) (2005) *Informatics in Proteomics*, Marcell-Dekker
17 Sullivan Pepe, M. (2001) Phases of biomarker development for early detection of cancer. *J. Natl. Cancer Inst.* 93, 1054–1061
18 Shibata, A. *et al.* (1998) Prostate cancer incidence and mortality in the United States and the United Kingdom. *J. Natl. Cancer Inst.* 90, 1230–1231
19 Oliver, S. *et al.* (2000) Comparison of trends in prostate-cancer mortality in England and Wales and the USA. *Lancet* 355, 1788–1789
20 Thompson, I.M. *et al.* (2004) Prevalence of prostate cancer among men with a prostate-specific antigen level. *N. Engl. J. Med.* 35, 2239–2246
21 Kelloff, G.J. *et al.* (2004) Prostate-specific antigen doubling time as a surrogate marker for evaluation of oncologic drugs to treat prostate cancer. *Clin. Cancer Res.* 10, 3927–3933
22 Marrero, J.A. *et al.* (2003) Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 37, 1114–1121
23 Marshall, E. (2004) Getting the noise out of gene arrays. *Science* 306, 630–631
24 Verma, M. and Srivastava, S. (2003) New cancer biomarkers deriving from NCI early detection research. *Recent Results Cancer Res.* 163, 72–84; discussion 264–266
25 Verma, M. *et al.* (2003) Proteomic analysis of cancer-cell mitochondria. *Nat. Rev. Cancer* 3, 789–795
26 Perou, C.M. *et al.* (2000) Molecular portraits of human breast tumours. *Nature* 406, 747–752
27 Alizadeh, A.A. *et al.* (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403, 503–511
28 Esteller, M. *et al.* (1999) hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. *Am. J. Pathol.* 155, 1767–1772
29 Esteller, M. *et al.* (1999) Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res.* 59, 793–797
30 Esteller, M. *et al.* (1999) Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res.* 59, 67–70
31 Laird, P.W. and Jaenisch, R. (1996) The role of DNA methylation in cancer genetic and epigenetics. *Annu. Rev. Genet.* 30, 441–464
32 Tycko, B. (2000) Epigenetic gene silencing in cancer. *J. Clin. Invest.* 105, 401–407
33 Ellerhorst, J.A. *et al.* (2003) Heterozygosity or homozygosity for 2 HLA class II haplotypes predict favorable outcomes for renal cell carcinoma treated with cytokine therapy. *J. Urol.* 169, 2084–2088
34 Gal, I. *et al.* (2004) The 1100delAT BRCA1 and the 8765delAG BRCA2 mutations: occurrence in high-risk non-Ashkenazi Jews and haplotype comparison of Jewish and non-Jewish carriers. Fam. *Cancer* 3, 11–14
35 Osorio, A. *et al.* (2003) Over-representation of two specific haplotypes among chromosomes harbouring BRCA1 mutations. *Eur. J. Hum. Genet.* 11, 489–492
36 Wu, X. *et al.* (2002) p53 Genotypes and Haplotypes Associated With Lung Cancer Susceptibility and Ethnicity. *J. Natl. Cancer Inst.* 94, 681–690
37 Petricoin, E.F. *et al.* (2002) Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 359, 572–577
38 Vlahou, A. *et al.* (2003) A novel approach toward development of a rapid blood test for breast cancer. *Clin. Breast Cancer* 4, 203–209
39 Adam, B.L. *et al.* (2002) Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. *Cancer Res.* 62, 3609–3614
40 Xiao, X. *et al.* (2003) Development of proteomic patterns for detecting lung cancer. *Dis. Markers* 19, 33–39
41 Zhang, Z. *et al.* (2004) Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res.* 64, 5882–5890
42 Ye, B. *et al.* (2003) Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic profiling and mass spectrometry. *Clin. Cancer Res.* 9, 2904–2911
43 Chatterjee, B. (2003) The role of the androgen receptor in the development of prostatic hyperplasia and prostate cancer. *Mol. Cell. Biochem.* 253, 89–101
44 Kiyama, S. *et al.* (2003) Castration-induced increases in insulin-like growth factor-binding protein 2 promotes proliferation of androgen-independent human prostate LNCaP tumors. *Cancer Res.* 63, 3575–3584
45 Lee, S.O. *et al.* (2003) Interleukin-6 promotes androgen-independent growth in LNCaP human prostate cancer cells. *Clin. Cancer Res.* 9, 370–376
46 Pfeil, K. *et al.* (2004) Long-term androgen-ablation causes increased resistance to PI3K/Akt pathway inhibition in prostate cancer cells. *Prostate* 58, 259–268
47 Stewart, J.R. and O'Brian, C.A. (2004) Resveratrol antagonizes EGFR-dependent Erk1/2 activation in human androgen-independent prostate cancer cells with associated isozyme-selective PKC alpha inhibition. *Invest. New Drugs* 22, 107–117
48 Wagner, J.A. and Srivastava, S. (2002) Overview of biomarkers and surrogate endpoints in drug development. *Dis. Markers* 18, 41–46
49 Lathia, C.D. (2002) Biomarkers and surrogate endpoints: how and when might they impact drug development? *Dis. Markers* 18, 83–90
50 Bucher, H.C. *et al.* (1999) Users' guides to the medical literature: XIX. Applying clinical trial results. A. How to use an article measuring the effect of an intervention on surrogate end points. Evidence-Based Medicine Working Group. *JAMA* 282, 771–778
51 Herrington, D.M. and Howard, T.D. (2003) From presumed benefit to potential harm–hormone therapy and heart disease. *N. Engl. J. Med.* 349, 519–521
52 Kelloff, G.J. *et al.* (2004) Colorectal adenomas: a prototype for the use of surrogate end points in the development of cancer prevention drugs. *Clin. Cancer Res.* 10, 3908–3918
53 Davide, T. (2003) Surrogate end points of quality of life assessment: have we really found what we are looking for. In *Health and Quality of Life Outcomes* (Vol. 1), pp. 71
54 Anonymous (1996) Outcomes of cancer treatment for technology assessment and cancer treatment guidelines. American Society of Clinical Oncology. *J. Clin. Oncol.* 14, 671–679
55 Johnson, J.R. *et al.* (2003) End points and United States Food and Drug Administration approval of oncology drugs. *J. Clin. Oncol.* 21, 1404–1411
56 Colburn, W.A. (2000) Optimizing the use of biomarkers, surrogate endpoints, and clinical endpoints for more efficient drug development. *J. Clin. Pharmacol.* 40, 1419–1427
57 Kelloff, G.J. *et al.* (2004) Biomarkers, surrogate end points, and the acceleration of drug development for cancer prevention and treatment: an update prologue. *Clin. Cancer Res.* 10, 3881–3884
58 Kelloff, G.J. *et al.* (2003) Counterpoint: Because some surrogate end point biomarkers measure the neoplastic process they will have high utility in the development of cancer chemopreventive agents against sporadic cancers. *Cancer Epidemiol. Biomarkers Prev.* 12, 593–596
59 Kelloff, G.J. *et al.* (2000) Perspectives on surrogate end points in the development of drugs that reduce the risk of cancer. *Cancer Epidemiol. Biomarkers Prev.* 9, 127–137
60 Lesko, L.J. and Atkinson, A.J., Jr (2001) Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. *Annu. Rev. Pharmacol. Toxicol.* 41, 347–366
61 Park, J.W. *et al.* (2004) Rationale for biomarkers and surrogate end points in mechanism-driven oncology drug development. *Clin. Cancer Res.* 10, 3885–3896
62 Armstrong, W.B. *et al.* (2003) Point: Surrogate end point biomarkers are likely to be limited in their usefulness in the development of cancer chemoprevention agents against sporadic cancers. *Cancer Epidemiol. Biomarkers Prev.* 12, 589–592
63 Shak, S. (1999) Overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2-overexpressing metastatic breast cancer. Herceptin Multinational Investigator Study Group. *Semin. Oncol.* 26(4, Suppl 12), 71–77
64 Slamon, D.J. *et al.* (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J.* Med. 344, 783–792
65 Alikhan, M.A. *et al.* (2004) Re: Randomized trial of adjuvant therapy in colon carcinoma: 10-year results of NSABP protocol C-01. *J. Natl. Cancer Inst.* 96, 1794; author reply 1794
66 Cunningham, D. *et al.* (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* 351, 337–345
67 Holmer, A.F. (2004) Cetuximab in colon cancer. *N. Engl. J. Med.* 351, 1575–1576
68 Choy, H. *et al.* (2000) A Phase I Trial of Outpatient Weekly Docetaxel and Concurrent Radiation Therapy for Stage III Unresectable Non Small-Cell Lung Cancer: A Vanderbilt

Cancer Center Affiliate Network (VCCAN) Trial. *Clin. Lung Cancer* 1(Suppl. 1), S27–S31
69 Herbst, R.S. (2003) Dose-comparative monotherapy trials of ZD1839 in previously treated non-small cell lung cancer patients. *Semin. Oncol.* 30(1, Suppl. 1), 30–38
70 Terry, K.L. *et al.* (2004) Blood and urine markers for ovarian cancer: A comprehensive review. *Dis. Markers* 20, 53–70
71 Jiang, Z. *et al.* (2005) Using an AMACR (P504S)/34betaE12/p63 cocktail for the detection of small focal prostate carcinoma in needle biopsy specimens. *Am. J. Clin. Pathol.* 123, 231–236
72 Rogers, C.G. *et al.* (2004) Prostate cancer detection on urinalysis for alpha methylacyl coenzyme a racemase protein. *J.Urol.* 172, 1501–1503
73 Umar, A. and Srivastava, S. (2004) The promise of biomarkers in colorectal cancer detection. *Dis. Markers* 20, 87–96
74 Maruvada, P. *et al.* (2005) Biomarkers in molecular medicine: cancer detection and diagnosis. *Biotechniques* 38, S9–S15
75 Jeronimo, C. *et al.* (2001) Quantitation of GSTP1 methylation in non-neoplastic prostatic tissue and organ-confined prostate adenocarcinoma. *J. Natl. Cancer Inst.* 93, 1747–1752
76 Petko, Z. (2000) Aberrantly methylated CDKN2A, MGMT, and MLH1 in colon polyps and in fecal DNA from patients with colorectal polyps. *Clin. Cancer Res.* 11, 1203–1209
77 Schneider, A. *et al.* (2000) Evaluation of microsatellite analysis in urine sediment for diagnosis of bladder cancer. *Cancer Res.* 60, 4617–4622
78 Mita, Y. *et al.* (1998) The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocelluar carcinoma. *Cancer* 82, 1643–1648