



Ex vivo biomarkers: functional tools to guide targeted drug development and therapy

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Most of the currently utilized predictive biomarkers for therapeutic decision-making provide information regarding the presence or absence of the drug target but reveal little about the functional circuitry of the signaling network that the drug must also impact. *Ex vivo* biomarkers are dynamic molecular markers evoked from living tumor cells after removal from the patient. Such *ex vivo* biomarkers provide valuable mechanistic information that may facilitate drug development and guide the clinical selection of targeted therapeutics.

KEYWORDS: biomarker • drug development • oncology • predictive marker • signal transduction • targeted therapy

With the emergence of numerous molecularly targeted agents (MTAs) for cancer therapy comes the responsibility of personalizing their application through the use of predictive biomarkers. At the same time, there is growing awareness that the current classes of biomarkers may not be sufficient. To date, the most effective predictive markers for solid tumors include those for HER2 overexpression and/or gene amplification, EGF receptor (*EGFR*) mutations and *KRAS* mutations. Unfortunately, even these predictive markers have limitations. HER2 overexpression, one of the first predictive biomarkers for a targeted therapy (trastuzumab) in solid tumors, is detected in approximately 20% of breast cancers but, at most, 50% of these women have an objective response to trastuzumab, and many of these are partial responses [1–4]. *EGFR* mutations, which are predictive markers for response to *EGFR* tyrosine kinase inhibitors (e.g., gefitinib and erlotinib) in non-small-cell lung cancer, are only detected in approximately 10% of lung cancer patients in the USA and, of these, only 55–75% of patients actually demonstrate a complete or partial response in prospective studies [5,6]. In addition, erlotinib has demonstrated prolonged survival in some patients without *EGFR* mutations [7]. *KRAS* mutations are found in 45% of colorectal cancers, and it has recently been found that patients whose tumors harbor *KRAS* mutations will not benefit from therapy with *EGFR*-targeting monoclonal antibodies, such as cetuximab and panitumumab. This has

led the American Society of Clinical Oncology (ASCO) to recommend *KRAS* mutation testing in all colorectal cancer patients who are candidates for anti-*EGFR* antibody therapy [8]. Unfortunately, the response rate of *KRAS* wild-type tumors is less than 15% and confers only a small overall survival benefit compared with supportive care [9]. The limiting factors for the development of better predictive biomarkers are multiple but include biospecimen quality and the information contained within the biomarker test.

One major limitation of the existing classes of biomarkers is the lack of functional information. Static biomarkers, such as *EGFR* mutation analysis or HER2 immunohistochemistry, identify drug targets but they do not reveal the complexity of the signal transduction network downstream of the target. For example, it has been experimentally determined that the ErbB family members, including *EGFR* and HER2, may directly interact with as many as 59 different proteins, comprising just a portion of the receptor's 'interactome' [10–12]. Consequently, a tumor cell's response to a targeted therapeutic drug is dependent not only on the presence of the target but also to the multitude of molecules and their variants, within the signaling network. Many investigators have attempted to utilize array technology and sophisticated bioinformatics tools to infer function from protein or microarray datasets derived from fixed tissues [13,14]; however, only functional assays on

living tumor cells reveal the synthesis of this variation without the need to identify and understand every molecular variant within the network.

Such sophisticated analysis may require specialized handling of tumor samples [15]. In fact, current specimen handling procedures may be impeding, rather than facilitating, our progress in this area, owing to antiquated tissue handling and processing protocols. The foundation for most tissue-based biomarkers remains formalin-fixed, paraffin-embedded tissues that are generated using techniques that were developed in the mid-19th Century [16]. These techniques, which were developed to optimize the microscopic appearance of tissues, kill cells, risk contamination and damage biomolecules, thus robbing the sample of the valuable information content [17,18]. The US National Cancer Institute (NCI) has recognized the magnitude of this problem and has responded through the creation of an Office of Biorepositories and Biospecimen Research (OBBR) [10]. One stated challenge for the OBBR is:

“...bridging the gap between existing clinical practice for biospecimens and emerging technologies for personalized diagnostics and therapies.”

One such bridging opportunity is presented by the *ex vivo* biomarker. The term ‘*ex vivo* biomarker’ (Latin *ex-*, *e-*, meaning ‘out of, from’ and Latin *vo*, ablative of *vvus*: living, a living body) has been used to define a novel class of biomarkers – those that are evoked by live tumor cells after they have been removed from the patient (Box 1). In the context of molecular biomarkers, this refers to the process of removing viable cells from a patient through peripheral blood or bone marrow collection during surgery or through a minimally invasive biopsy, such as a fine-needle aspiration (FNA) biopsy (Figure 1). The viable sample is then stimulated *in vitro*. *Ex vivo* stimulation of peripheral blood mononuclear cells has been previously utilized in several settings, particularly in immunology. For example, the QuantiFERON-TB assay measures *ex vivo* IFN- γ production by peripheral blood mononuclear cells in response to antigen stimulation as an indicator of previous mycobacterial

exposure [19]. In oncology applications, these stimuli are growth factors, such as EGF, which are relevant to the signal transduction networks targeted by new therapeutic drugs. The biomarkers themselves can represent any dynamic biomolecule but, typically, are newly modified phosphoproteins or newly expressed mRNAs in the signaling network. Cellular events occurring rapidly (minutes) after *ex vivo* stimulation, such as protein phosphorylation events, are often considered ‘proximal’ to the stimulus and may be most valuable in determining the dominant signal-transduction pathways utilized by the tumor. Events occurring later following *ex vivo* stimulation (minutes to hours), such as new mRNA transcription, are considered ‘distal’ markers and may be more useful in assessing a composite view of the signal transduction events and their impact on cellular functions, such as proliferation or apoptosis. Multiplexed panels of such phosphoproteins, or gene expression microarrays, may facilitate the generation of comprehensive functional profiles that are distinct from, and more informative than profiles generated from fixed tissues. In some cases, the effect of a MTA on the pathway can be monitored *ex vivo* by stimulating the sample in the presence of a modulator, such as a chemical pathway inhibitor or the MTA itself. Overall, *ex vivo* biomarkers offer the possibility of functional assays that interrogate entire signal transduction networks. Such assays offer two exciting possible applications: patient stratification based on functional information to inform clinical trial design or clinical management and novel pharmacodynamic assays for use in the development of targeted therapies. This article will summarize the published literature on *ex vivo* biomarkers, with an emphasis on those that have been applied to actual human tumors or to model systems that mimic clinical samples from human tumors. Opportunities for future application will also be explored.

***In vitro* chemotherapy sensitivity & resistance assays**

In vitro chemotherapy sensitivity and resistance assays (CSRAs), in which a sample of a patient’s tumor cells are directly tested, *ex vivo*, for sensitivity or resistance to a panel of chemotherapeutic agents, would seem to be the ideal approach to personalized cancer therapy. Indeed, numerous different approaches to such testing

Box 1. Glossary of functional biomarker terminology.

- Biomarker: a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention [42]
- *Ex vivo* biomarker: a biomarker that is evoked from a sample after it has been removed from an animal or a human. This may represent a newly phosphorylated protein or newly expressed mRNA, among other dynamic molecules
- Modulator: an agent (e.g., a molecularly targeted agent) that is applied to sample after it has been removed from an animal or a human to block or modify an *ex vivo* biomarker response to a stimulus
- Pharmacodynamic biomarker: a biomarker that determines whether a drug is having the desired effect on a molecular target or pathway
- Predictive biomarker: a biomarker that determines whether a person’s cancer will respond to a specific treatment
- Prognostic biomarker: a biomarker that assesses an individual’s likelihood of disease progression or death
- Signaling profile: a collection of molecular features from a tumor’s signal transduction network that defines a specific group with known biological behavior
- Stimulus: an agent (e.g., granulocyte–macrophage colony-stimulating factor or EGF) that is applied to sample after it has been removed from an animal or human to evoke a measurable response through an *ex vivo* biomarker

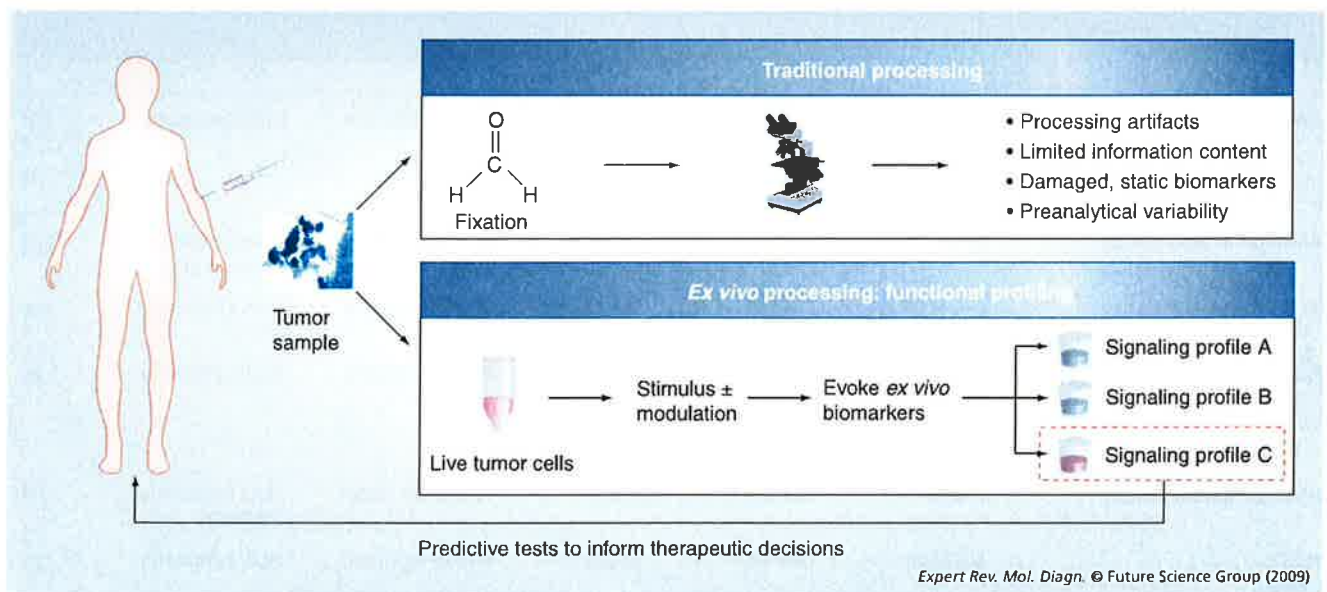


Figure 1. Ex vivo biomarker approach to functional profiling to guide cancer therapy. Live tumor cells are procured from a patient and are exposed to a stimulus, such as a growth factor, sometimes in the presence of a modulator like a drug, to evoke ex vivo biomarkers. These biomarkers are then assembled into a signaling profile that provides functional information about the tumor that is not available using traditional processing. Such functional profiles are then used to inform drug development and therapeutic decisions. Adapted with permission from [102].

have been developed [20]. Each involves either short- or long-term *in vitro* culture of a tumor sample, usually from a surgically excised tumor (or ascites), exposure to chemotherapeutic drugs, and analysis of a metric of sensitivity or resistance. These assays have measured a variety of outcomes, including colony formation in soft agar, differential staining of viable and dead cells, metabolic activity, or uptake of tritiated thymidine. Each of these is essentially a direct measure or surrogate for tumor cell proliferation or cell viability in the presence of cytotoxic drugs. Despite the appeal of this approach, there are significant limitations. As summarized by an ASCO technology assessment of CSRAs:

"Limitations in the literature include small sample sizes, the lack of prospective studies, the generally low yield of assays, and the availability of newer chemotherapy and biologic agents since the advent of these trials" [21].

These limitations prompted ASCO to determine that the use of CSRAs was not recommended outside the clinical trial setting. However, the CSRA approach has the:

"...opportunity for further research, particularly with newer therapies."

One intriguing variation involves the propagation and drug testing of patient tumor samples in murine xenografts. This has been undertaken by several groups using pancreatic and other cancers [22]. Such an approach creates valuable research and drug development tools that may more accurately represent the spectrum of human tumors than collections of cell lines; however, this approach is unlikely to be sufficiently cost effective for widespread clinical application.

Ex vivo analysis of hematopoietic malignancies

The concept that *ex vivo* stimulation of tumor cells can reveal functional or mechanistic features of patient samples that are clinically relevant has been extensively pursued by the Nolan laboratory, among others [23]. The Nolan laboratory has optimized protocols for the *ex vivo* analysis of signal transduction network function in hematopoietic cells using phosphoprotein-specific antibodies and flow cytometric analysis [24]. Similar to many molecular studies on human tumors, progress in this area has been facilitated by the relatively easy access to homogeneous, abundant populations of blood-based tumor cells, the single-cell nature of the tumor, and the availability of a clinically available testing platform (flow cytometry). Using these techniques, this laboratory has studied the signaling network profiles in patient samples of several hematologic malignancies, including AML, follicular lymphoma, juvenile myelomonocytic leukemia (JMML), and other myeloproliferative disorders (MPDs; TABLE 1). In studies of AML this group found heterogeneity among patients in the signaling profiles generated by *ex vivo* granulocyte colony-stimulation factor stimulation and p-STAT3 or p-ERK1/2 expression [25]. Biosignatures derived from this analysis enabled the grouping of AML patients that correlated with known clinical prognostic markers. In another study of AML patients, distinctive *ex vivo* STAT and MAPK signaling signatures were associated with a subset of patients with high levels of Bcl-2 expression and a specific mutation in Flt3 [26]. In addition, *ex vivo* induction of DNA damage using idarubicin revealed that wild-type p53 was phosphorylated following DNA damage, suggesting a blockage of apoptosis downstream of p53. In an analysis of pediatric MPD patients suspected of having JMML, the *ex vivo* response of

Table 1. Summary of published predictive *ex vivo* biomarkers.

Tumor type (source)	Biomarker	Stimulus	Inhibitor	Molecular class	Detection	Ref.
AML (peripheral blood)	p-STAT1,3,5 p-ERK1/2	G-CSF GM-CSF IL-3 IFN- γ	None	Phosphoprotein	Flow cytometry	[25]
AML (peripheral blood)	p-ERK1/2	PMA	CI-1040	Phosphoprotein	Flow cytometry Western blot	[29]
AML (peripheral blood)	p-ERK1/2	PMA SCF	Sorafenib	Phosphoprotein	Flow cytometry	[30]
Follicular lymphoma (lymph node)	p-Btk p-p38 p-Syk p-ERK1/2	α -IgM/IgG F(ab') ₂ + H ₂ O ₂	None	Phosphoprotein	Flow cytometry	[28]
AML (peripheral blood)	p-p53	Idarubicin	None	Phosphoprotein	Flow cytometry 2D PAGE	[26]
JMML CMML M4/M5 AML (bone marrow, peripheral blood)	p-STAT5	GM-CSF	XL019	Phosphoprotein	Flow cytometry	[27]
Cholangiocarcinoma cell line (xenograft FNA)	FOS	EGF	Erlotinib	mRNA	RT-PCR	[33]
Pancreatic cancer (xenograft FNA)	p-ERK1/2	FCS	CI-1040	Phosphoprotein	Western blot	[22]
Pancreatic cancer (xenograft FNA, human FNA)	p-S6 RP p-ERK1/2	FBS	Temsirolimus Erlotinib	Phosphoprotein	Western blot	[34]
Pancreatic cancer cell lines; pancreatic cancer (xenograft FNA)	Cyclin B1	FBS	ON 01910.Na	mRNA	RT-PCR	[35]

AML: Acute myeloid leukemia; CMML: Chronic myelomonocytic leukemia; FBS: Fetal bovine serum; FCS: Fetal calf serum; FNA: Fine-needle aspiration; G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; JMML: Juvenile myelomonocytic leukemia; M4/M5 AML: Myeloid leukemia with myelomonocytic and monocytic differentiation; PMA: Phorbol 12-myristate 13-acetate; RT: Real time; SCF: Stem cell factor; XL019: Oral JAK2 inhibitor (Exelixis).

p-STAT5 to granulocyte-macrophage colony-stimulating factor enabled the distinction of true JMML patients from clinically similar MPDs [27]. These patients had a hypersensitive response relative to normal bone marrow or other MPDs. This signature was also found in the similar adult diseases, chronic myelomonocytic leukemia and M4/M5 AML. Using a different combination of stimuli and phosphoprotein responses related to B-cell receptor stimulation (TABLE 1), samples of follicular lymphoma were shown to differ from nontumor B cells from the same patients [28]. In applying this assay to lymphoma cells, B-cell receptor signaling occurred more rapidly, achieved higher levels and was sustained for longer than in nontumor B cells.

Two other groups have used the flow cytometric phosphoprotein analysis method as an *ex vivo* pharmacodynamic tool to assess the effects of targeted therapies on patient samples. In one example, the MEK inhibitor CL-1040 was applied to cells that were stimulated *ex vivo* with phorbol 12-myristate 13-acetate and assessed for ERK1/2 phosphorylation using flow cytometry [29]. AML samples contained high basal levels of p-ERK relative to normal bone marrow and *ex vivo* treatment of AML samples with CL-1040, significantly reduced p-ERK levels and was associated with growth arrest. In a second study of the raf and c-kit

inhibitor, sorafenib, in AML, a pharmacodynamic assay involving *ex vivo* stimulation of AML cells with stem cell factor and flow cytometric analysis of p-ERK revealed marked c-kit inhibition using high doses of sorafenib [30].

Ex vivo signaling profiles in solid tumor samples from patients & biopsies of xenografts

Hematologic malignancies lend themselves to *ex vivo* manipulation and analysis because of their single-cell nature and relative abundance of tumor cells in easily obtained clinical samples, such as peripheral blood and bone marrow aspirates. On the other hand, solid tumors present several technical challenges to *ex vivo* analysis. As with any molecular diagnostic test based on a sample of a tumor (indeed, no tissue-based molecular diagnostic test is based on an entire tumor), the impact of tumor cell heterogeneity on the test must be considered. Solid tumors have cell-cell junctions composed of adherens junctions, tight junctions, gap junctions and/or desmosomes, which result in variable degrees of cell clustering. In addition, solid tumor cells also express cell adhesion molecules, such as integrins, that mediate their association with components of extracellular matrix, such as collagen. Dissociation methods are available to

facilitate single-cell suspension of solid tumor cells but these typically employ harsh treatments, such as vigorous mechanical disruption, or protease and collagenase treatments, which may impact the biology of the disrupted cells. Consequently, analysis platforms other than flow cytometry may be more suitable for *ex vivo* analysis of solid tumors. Circulating tumor cells (CTCs) represent a theoretical source of isolated tumor cells for *ex vivo* analysis; however, they present several challenges. The effect of various isolation methodologies on the cell biology and signal-transduction pathways of CTCs will have to be studied carefully. For many tumor types, the number of obtainable CTCs may be too limited for *ex vivo* analysis. Finally, similar to any molecular test based on CTCs, the relevance of the CTCs to the actual tumor must be studied to ensure that the CTC subset is representative of the entire tumor.

Another challenge presented by solid tumors is their heterogeneous cellular composition. While leukemic cells may be obtained from peripheral blood relatively free of any contaminating cells, solid tumors are much more heterogeneous, containing variable amounts of infiltrating lymphocytes, histiocytes, fibroblasts and vessels [31]. Symmans *et al.* have shown that FNA biopsy samples of primary breast cancers are relatively enriched for tumor cells relative to needle core biopsies of the same lesions [32]. Consequently, the FNA biopsy procedure itself may provide some tumor cell enrichment of samples; however, this needs to be further studied. In addition, the mechanical effects of the FNA procedure on live cells that are being cultured *ex vivo* warrants further study. Despite this, FNA has proven to be one effective means for obtaining live solid tumor cells from murine xenografts or individual patients for *ex vivo* analysis.

Despite technical challenges, several groups have successfully utilized *ex vivo* analysis of solid tumors to predict responsiveness to targeted therapies (TABLE 1). In one study of human cholangiocarcinoma xenografts, FNA samples of these tumors were stimulated *ex vivo* with EGF and subsequently examined for *FOS* mRNA expression as a biomarker of EGFR pathway activation [33]. *FOS* is an immediate early gene whose expression is rapidly upregulated in response to growth factor stimulation and is mediated by ERK, among other upstream signaling molecules. In this study, FNA samples of CAL27 xenografted tumors were stimulated with EGF *ex vivo*, in the presence or absence of the EGFR antagonist erlotinib. *In vivo* growth curves of CAL27 reveal that this tumor is sensitive to erlotinib treatment. This is predicted in the *ex vivo* setting, where erlotinib successfully abrogated the *FOS* upregulation seen with EGF stimulation. A second study employed xenografts derived from primary pancreatic cancers and p-ERK as a biomarker of pathway activation in the presence of the MEK inhibitor CI-1040 [22]. All three tumors tested showed *ex vivo* pharmacodynamic evidence of CI-1040 activity, as shown by blockage of p-ERK activation. However, only one of the three tumors was susceptible to CI-1040 tumor growth inhibition *in vivo*, representing a disconnection between the pharmacodynamic activity and the proliferative response. This work was extended in another study to a panel of eight different primary pancreatic xenografts as well as three human

pancreatic carcinoma FNA samples [34]. In this study, FNA samples were treated *ex vivo* with erlotinib and then assayed for its effect on pERK expression or treated *ex vivo* with the mTOR inhibitor, temsirolimus, and then assayed for its effect on its downstream phosphorylation target, p-S6-RP. The *ex vivo* erlotinib and temsirolimus assays successfully predicted the *in vivo* response to erlotinib and temsirolimus in all cases. Jimeno *et al.* used a similar *ex vivo* approach to develop predictive *ex vivo* biomarker tests for the polo-like kinase inhibitor ON 01910.Na [35]. Of the candidate biomarkers studied, cyclin B1 mRNA evaluation in the presence or absence of inhibitor *ex vivo* was the best predictor of the *in vivo* response. Specifically, in a panel of nine different pancreatic xenograft FNAs the cyclin B1 *ex vivo* assay correctly predicted two out of two sensitive tumors and six out of seven resistant tumors.

Future challenges & opportunities

In considering the future of *ex vivo* biomarkers, it is useful to put them in the context of the developmental pathways recently proposed by the NCI's Translational Working Group, specifically the Developmental Pathway for Biospecimen-based Assessment Modalities [36]. First, it is important to define the clinical goals that are driving *ex vivo* biomarker development. One obvious goal is the development of prognostic tests to determine outcome, and tests predictive of response to targeted therapies. In addition, *ex vivo* biomarkers offer the possibility of contributing to the drug development process. For example, *ex vivo* biomarkers from model systems, such as murine xenografts, could assist with drug target validation and lead agent optimization [37]. In clinical trials, *ex vivo* biomarkers could provide valuable mechanistic information or identify potential responder subpopulations.

Once specific *ex vivo* biomarker goals have been defined, a credentialing step is necessary to determine which fundamental research observations yield candidate *ex vivo* biomarkers with clinical potential. These will typically include molecules downstream of drug targets that reflect pathway activation or a specific biological process within the cell, such as apoptosis. There are already dozens of molecular targets for which there is an approved or emerging MTA. Many of these targets fall into the ErbB signaling pathway or other receptor tyrosine kinase pathways, but also included are such diverse targets as apoptosis-associated molecules and kinases that regulate the cell cycle. As noted previously, only EGFR, HER2 and *KRAS* have commercially available, validated predictive biomarkers, exposing a clear unmet need. *Ex vivo* biomarkers can be considered in two broad classes: phosphoproteins and mRNAs. Obvious phosphoprotein candidates include effector molecules produced during signal transduction in pathways, such as MAPK, JAK-STAT and AKT/mTOR, while others regulate key cellular processes, such as apoptosis, the cell cycle or cellular metabolism. The mRNA candidates could be considered in three major classes: the immediate responders to signal transduction activation, delayed responders that regulate signal transduction, and effectors of cellular responses such as the cell cycle.

The next step in the developmental pathway is the identification of technical challenges that might require new enabling technologies. For hematopoietic malignancies, the use of flow cytometric instrumentation and reagents is already well established in both research and clinical settings, which represents an obvious technology platform to utilize for *ex vivo* analysis of lymphoma and leukemia. The *ex vivo* analysis of solid tumors is associated with various additional challenges. Solid tumor samples are typically frozen or fixed immediately upon procurement, which eliminates the opportunity for *ex vivo* analysis. To date, most *ex vivo* biomarker studies on solid tumors have been performed through manual manipulation of the sample by technicians at the point of biopsy. Widespread implementation and standardization of *ex vivo* biomarker tests will require the development of automated specimen-handling platforms. In addition, in order to utilize specimens that can be obtained from minimally invasive biopsies, such as FNAs, analytical technologies that are highly sensitive and permit multiplexing will be required.

In order to move credentialed *ex vivo* biomarkers forward, an assessment modality, or biomarker assay, will then need to be developed. For traditional biomarkers this often utilizes archival specimens for retrospective studies. Such archival specimens are not an option for *ex vivo* biomarkers since they require live cells. Model systems, such as murine xenografts, may have to be utilized instead to analytically validate biomarker assays. Once this analytical validation is complete the biomarkers are ready for clinical validation in clinical trials.

The use of any type of molecular biomarker in oncology drug development and clinical decision-making has been met with both enthusiasm and skepticism. Skeptics point to historical failures of biomarkers to improve the efficiency or efficacy of early-phase clinical trials [38]. The paucity of oncology drug–diagnostic combinations that are required or recommended by the US FDA point to deficiencies in biomarker integration into late-stage clinical trials and clinical practice [39]. Possible explanations for these failures include pre-analytical instability of biomarkers within biospecimens, imprecise analytical tests and flawed study design and statistical analysis. Enthusiasts of biomarkers argue that analytically validated biomarkers used on carefully handled biospecimens offer the possibility of useful pharmacodynamic information and patient selection through predictive biomarkers [40]. Economic modeling suggests that biomarkers may enable an increase of pricing and a growth in market share for pharmaceutical companies [41]; however, this may require better alignment of the drug development and diagnostic industries. Ultimately, the realization of biomarker enthusiasm may be limited by the very nature of biomarkers derived from dead, fixed tissue. Indirect assessments of biological function through measurement of individual, static molecules may not contain sufficient biologically relevant information, or may be too inferential, to provide predictive value. The successful predictive tests of the future may require functional analysis of signal transduction networks through the sophisticated testing of *ex vivo* biomarkers.

Expert commentary

Molecularly targeted agents have rapidly become the focus of cancer drug development and clinical therapy. The rational development and personalized application of MTAs to cancer patients will rely on molecular biomarkers. Such biomarkers should provide information about the presence of the drug target and also the functional cell circuitry surrounding the target. Unfortunately, we currently have too few biomarkers to accurately identify targets and virtually none that reveal functional profiles of tumors. This is partly owing to a reliance on an anatomic pathology infrastructure that is rooted in the distant past and designed to facilitate the microscopic analysis of tumors rather than their molecular analysis. In fact, current tissue handling and processing methods based on formalin-fixation and paraffin-embedding techniques relieve samples of information at every step. Killing the cells through fixation eliminates the possibility of gathering valuable functional information from the tumor cells. The emerging utilization of *ex vivo* biomarkers will provide important functional information to guide drug development and therapeutic choices. This will probably begin in lymphoma and leukemia and then be extended to solid tumors. Such progress is essential but may require significant changes in the existing pathology infrastructure.

Five-year view

Optimistically, in 5 years, oncologists and their patients will have many more approved indications for the use of existing MTAs, more approved MTA combinations and access to between ten and 25 new approved MTAs. Scientists will have discovered virtually every possible mutation within the genomes of the most common cancers and the field of tumor cell-signal transduction will have advanced, largely owing to progress in bioinformatics and systems biology. Our knowledge regarding biospecimens will also have advanced to provide a better foundation for the application of cancer biology knowledge. Technological advances will be made enabling vast amounts of molecular information to be gathered from small tumor samples. This will motivate pathologists, oncologists and other healthcare providers to begin to transform the system utilized to characterize biopsy samples. Every tumor will demand a comprehensive molecular analysis that will include functional profiling of live cells to individualize the patient's therapy. For the most common cancers, a patient's therapy will increasingly be monitored for success early in the course of treatment through minimally invasive biopsies that are coupled with robust biomarkers. We will, indeed, be practicing personalized cancer care.

Financial & competing interests disclosure

Douglas P Clark is entitled to a share of equity as a founder of BioMarker Strategies, LLC and is currently an officer for the company. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Key issues

- Molecularly targeted agents have become the major focus for oncology drug development and therapy.
- Molecular biomarkers are essential to guide the development and therapeutic selection of molecularly targeted agents.
- The ideal biomarkers reveal the presence of the molecularly targeted agents target, as well as the functional cellular circuitry surrounding the target.
- There are currently an insufficient number of biomarkers for cancer detection.
- *Ex vivo* biomarkers are functional biomarkers that are evoked from live tumor cells after they have been removed from a patient.
- *Ex vivo* biomarkers may be newly expressed phosphoproteins, mRNAs or other molecules that reveal details of an individual tumor's signal transduction network.
- *Ex vivo* biomarkers may be evoked by stimuli, such as growth factors, including granulocyte–macrophage colony-stimulating factor and EGF.
- *Ex vivo* biomarkers in leukemias and lymphomas may be monitored through flow cytometric platforms, but solid tumor analysis may require novel technology platforms.
- The functional signaling profile derived from individual tumors promises to be an essential tool to guide oncology drug development and therapy.

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