PERSPECTIVE

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# Functional precision cancer medicine—moving beyond pure genomics

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**The essential job of precision medicine is to match the right drugs to the right patients. In cancer, precision medicine has been nearly synonymous with genomics. However, sobering recent studies have generally shown that most patients with cancer who receive genomic testing do not benefit from a genomic precision medicine strategy. Although some call the entire project of precision cancer medicine into question, I suggest instead that the tools employed must be broadened. Instead of relying exclusively on big data measurements of initial conditions, we should also acquire highly actionable functional information by perturbing—for example, with cancer therapies—viable primary tumor cells from patients with cancer.**

'Precision medicine' is a hot phrase these days—its use has gone beyond the biopharmaceutical and academic medical center communities into the lay press and political discourse. Everyone agrees that it is a good thing and that more of it is needed. However, it is important to understand what is meant by precision medicine if investment in it is going to be increased. For the purposes of this Perspective, I define precision medicine as the process of matching an individual patient with the medicines that are best for them using any method. In cancer precision medicine, this usually means identifying the treatment(s) that will best decrease tumor size or eradicate the patient's cancer.

How has precision cancer medicine been doing recently? Many new initiatives have embraced it, including the US federal government's 2016 Precision Medicine Initiative, as well as the Cancer Moonshot effort championed by former Vice-President Joe Biden. Most major cancer centers have an institutional precision medicine program. However, some investigators have expressed grave concerns about the use of precision medicine in cancer treatment, responding to recent reports of precision medicine trials demonstrating a level of patient benefit that is disappointingly low[1–6]. Given the significant public and private investment in precision cancer medicine, this seems to be a good time to examine what tools have been used in precision cancer medicine, what results have been obtained, and what opportunities there are for improving precision cancer medicine in the future.

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## Is precision medicine equivalent to genomic medicine?

Precision cancer medicine, as it is practiced today, is nearly synonymous with 'genomic medicine' (refs. 5,7). A scan of presentation titles at the upcoming 2018 Keystone Precision Medicine in Cancer meeting provides a good example: at least 24 of the 27 listed presentations apparently involve genomics. This is typical of recent meetings on cancer precision medicine. The website describing the association of the National Cancer Institute (NCI) with the Precision Medicine Initiative (see URLs) states: "Precision medicine uses the genetics of disease to identify effective therapies, and, thanks in large part to NCI-supported research, we know that cancer is a disease of the genome." The website refers to genomics alone as the tool to be deployed for precision medicine in cancer treatment. No functional approaches are mentioned.

The fundamental idea in genomic medicine is that somatic genetic alterations (including point mutations, deletions, amplifications, translocations, and quantitative chromosomal abnormalities) can be identified and matched with drugs targeting those abnormalities for a patient's benefit. What success has this approach attained? In some cases, large groups of patients with tumors bearing a particular mutation have been treated very successfully with a single drug. The first example was the use of imatinib to treat patients with chronic myeloid leukemia (CML) bearing the t(9;22) translocation that creates a BCR-ABL fusion kinase[8]. Not only were lives saved and clinical practice in CML dramatically changed, but a new translational paradigm was also born in cancer research: cancer biologists would identify somatic genetic alterations, drugs would be made to target those cancer-specific alterations, and cancer would thus be controlled. In subsequent years, there have been numerous other successes via identification of somatic mutations in cancers that were then targeted with drugs. In just two prominent examples, lung cancers bearing mutant epidermal growth factor receptor (EGFR) usually respond to EGFR inhibitors, and melanomas bearing mutated BRAF usually respond to BRAF inhibitors[9–12]. More recently, an important opportunity for immune checkpoint blockade was identified in classical Hodgkin's lymphoma through genetic means[13,14], and microsatellite instability was suggested as a marker for sensitivity to checkpoint blockade in colon cancer[15]. Although it is true that the benefit realized in most cases is less complete and less durable than was the case for imatinib treatment of CML, this should not be interpreted as a failure of genetics as a predictive biomarker. Clinical genetics achieved the goal of precision medicine in these cases by identifying a drug that was better than anything else available for these patients.

There are also moving stories of individual patients for whom a targeted drug produced exceptional, life-changing responses when relevant tumor-specific somatic mutations were identified[16–18]. So it is clear that using clinical genetics and genomics for precision medicine has provided

tremendous benefit and saved lives for select patients with cancer[19]. However, I want to convince the reader that using genomics alone to do the job of precision cancer medicine is a big mistake. The problem is that when exceptional responders and somatic tumor mutations are reported, for instance, the numerator (cases of successful treatment) is considered but not the denominator (total cases treated with a genetic approach). For every patient with lung cancer harboring a targetable alteration in EGFR or ALK, there are four who lack such an indicator[20]. Indeed, although tens of thousands of patient tumors have been studied with some kind of genomic platform, some have suggested that there are only dozens of these types of exceptional responses reported, which are often held up as a paradigm[3]. Simply put, most patients with cancer do not currently benefit from a genomic approach.

Although one can easily find impressive rates of identification of 'actionable' mutations by genomic panels, evidence of actual clinical benefit to patients with cancer is harder to come by[3,6,21–24]. Where clinical benefit has been identified, it has so far been on the basis of results from non-randomized retrospective analyses and, even then, is relatively modest[25–27]. The recent report from one randomized controlled trial on the therapeutic benefit of a 'basket trial' approach is sobering[28]. The SHIVA trial was a prospective, randomized, controlled phase 2 clinical trial at eight French academic centers. There were 741 tumor samples from patients with advanced solid tumors screened for genetic alterations. Of these tumors, 40% bore alterations in genes mapping to a pathway that could be linked to putative activity of a molecularly targeted agent. Patients with such tumors were randomized and received either a molecularly targeted agent as directed by genetic tests (experimental group) or a treatment of the physician's choice whose selection was not guided by genetic tests (control group). The primary outcome was progression-free survival, which did not differ between the experimental and control groups (2.3 versus 2.0 months, $P = 0.41$). The study had limitations, such as the handling of simultaneous mutations that might be expected to render monotherapy ineffective and the use of certain targeted agents that might not currently be considered optimal[29]. The authors nonetheless concluded that "the use of molecularly targeted agents outside their indications does not improve progression-free survival compared with treatment at physician's choice in heavily pretreated patients with cancer." They reasonably suggest that continued enrollment in clinical trials to test predictive biomarkers is important. It is also imperative that these results temper the expectations of genomic precision medicine approaches in cancer, both in conversations with individual patients and public policy discussions.

Other trials have not yet evaluated clinical benefit, but have assessed the rate of assigning patients to targeted therapies via genomic approaches. The Molecular Analysis for Therapy Choice (NCI-MATCH) trial released an interim analysis in May 2016, which showed that there was good analytical performance of their genetic screening strategy, with testing completed in 87% of the 739 samples submitted (see URLs). However, only 9% of patients for whom testing was completed had tumors that bore a mutation that could direct them to one of ten targeted therapy arms, and only 2.5% actually entered a treatment arm (**Fig. 1**). Reasons for the discrepancy between the percentage of patients with putative actionable mutations and the percentage of patients who actually underwent treatment include ineligibility (possibly including inadequate performance status, laboratory abnormality, or excluded prior therapy), starting other treatment, disease progression, and death. In response to the issues identified in this progress report, the NCI-MATCH trial has proceeded with modifications. In a study at the University of Texas MD Anderson Cancer Center, 2,000 patient tumors were tested with multiplex platforms containing 11, 46, or 50 genes. Of these tumors, 789 (39%) harbored potentially actionable

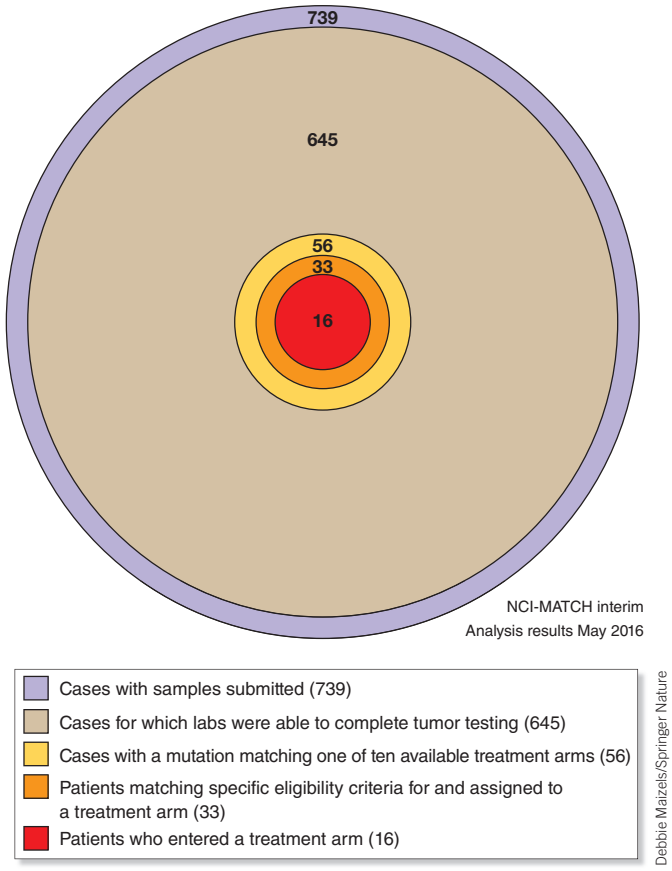


**Figure 1** NCI-MATCH interim analysis as of May 2016.

alterations, but only 83 (4.2%) of the corresponding patients enrolled in a genotype-matched trial[30]. Clinical response was not reported. A more recent report from the Dana-Farber Cancer Institute indicates that, of 3,727 patient tumors successfully tested with a hybrid capture and massively parallel sequencing assay including 282 genes, 73% harbored an "actionable or informative alteration" (ref. 22). Included in this paper are individual cases in which clinical benefit appears clear, but no systematic evaluation of clinical benefit is reported.

The identification of actionable mutations is presented as an important metric of success in many genomic ventures, both commercial and academic. The careful reader will note that actionable is often not clearly defined within reports. Moreover, there are many problems in attaining a stable, standard definition for this term across different studies. What actionable means often depends on how the mutation is identified, how frequent it is in the tumor, the histologic context, whether the perspective is that of the patient, the clinician, or the cancer biologist, and what treatments are readily available[31,32]. Some have suggested further qualification of actionable mutations into tiers generally on the basis of the likelihood that the mutation truly identifies a vulnerability that is exploitable by a drug to induce clinical response[31–36]. Intentionally or not, the use of 'actionable mutation' is often misleading. A plain-English reading of actionable mutation would lead one to assume that what was being identified was a mutation for which there was a specific drug available that a doctor could actually give the patient with a high likelihood of inducing a response. As indicated by the clinical studies mentioned above, this is usually not the case.

Of course, the lack of good drugs is partly responsible for the fact that genomic-based cancer precision medicine has so far benefited a small

**Table 1 Comparison of functional precision medicine methods**

| Method | Number of drugs testable | Model cost per patient sample | Incremental cost per drug tested | Time until result | Specialized equipment/procedure |
|---|---|---|---|---|---|
| 2D culture | High (thousands) | Low | $ | Days to weeks | No |
| 3D culture | High (thousands) | Medium | $ | Weeks to months | No |
| PDX models | Low (up to a dozen) | High | $$$ | Months | Yes |
| Dynamic BH3 profiling | High (thousands) | Low | $ | Hours to days | No |
| Mass accumulation rate | Low (up to a dozen) | Low | $ | Days | Yes |
| Implantable devices | Medium (dozens) | High | $ | Days | Yes |
| CIVO | Medium (dozens) | High | $ | Days | Yes |

Values are the author's best estimates based on published information.



minority of patients with cancer. There simply are not that many potent, tolerable targeted agents in the clinic that can induce significant clinical responses. An increase in this number will make any predictive biomarker—genomic or otherwise—perform better as a clinical tool. One reason for the limited number of drugs, however, may be the reliance of precision medicine on genetics to define targets. The requirement that drugs be assignable via reference to specific genetic abnormalities is inherently limiting and highlights a major weakness of genomically driven precision medicine in drug development.

If one reflects on the history of medical oncology, the majority of the most effective drugs do not function by exploiting genetic mutations in individual tumors. Curative chemotherapy regimens in leukemia, lymphoma, and testicular cancer were discovered without any reference to genetic alterations. Indeed, one can wonder whether adoption of these curative regimens might have been slowed rather than quickened if today's capacity for and dependence on assaying the genome were available in earlier decades, given the paucity of genomic predictive biomarkers associated with standard cancer therapy. Consider the very modern example of chronic lymphocytic leukemia (CLL), in which new targeted agents are rapidly changing clinical practice at all stages of the disease. Drugs targeting CD20, phosphoinositide 3-kinase (PI3K), Bruton's tyrosine kinase (BTK), and B cell lymphoma 2 (BCL-2) have all received US Food and Drug Administration (FDA) approval in the last few years[37]. In addition to excellent clinical activity, what these agents have in common is that they target products of genes and members of pathways for which somatic genetic alterations have not been identified in CLL. In all of these cases, drugs are exploiting biology that is driven by the B cell lineage of the cell of origin or by properties of oncogenesis that are not captured by genomics. It seems a reasonable conjecture that similar, nongenetic vulnerabilities exist in many other cancers, which will require nongenetic means to be identified.

In support of this conjecture, a recently published unbiased investigation across 501 diverse cancer cell lines identified 769 vulnerabilities using RNA interference (RNAi)[38]. The researchers looked for predictive factors associated with these 769 vulnerabilities using unbiased nonlinear regression against many features, including genetics and gene expression, of this heavily annotated group of cell lines. Only 289 (38%) vulnerabilities could be paired with a static predictive marker. Of these 289, only 18% had genetic predictive markers. This means that, in a broad and unbiased investigation of vulnerabilities in cancer cells, <7% of the vulnerabilities had a genetic predictive biomarker. This result puts a sobering rough upper bound on expectations for the population-wide utility of genomic-based precision cancer medicine. To reach a point at which precision medicine routinely matches the majority, rather than the minority, of patients with cancer to an effective drug, approaches that detect not just somatic cancer mutations but rather somatic cancer vulnerabilities must be pursued.

## Functional precision cancer medicine

Functional precision cancer medicine has the potential to be a powerful ally to current genomic approaches. The unifying principles behind functional precision cancer medicine are that perturbing a cancer cell will yield important information that is difficult to capture by static measurements of initial conditions and that the most important perturbation is exposure to the cancer drug.

The first principle is one that is backed by abundant experience in many sciences. In simple systems, one can often predict the future behavior of the system by measurements of initial conditions. For instance, one might measure the height at which a ball is dropped and use straightforward Newtonian mechanics to make a very accurate prediction of its velocity when it hits the ground. However, in complex systems, prediction of evolution of the system over time following a perturbation may be very difficult. The behavior of systems containing even just three interacting bodies is extremely hard to predict from initial conditions. In this respect, it is worth noting that intracellular signaling responses to drugs can be very complex and involve vastly more than three interacting molecules.

In complex systems, therefore, an alternative approach often employed in physical sciences is simply to perform a relevant perturbation in the system and carefully measure what happens next. Consider trying to predict what will happen if you poke a dog with a stick. To draw an analogy from this scenario to current approaches in precision medicine, we might kill the dog, sample its tissues for genomic, proteomic, and metabolomic measurements at the initial condition, and somehow use these terabytes of analytically accurate information to make a prediction. The functional approach is to poke the dog with a convenient stick and see what happens. There are far fewer bits of data in the latter approach, but the data acquired are exquisitely relevant and actionable. Note that, in functional approaches, the 'stick' used to poke cancer cells may be composed of more than one drug so that responses to combinations of drugs can be directly observed, providing a distinct advantage over genetic approaches.

The practical questions for functional precision medicine are the following: how does one apply the drug to the tumor cell, and what does one measure after drug exposure? I outline a few different approaches to resolving these questions (**Table 1**). None of these approaches have yet completed prospective testing in clinical trials, but either singly or in combination they offer a way forward for assigning effective therapies to patients with cancer, even when somatic genetic indicators are not available.

## Aggregate cell-based measurements in 2D culture

For decades, the gold-standard test for assigning antibiotics to patients has relied on *ex vivo* culture of the bacterium followed by direct exposure of the microbe to a panel of antibiotics and subse-

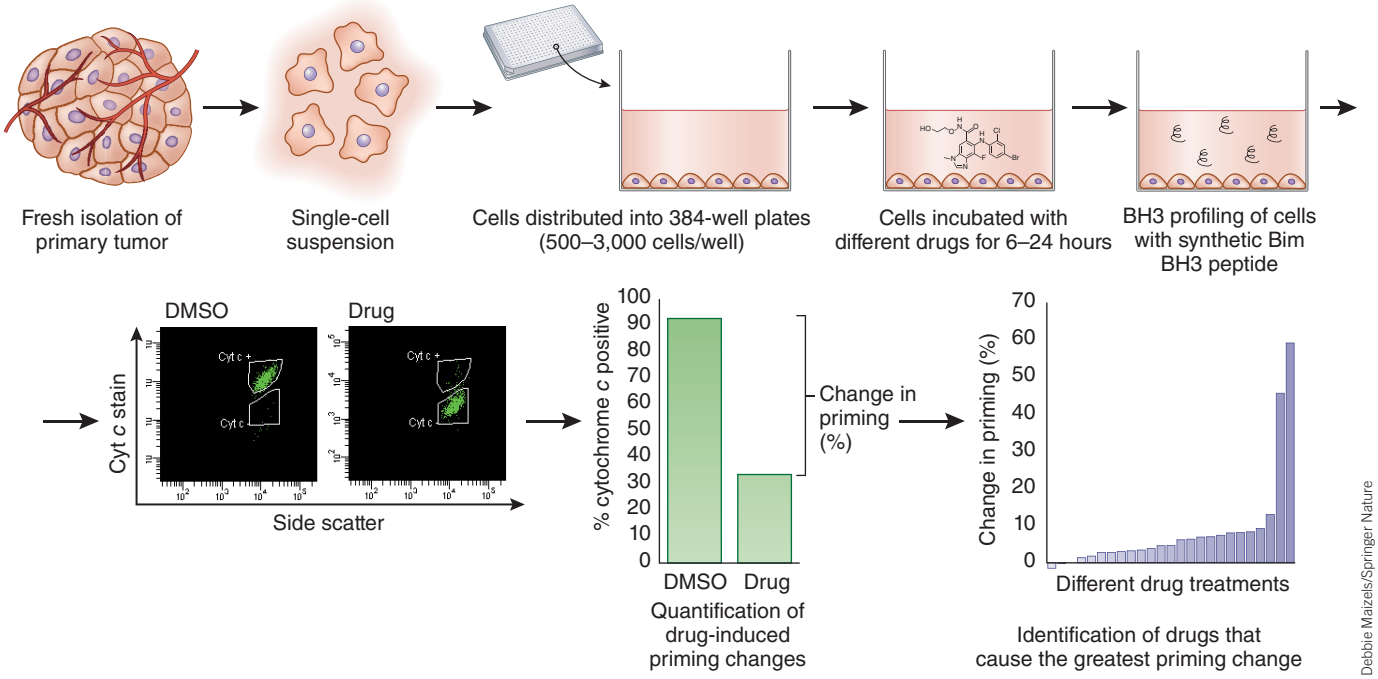


**Figure 2** Dynamic BH3 profiling. In dynamic BH3 profiling, a single-cell suspension is made from tumors and distributed into the wells of a 384-well plate without further purification. Cells are exposed to a panel of up to thousands of drugs of interest, including combinations. After 6–24 hours of exposure, BH3 profiling is performed. Cells are gently permeabilized with digitonin, and BH3 peptides are added for 30–90 minutes. Cells are then fixed with paraformaldehyde and immunofluorescently stained for loss of cytochrome *c* (cyt *c*). Additional stains to discriminate tumor from stroma as well as to distinguish heterogeneous cancer subpopulations can be added. Cytochrome *c* staining is then evaluated in tumor cells via FACS (for non-adherent cells) or microscopy (for adherent cells). When BH3 peptide–induced loss of cytochrome *c* (MOMP) is augmented by drug treatment in particular wells, this indicates that these wells contain active drugs that induce proapoptotic signaling in tumor cells. Protocols and technical details are available (see URLs).

quent measurement of bacterial growth inhibition. Analogous efforts in the *ex vivo* culture of tumor cells and exposure to antitumor agents have been challenged by the difficulty of *ex vivo* culture of primary tumor cells and the nonspecific nature of many of the readouts. Many of these readouts measure metabolites or metabolic activity, such as MTT assays, [$^3$H]thymidine incorporation, or ATP abundance. As such, they are not designed to distinguish among cell death, cell cycle arrest, and adaptive variations in metabolism. Although prospective and retrospective comparison of assay results to clinical results has in some cases found correlations with some aspects of clinical response[39,40], the guidance provided by these standard *ex vivo* chemosensitivity assays was not considered sufficient for their application to be recommended for clinical use outside of clinical trials according to an American Society of Clinical Oncology clinical practice guideline published in 2011. This was the last time that organization visited this issue.

However, some investigators have revisited the value of simply exposing primary cancer cells to a drug to detect the drug's effect on cells in 2D culture. At the Oregon Health & Science University, investigators examined their ability to measure drug response based on *ex vivo* exposure of primary leukemia cells from patients to a panel of targeted therapies that originally numbered 66 but currently numbers over 170 (J. Tyner, Oregon Health & Science University, personal communication). They used standard colorimetric measurements of viable cell number to evaluate drug effect and demonstrated the ability to identify alterations in patient cell viability induced by the drug panel across 151 patient samples. Use of this *ex vivo* assay identified activity of kinase inhibitors in at least 70% of cases, whereas only 13% of cases could be assigned to a kinase inhibitor on the basis of genetic and/or cytogenetic findings (*BCR-ABL* or *FLT3-ITD* fusion or point mutations in *FLT3*, *KIT*, *JAK2*, or *MPL*). Moreover, they have reported examples supporting the ability of this system to predict clinical response[37,39]. Their approach to predicting clinical response to therapy is currently being tested in prospective, but not randomized, clinical trials in acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and CLL (NCT02779283, NCT01620216, and NCT01441882, respectively).

Wennerberg and colleagues[41–43] in Finland have used a similar *ex vivo* testing approach, again mainly in the study of leukemias. In their Drug Sensitivity and Resistance Testing (DSRT) platform, they employ luminescent measurements of cell viability in combination with an algorithm based on the area under dose–response curves to more specifically identify sensitivity to individual drugs in cancer cells in comparison to control normal, nonmalignant mononuclear blood cell samples. A similar approach to the study of drug sensitivity in ALL has been reported recently by Frismantas and colleagues[44] in Zurich. Although this strategy is being used to assign therapy to patients in some cases, it awaits validation in prospective clinical trials. *Ex vivo* testing for evaluation of drug sensitivity in cells from solid tumors has also been reported but, perhaps owing to greater challenges in sample acquisition, is apparently not as systematized in a clinical format as the hematological malignancy efforts described above[45,46].

## Spheroids

Numerous methods for 3D culture of primary solid tumor cells have been described. These platforms have the advantage of expanding sometimes modest amounts of starting material so that many different perturbations and conditions may be tested. Depending on the specific approach, cells from the tumor microenvironment may be

included in the culture, which might help replicate the *in vivo* context. However, these methods typically require weeks of *ex vivo* culture, a time period in excess of what is sometimes needed for clinical decision-making. This extended period lends itself to adaptation of tumors to an artificial environment and to selection for clones that might not be representative of the primary tumor. Indeed, the rate of success in establishing these cultures can vary considerably with histology. Still, there is evidence that these approaches can anticipate *in vivo* response to drugs, including a breast cancer study that showed good correlation between *in vitro* sensitivity and pathological complete response to neoadjuvant therapy[47].

### Patient-derived xenograft models

There is much valuable information to be gained from the study of patient-derived xenografts (PDXs)[48,49]. Immunodeficient mice grafted with primary human tumor material can be used as probes for tumor heterogeneity and the phenotype of the tumor-initiating cell. Moreover, they provide the opportunity for a rough estimation of a therapeutic index, as any treatment of a patient's tumor in a PDX occurs in the context of an entire organism. Using these 'avatars', the same patient tumor can be treated many different ways in an *in vivo* setting, a potentially valuable method of estimating patient response to a given treatment. However, there are some major challenges for the use of PDX models as clinical decision-making tools. One such challenge is the time it takes to create a PDX and serially passage and expand the tumor it bears to a sufficient number of mice to perform interpretable experiments. Depending on the model, this expansion alone can take 4–8 months, a time period that then must be followed by the actual *in vivo* experiment. This is longer than most clinicians and patients are willing to wait for a treatment decision. Another challenge is the variability in engraftment rate, which can be 13–90% depending on tumor type and technique. Another challenge is cost. Although difficult to estimate, if costs for personnel and animal housing are included, the total cost is likely orders of magnitude higher than that of *ex vivo* functional approaches. Although PDX models undoubtedly capture important aspects of patient tumor biology and as such are very valuable hypothesis-testing tools, these challenges render them inefficient for making clinical decisions for individual patients at present[49,50].

### Dynamic BH3 profiling

Dynamic BCL-2 homology domain (BH3) profiling is a strategy based on measuring the rapid induction of proapoptotic signaling by drugs (**Fig. 2**)[51]. The BCL-2 family of proteins regulates the programmed cell death pathway of apoptosis by controlling mitochondrial outer-membrane permeabilization (MOMP)[52]. Essentially, when proapoptotic BCL-2 family proteins overwhelm antiapoptotic BCL-2 family proteins, MOMP ensues. Synthetic BH3 peptides derived from the α-helical BH3 domains of proapoptotic BCL-2 family proteins can replicate the proapoptotic function of the intact proteins and can tilt the balance of a mitochondrion toward MOMP. BH3 profiling enables measurement of how close the mitochondria of a cell are to the threshold of apoptosis. This is done by titrating BH3 peptides and testing how much is required to induce MOMP[53–57]. For cells highly primed for apoptosis, little BH3 peptide is required. For relatively unprimed cells, a large amount of BH3 peptide is necessary to induce MOMP. BH3 profiling identified clinical sensitivity of CLL and AML to the BCL-2 inhibitor venetoclax[57–59].

Dynamic BH3 profiling measures changes in apoptotic signaling induced by short-term exposure of cells to drugs. A tumor sample is dissociated into a single-cell suspension and distributed in a 384-well plate. Drugs, singly or in combination, are then applied to the wells. After an incubation period of 6–24 hours, apoptotic priming is measured via BH3 profiling. Drugs added to wells in which there is an increase in BH3 peptide–induced MOMP in comparison to untreated control wells have induced proapoptotic signaling. An increase in priming is very informative, as it not only implies that the target of the drug is present in the cell and that the drug reached the target, but also that the cell was somehow dependent on the target, such that target inhibition caused proapoptotic signaling.

The assay requires only relatively brief exposure to drugs because the readout is of early death signaling, which often occurs several days before actual cell death. Therefore, it does not require establishment of multiday cultures, which has previously been a considerable barrier (as described above) in the reliable study of cancer cells. Moreover, the use of a multiwell system has proven amenable for the simultaneous testing of thousands of different drugs on the same sample, with data available within roughly 48 h of biopsy. We have demonstrated that the early measurement of drug-induced proapoptotic signaling via dynamic BH3 profiling is a good predictor of the *in vivo* efficacy of a drug in both mice and humans for liquid and solid tumors, and for single agents and drug combinations[49,51,56,60,61]. Note that, like many of the other functional approaches described in this article, dynamic BH3 profiling can incorporate markers of cell identity not only to discriminate cancer cells from stroma, but also to discriminate among heterogeneous cancer cells, including discrimination of cancer cells thought to have a more stem cell–like phenotype.

### Mass accumulation rate measurement

Manalis and colleagues[62,63] have developed an alternative approach to rapidly assess drug effect without prolonged *ex vivo* culture of primary cells. Cells enduring exposure to a toxic agent rapidly lose the ability to increase their mass. A microfluidic device can very precisely measure the mass of single cells on the basis of their interaction with a suspended microchannel resonator. This device was used to predict the sensitivity of patient-derived glioblastoma cell lines and primary murine leukemia cells to targeted agents. Although this approach has the challenge of relatively low throughput, it has the advantages of rapid turnaround (hours) and low cell number requirements. Detection of drug-induced changes in mitochondrial apoptotic priming or mass accumulation rate (MAR) indicates that the drug induces pro-death or antiproliferative signaling, even when the mechanism of action of the drug is unknown.

### *In vivo* exposure

An alternative approach that completely eliminates any problems associated with *ex vivo* cell culture or maintenance is to expose cancer cells to drugs *in situ* in the living patient. Two independent efforts have developed devices that deliver a panel of drugs to tumor tissues *in vivo*[64–68]. The CIVO platform, deployed by Presage Biosciences, uses a device to introduce up to eight different drugs into a tumor via transcutaneous injection. This platform is thus suitable only for relatively superficial tumors. Jonas and colleagues[63–65] and Kibur Medical report an implantable device that delivers up to dozens of drugs to a tumor. This can theoretically be implanted into any location in the body, but access to internal structures would obviously require a surgical or interventional radiology procedure. A pilot clinical study using this device for breast cancer treatment is underway (NCT02521363). For both of these approaches, the device and surrounding tissue are removed following exposure of the tumor to drugs, and histological analyses of the tumor are used to infer the effects of individual drugs. Histological analyses can include conventional H&E staining, as well as any desired immunohistochemical signal. For instance, cleaved caspase-3 can be used as a readout of apoptotic signaling. Initial descriptions

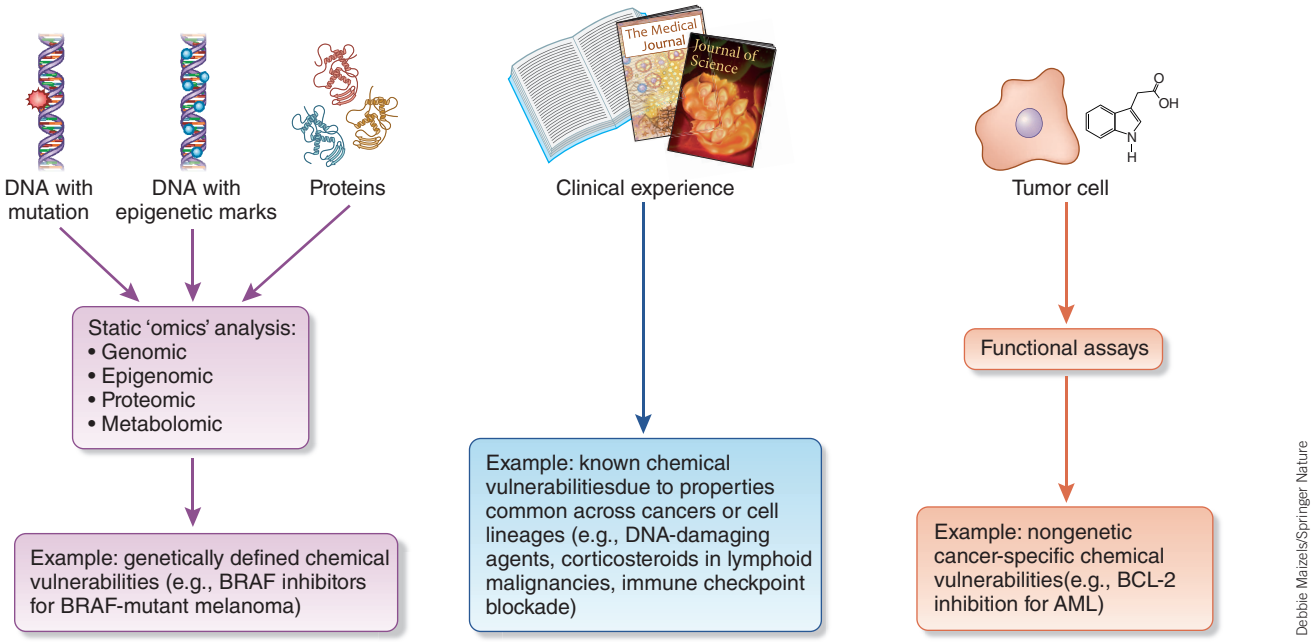


**Figure 3** Multipronged precision medicine approach to rationally assembling combination regimens. Agents are selected on the basis of genetic and functional tests performed directly on the individual patient's tumor sample and are combined with agents of known broad activity in the histology of the specific cancer being tested.

of these devices provided evidence of their ability to predict *in vivo* response in mouse models. Although there are clinical and financial challenges in requiring two procedures for a diagnostic—one for insertion of the device and one for device removal—a considerable appeal of this approach is the inarguable validity of the microenvironmental conditions of the tumor during drug exposure. All other methods described above, whether *ex vivo* or in an immunocompromised mouse, can offer only approximations of the true tumor microenvironment in the patient.

Perhaps an even simpler strategy is to administer the drug systemically and then take post-treatment biopsies to evaluate its effect. This is particularly important if a drug is coordinating a systemic response that goes beyond the cancer cell alone. Chen and colleagues[69] used such an approach to anticipate response to immune checkpoint blockade, an exciting therapeutic strategy greatly in need of predictive biomarkers. Pretreatment biopsies showed no difference between responders and non-responders to CLTA-4 blockade in a 12-marker immunohistochemistry (IHC) panel. However, early post-treatment biopsies demonstrated a significantly higher density of $CD8^+$ T cells in responders as compared to nonresponders. They similarly found that IHC analyses of post-treatment biopsies from patients treated with PD-1 blockade provided better prediction of response than corresponding analyses of pretreatment biopsies did. Such an approach is limited to a single treatment at a time and is not adaptable to making a decision among a panel of options. Although these results blur the distinction between predictive and pharmacodynamic biomarkers, this report nonetheless offers an excellent example of the valuable information that can be obtained when relevant measurements follow drug exposure.

## The future of precision medicine in cancer

Although I emphasized the shortcomings of a purely genomic approach to precision cancer medicine, these shortcomings will be shared by almost any measurement of static initial conditions. There are advantages in the analytics of static tests—and no doubt a great abundance of information can be obtained from them—but much of this information does not help identify good treatments for patients. Indeed, it is a lot to ask of genomics to, for instance, make the finer distinctions between a lung cancer cell that is sensitive to MEK inhibition and one that is resistant when genomics cannot even distinguish between a neutrophil, a cardiac myocyte, and an odontoblast. It should also be remembered that genomics can teach us interesting things about cancer, such as by elucidating mechanisms of tumorigenesis, documenting intratumoral heterogeneity, and detecting clonal evolution in therapy, even when it is not useful in assigning therapies to patients.

The goal of this Perspective is not to derail the genomics project in precision medicine, but to make it absolutely clear that additional functional approaches are badly needed to identify new drugs and assign existing drugs to larger numbers of patients with cancer. Indeed, functional approaches have lagged behind genomic approaches and thus lack prospective clinical validation, just as genomic approaches do. There are several reasons for this. One is the challenge of tissue acquisition. Functional approaches demand that the tissue under study be fresh or viably frozen, a handling protocol that is not standard for clinical pathological specimens. However, all biopsies start out viable, and the main reason to get a biopsy is to direct therapy. If functional precision medicine approaches can demonstrate superior efficacy in proof-of-principle clinical studies, these results will provide an impetus to implement the logistical procedures needed to ensure that viable tissues are procured for this purpose. Second, I believe there is an outdated perception that functional approaches are somewhat crude and unsophisticated. Whether or not this is true, it is completely irrelevant. What matters is whether functional approaches work. Advocates of these approaches must perform prospective clinical trials in the coming years to demonstrate utility. It is important to note that, while important prospective clinical validation studies have yet to be

performed for functional predictive biomarker platforms, they likewise remain to be performed for the many genomic platforms that are already deployed and heavily supported across the field of oncology. Finally, there has been a relative lack of resources applied to research in functional approaches. Genomics has clearly dominated all discussion of precision cancer medicine for the last decade and, likewise, both public and private funding.

A reigning paradigm of cancer research is that the basic mechanisms of cancer, including genetics and signaling pathways, must be understood before new therapies can be moved forward. It has become quite clear to me that, to strict adherents of this paradigm, functional precision medicine is deeply unappealing because it identifies therapeutic opportunities without necessarily illuminating the underlying mechanism. I would like to make it clear that understanding therapeutic mechanisms and functional precision medicine are not mutually exclusive endeavors. To the contrary, functional precision medicine provides immensely important grist for the mechanistic mill, as it will be informative and immediately relevant to understand why effective drugs work, even if their effectiveness is identified before the relevant pathways are. However, I do not think that clinical progression of active drugs should be halted while awaiting full understanding of their mechanisms. Indeed, I would assert that the mechanisms of the therapeutic indexes for the curative regimes we have exploited for years in ALL, promyelocytic leukemia, and testicular cancer, for instance, remain poorly understood yet are among the most striking successes in medical oncology.

A mature approach to precision cancer medicine must combine prior knowledge of broad cancer vulnerabilities with vulnerabilities private to an individual tumor based on both static 'omics' approaches and functional approaches, and I am confident that it will within the next decade. A rough schema of how these approaches could be combined to generate badly needed individualized combination regimens is presented in **Figure 3**. For this to happen, the definition of precision medicine at the NCI and other major cancer institutions must move beyond pure genomics and resources must follow. If information about what happens when a patient's actual cancer cell encounters an actual drug is not included in the cancer precision medicine strategy, we will be fighting cancer with one hand tied behind our backs.

**URLs.** NCI association with the Precision Medicine Initiative, https://www.cancer.gov/research/key-initiatives/precision-medicine/; NCI-MATCH, http://ecog-acrin.org/nci-match-eay131/interim-analysis/; BH3 profiling and technical details, http://letailab.dana-farber.org/bh3-profiling.html/.

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COMPETING FINANCIAL INTERESTS

The author declares competing financial interests: details are available in the online version of the paper.



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