

Perspective

Functional precision oncology: Testing tumors with drugs to identify vulnerabilities and novel combinations

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SUMMARY

Functional precision medicine is a strategy whereby live tumor cells from affected individuals are directly perturbed with drugs to provide immediately translatable, personalized information to guide therapy. The heterogeneity of human cancer has led to the realization that personalized approaches are needed to improve treatment outcomes. Precision oncology has traditionally used static features of the tumor to dictate which therapies should be used. Static features can include expression of key targets or genomic analysis of mutations to identify therapeutically targetable “drivers.” Although a surprisingly small proportion of individuals derive clinical benefit from the static approach, functional precision medicine can provide additional information regarding tumor vulnerabilities. We discuss emerging technologies for functional precision medicine as well as limitations and challenges in using these assays in the clinical trials that will be necessary to determine whether functional precision medicine can improve outcomes and eventually become a standard tool in clinical oncology.

INTRODUCTION

Targeted cancer therapies are capable of inhibiting specific oncogenic proteins. Many of these targeted therapies are administered following a positive companion diagnostic predicting therapy outcomes. For example, the presence of HER2 amplification in breast cancer predicts response to HER2-targeted therapy, and mutation of EGFR in lung cancer predicts response to EGFR-targeted therapeutic agents. The success of several targeted therapies, along with a comprehensive understanding of molecular diversity in cancer, gave rise to the tantalizing idea that cancer therapy could be personalized, leading to the concept of precision oncology. Precision oncology is an approach where properties specific to an individual's tumor are used to direct effective therapy. Indeed, precision oncology has been conducted for decades in some types of cancer (e.g., hormone therapy in estrogen receptor-positive breast cancer and imatinib in Philadelphia chromosome-positive leukemia). The field has expanded vastly since several large cancer genome projects (Hudson et al., 2010) revealed common molecular features of tumors that could be targeted. This perspective describes how precision oncology can be augmented by functional approaches to help realize the promise of personalized cancer treatment.

We contrast diagnostic approaches that are “functional” (measuring response to perturbations of living cells) with those that are “static” (measuring a fixed property). Current approaches to precision oncology have been dominated by genomics, a static diagnostic. Notable successes in identification of

genomic variants that portend effective treatment with targeted therapies, in addition to those already mentioned, include targeting EGFR or ALK in lung adenocarcinoma, c-KIT in gastrointestinal stromal tumors, BRAF in melanoma and non-small cell lung cancers, NTRK in a variety of rare tumors, PD-1 in microsatellite instability-high tumors, and PIK3CA in breast cancer. These successes, along with knowledge gleaned from The Cancer Genome Atlas (TCGA) data, launched the largest precision oncology trial to date: the National Cancer Institute's Molecular Analysis for Therapy Choice (NCI-MATCH) trial. This trial features nearly 40 treatment arms, with at least 35 individuals per arm, with tumors harboring a specific genetic alteration. A recent report on NCI-MATCH demonstrated the feasibility of rapid large-scale enrollment of affected individuals, DNA sequencing, and matching individuals to trial arms (Flaherty et al., 2020); however, the clinical benefit is so far underwhelming. 38% of participants were matched to a targeted therapy, and 18% had access to the relevant therapy through one of the treatment arms. Unfortunately, a minority of the >6,000 participants experienced clinical benefit: overall response rates in individual arms ranged from 0%–38%, with most on the low end of the spectrum (Flaherty et al., 2020; Commentary, 2021). Thus, the data from this large national trial essentially revealed that the current “upper limit” for individuals with targetable genetic alterations is 38% but that many tumors with predicted genetic vulnerabilities failed to respond to the matched targeted therapy. If all individuals who received genetic analysis in NCI-MATCH are considered the denominator, then the response rate in an intention-to-treat analysis



would be well below 5%. Several other precision oncology trials, comprising more than 7,000 additional individuals, have resulted in similar conclusions (Chen et al., 2021; Le Tourneau et al., 2015; Massard et al., 2017; Middleton et al., 2020; Sicklick et al., 2019; van Tilburg et al., 2021).

Given the broad use of genomics as a precision medicine tool, it is worth considering why genomics have not had a greater impact in providing individuals with cancer with effective therapies. We think that there has been an underestimation of the degree to which cancer phenotypes are driven by non-genetic mechanisms. If one were to perform whole-genome sequencing on the fingernails, retinas, neutrophils, and hepatocytes of an individual, the same result would be obtained for each tissue, but all researchers would surely agree that there are physiologically important differences between the tissues. To put it another way, the reason why individuals with lymphoma can often be cured by the R-CHOP drug combination regimen and those with pancreatic cancer cannot is not mainly because of mutations distinct to one of the tumor types but, rather, due to non-genetic mechanisms determining cell type and cellular response to drug treatment. There are many discrete, at least metastable, states of cancer cells that are determined non-genetically and, in many cases, epigenetically (Baylin and Jones, 2016). In fact, there is a growing body of literature across many treatments and many tumor types showing that resistance can often be attributed to establishment of an epigenetically determined “persister” state (Vallette et al., 2019). Thus, it is clear that many cancer phenotypes, including those that influence response to therapy, are determined by non-genetic mechanisms in addition to the mutation-driven mechanisms that are commonly considered.

An additional challenge for genomics-only approaches is that it is rare for a genomic strategy to identify an active single agent and even rarer for it to identify more than one. In a pattern repeated many times across many cancers, single agents, with only rare exceptions, are not capable of causing a sustained response. An excellent example is pediatric ALL. When early anti-metabolites were applied as single agents, there was a benefit for affected individuals, but it was transient. It was only after several active drugs were combined that sustained responses and cures were obtained. Combinations were what made a previously incurable disease over 90% curable today. To assemble the type of novel combinations needed to make a substantial difference in cancer care, alternative precision methods are needed.

FUNCTIONAL PRECISION MEDICINE IN ONCOLOGY

Functional precision medicine (FPM) is an approach based on direct exposure of tissues derived from affected individuals to drugs (Letai, 2017). This approach generates dynamic, functional data that may encompass key vulnerabilities, including those conveyed by altered epigenetic states and/or altered signaling pathways not necessarily driven by distinct genomic aberrancies. A growing appreciation of the heterogeneity of human cancers, along with new enabling technologies, and a rapidly expanding set of active drugs has renewed the push for models derived from affected individuals in research. Use of pre-clinical models, such as patient-derived xenografts (PDXs) and patient-derived organoids (PDOs), representing tumors

from affected individuals, may refine drug discovery and improve success of new therapies in the clinic. Improved feasibility of making models derived from affected individuals on a large scale for research has also made these models accessible for personalized therapy. For example, the realization that engraftment of breast and other tumors as PDXs predicted metastatic relapse (DeRose et al., 2011; du Manoir et al., 2014; Garrido-Laguna et al., 2011; Kleine, 1986; Nemati et al., 2010) spawned the idea that the models could be used to personalize therapy upon recurrence, which led to the Functional Precision Oncology for Metastatic Breast Cancer study (FORESEE; ClinicalTrials.gov: NCT04450706).

FPM is not a new concept. *In vitro* culture or growth of cancer cells in mouse models for chemosensitivity testing began more than half a century ago using proliferation, viability, or clonogenic assays as readouts for drug efficacy. It is worth noting that nearly every curative cytotoxic regimen used today in oncology was derived based on information originating from these crude but informative assays (DeVita and Chu, 2008). The question arose whether these assays could be used to personalize therapy. However, two editorials in the New England Journal of Medicine in 1983 (Selby et al., 1983; Von Hoff, 1983) and later position papers of the American Society for Clinical Oncology (Burstein et al., 2011; Schrag et al., 2004) cast doubt on the utility of such assays to personalize cancer care. Problems included lack of tumor cells surviving in *ex vivo* cultures, unsophisticated culture conditions, and uninformative assays. Moreover, there were simply not many drugs to choose from so that the utility of the information, even when accurate, was limited. In addition, there was a lack of prospective data demonstrating clinical utility.

The past few decades have yielded dramatic increases in biological knowledge, available technology, and the number of drugs available and re-kindled interest in *ex vivo* analysis of tumor cells to guide therapy. Advances in three-dimensional culture technology have accelerated work with tumors from affected individuals grown *ex vivo* as organoids for feasible drug screening (Liu et al., 2021; Tuveson and Clevers, 2019; Vlachogiannis et al., 2018); this was reviewed by Veninga and Voest (2021). The advent of new technologies allows comprehensive assessment of drug sensitivity or resistance on tumor samples from affected individuals not only for evaluation of proliferation, survival, and metabolic activity but also by incorporating single-cell analysis to evaluate bulk or sub-clonal phenotypic effects. These “next-generation” functional tests (Friedman et al., 2015) hold great promise to integrate with genomic evaluation for an improved approach to FPM, whereby drugs could be prioritized based on how well they work functionally and whether there is a genomic explanation for the effect. FPM should also be useful for identifying which genomic abnormalities actually provide good targets. An example of this is the case of the previously mysterious lack of efficacy of RAF inhibitors in BRAF V600E-mutated colorectal cancer despite proven efficacy in melanomas with the same mutation. It was not until functional studies were performed that it was revealed that, in these colorectal cancers, RAF inhibition causes feedback activation of EGFR, which supports tumor growth (Prahallad et al., 2012).

A key aspect of successful FPM is development and validation of short-term or long-term models that retain fidelity to the human tumors from which they arise. A recent large analysis of

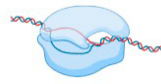
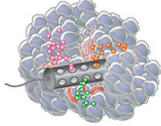
Patient derived model	Advantages	Disadvantages	Readouts to assess drug response
2D culture 	• Rapid expansion • Low cost • High throughput • Amenable to rapid testing	• Prolonged ex vivo culture; assay dependent • Missing many microenvironment components • Non-physiologic culture conditions	Cell Viability  
3D Organoids 	• Mimics the 3D tumor environment • Moderate cost • Moderate throughput • Amenable to rapid testing; assay dependent	• Prolonged ex vivo culture; assay dependent • Missing some microenvironment components • Non-physiologic culture conditions	Gene/protein expression  
Microfluidics & Engineered Microenvironments 	• Engineered tumor microenvironment • Amenable to rapid testing; assay dependent	• Prolonged ex vivo culture; assay dependent • Missing some microenvironment components • Generally low throughput	Tumor volume  
Microfluidics & Engineered Microenvironments 	• Engineered tumor microenvironment • Amenable to rapid testing; assay dependent	• Prolonged ex vivo culture; assay dependent • Missing some microenvironment components • Generally low throughput	Cell mass and morphology  
Patient Derived Xenografts 	• in vivo tumor environment • Incorporates drug pharmacokinetics • Clear toxicity readout	• Slow expansion • High cost • Low scalability • Tumor-host species differences • Lacking immune system	Mitochondrial priming  
Human Patient 	• Human microenvironment • Avoids model establishment	• Regulatory barriers • Biopsies required to rapidly assess response	Implantable microdevice  

Figure 1. Models and readouts in FPM

FPM directly exposes cancer cells derived from affected individuals (left panel) to therapeutic agents and measures drug response using various readouts (right panel). Material derived from affected individuals that is amenable to specific readouts is indicated by color codes in the right panel. Use of specific models derived from affected individuals in functional precision medicine has strengths and weaknesses, as outlined in the center panels.

>500 PDX models from various cancer types showed that they recapitulate human tumors with relatively high fidelity (Woo et al., 2021). PDXs also exhibit treatment responses that are concordant with those observed in the individuals from whom they are derived (Byrne et al., 2017; Zhang et al., 2013). *Ex vivo* cultures of tumors, such as PDOs, are less time consuming and less expensive than PDX models, and data are accumulating to show that organoids also have high fidelity to human tumor drug responses. Pancreatic and colorectal tumors in particular

have been modeled extensively as PDOs. They show strong genotypic and phenotypic concordance with samples from affected individuals (Gendoo et al., 2019; Larsen et al., 2021) and are being used to predict therapeutic responses (Narasimhan et al., 2020; Tiriac et al., 2018). Validation of each model prior to performing FPM assays is paramount, though, because aggressive stromal cells or normal cells can sometimes overgrow the cultures depending on the conditions (Dijkstra et al., 2020; Gao et al., 2017; Yu et al., 2017).

EMERGING MODELS AND TECHNOLOGIES FOR FPM

Development of new technologies at the intersection of engineering and biology can help meet some of the practical challenges of FPM (Figure 1). Notable advances have been made in technologies that aim to represent the tumor microenvironment, including extracellular matrices. Additionally, organ chips (also referred to as tissue chips or microphysiological systems) are being used to model cancer within its tumor microenvironment (Sontheimer-Phelps et al., 2019). These microfluidics devices, typically composed of tissue-specific cell types, extracellular matrix gels, and cancer cells, attempt to recapitulate the molecular and mechanical cues in human tissue (Ayuso et al., 2020; Choi et al., 2015; Hassell et al., 2017). Potential advantages of organ chips include the ability to model drug delivery, pharmacokinetics, pharmacodynamics, and drug toxicity (Barrile et al., 2018; Herland et al., 2020). Some obstacles to widespread use of organ chips in FPM are the low throughput of drug testing and the requirement of special expertise to manufacture and operate these devices (Ayuso et al., 2021).

Another approach to mimic the environment of tumors is to leave cells in their native environment during drug treatment. This includes drug treatment on tumor slices or core needle biopsies from patients, where immunofluorescent markers of cell death can be used as a readout of drug sensitivity (Horowitz et al., 2020; Majumder et al., 2015; Naipal et al., 2016). Another compelling approach for FPM is the use of drug-containing microdevices that can be implanted directly into tumors in individuals (Jonas et al., 2015; Klinghoffer et al., 2015). Drugs in these microdevices can act on the surrounding tissues and drug responses evaluated in the tumors upon resection. Although implantable microdevices may face higher regulatory and clinical barriers than *ex vivo* approaches, they may enable drug testing within the tumor *in situ*.

CHALLENGES OF FPM

Although there are academic and commercial efforts using FPM to assign drugs to individuals with cancer (Table 1), these are not yet in the mainstream of diagnostics. Next, we will discuss the challenges facing routine use of FPM in oncology.

Tissue handling

FPM methods by definition require viable tissues. All resections and biopsies start out viable until they are killed by time, fixation, or flash freezing. However, current diagnostic practice in oncology nearly always requires rapid fixation or destruction of tissue so that it can be subjected to static tests like histology or genomics without uncontrolled degradation. Clinicians apply established algorithms and experience to use this information to make treatment decisions. Therefore, for FPM to get a foothold, standard tissue handling must change. This is something of a catch-22. To change standard tissue handling so that valuable tissue is shunted to functional studies, pathologists and clinicians must feel confident that the information obtained will change management in a beneficial way. However, to provide evidence showing that FPM methods benefit affected individuals requires viable tissue in the first place. Many therapeutic clinical trials mandate storage of samples for evaluation of biomarkers

Table 1. Companies conducting FPM services

Company	Location	FPM assay(s)
2CureX	Copenhagen, Denmark	patient tumor <i>ex vivo</i> assays
Allcyte	Vienna, Austria	patient tumor <i>ex vivo</i> assays
Celcuity	Minnesota, USA	patient tumor <i>ex vivo</i> assays
Cellesce	Cardiff, Wales	patient tumor <i>ex vivo</i> assays
Certis	California, USA	PDX
Champions	New Jersey, USA	PDX
Crown Bio	California, USA	patient tumor <i>ex vivo</i> assays
Imprimed	California, USA	veterinary <i>ex vivo</i> assays
Kibur Medical	Massachusetts, USA	implantable drug delivery microdevice
Knownmed	Utah, USA	patient tumor <i>ex vivo</i> assays
Nagourney Cancer Institute	California, USA	patient tumor <i>ex vivo</i> assays
Notable Labs	California, USA	patient tumor <i>ex vivo</i> assays
Presage Bio	Washington, USA	implantable drug delivery microdevice
SageMedic	California, USA	patient tumor <i>ex vivo</i> assays
SEngine	Washington, USA	patient tumor <i>ex vivo</i> assays
SpeciCare	Georgia, USA	patient sample provider
Tempus	Chicago, IL	patient tumor <i>ex vivo</i> assays
Travera	Massachusetts, USA	suspended microchannel resonator
Vivia Biotech	Madrid, Spain	patient tumor <i>ex vivo</i> assays

so that, when the clinical response is known, a comparison can be made between biomarkers and clinical response. However, in our experience, it is nearly unheard of for samples to be stored in a viable fashion. Thus, nearly all of the existing tumor sample sets linked to completed and ongoing clinical trials are useless for testing FPM methods. Because biological signaling can change as the tumor is kept in the operating room, in banking facilities, or shipped to the laboratory, optimizing and standardizing storage and shipping conditions will be important for performing FPM clinical trials across different hospitals.

Another challenge when applying FPM is obtaining sufficient tumor cell yields from the types of samples that are acquired from individuals within current clinical practice. For many hematologic malignancies, bone marrow aspirates or blood draws can yield cancer cell quantities that enable chemical screens. For solid tumors, however, large resections are relatively rare in the setting of metastatic disease, which may be the most effective application of FPM. For most metastatic solid tumors, tissue sources typically include core needle biopsies, fine-needle aspirates, or circulating tumor cells, all of which produce low cell numbers. When cell numbers are low, establishment of the model can be a challenge, and FPM strategies that expand tissue prior to chemical screens are advantageous.

Calibration and decision making

Information obtained from most FPM methods is parametric, in contrast with genetic information, which is usually categorical.

Thus, for any drug applied to a tumor model, a number is obtained that might vary along a range, like 0–100. This is often a parameter like percentage dead cells or percentage loss of viability. Two main questions arise. First, at what value from the *ex vivo* test for a given drug is the desired clinical response obtained? Second, when obtaining results from different drugs, how can values be compared among drugs to derive a ranking of drugs according to predicted efficacy?

When considering the first question, the gold standard is simply empiric comparison of FPM results for an individual drug with clinical outcome after treatment with that drug. As one acquires more comparisons, the calibration can be updated continuously. As one acquires experience for multiple drugs in multiple tumors, it may be that patterns emerge that can accelerate this process. For instance, perhaps a value output from the diagnostic assay will emerge, common to many drugs, above which a response is predicted. For this approach, there is no substitute for experience.

Other strategies provide useful information while experience is being obtained. For instance, the assay signal of a drug on a tumor model can be compared with the response of that same drug on other models derived from affected individuals. Then one can rank the response according to percentage or SDs. One can then set a cutoff, initially arbitrary, above which a response is predicted. For instance, one might identify a particular tumor as being in the top 5% of *ex vivo* signals from an individual drug and thus classify it as a “hit.” Again, experience and clinical concordance data will refine how the output is interpreted.

A key consideration for interpretation of *ex vivo* FPM tests is the drug concentration and drug treatment duration used in such tests. Poor calibration of drug concentration in FPM tests may lead to selection of drugs that do not work or may cause effective drugs to be missed. Unfortunately, the drug concentration a tumor experiences in an affected individual is not easy to measure accurately and varies over time. One approach to identify the optimal drug concentration is to make empirical comparisons of *ex vivo* drug response and identify ranges of drug concentrations that correlate with response. Although attractive, this approach requires an ongoing clinical trial, which potentially limits the application of FPM.

Several pre-clinical trial strategies to calibrate *ex vivo* drug testing have emerged. One strategy involves comparing drug responses in 2D or organoid models with responses in matched *in vivo* models to determine whether the *in vitro* response is relevant (Bhola et al., 2020; Guillen et al., 2021; Lalazar et al., 2021). Another strategy involves comparing FPM tests on cancer cells and on healthy cells. Finally, leveraging inter-individual heterogeneity of drug response, it is possible to collect data at multiple drug concentrations across a range of tumors and identify drug concentrations where there is maximal variation between *in vitro* model responses. These “discriminating” drug concentrations are where most of the information would be obtained and, hence, would be the most useful for ranking predicted drug sensitivities.

Speed of FPM results

The clinical utility of FPM depends on how quickly results are returned to the clinician. FPM assays have various times to

generate drug responses (Figure 1). For example, FPM assays on cancers that do not require *ex vivo* expansion can typically be completed within several days. This is frequently found in hematologic cancers, where tumor cell yields from the affected individual are typically high. In contrast, many solid tumors, especially those starting from core needle biopsies, typically require expansion as cell lines, organoids, or PDX models prior to drug testing. This can take several weeks or months to establish, expand to sufficient quantity, and test. One strategy to deal with competing pressure between FPM speed and model expansion time is to collect samples prior to standard-of-care treatment, perform FPM, and then treat based on the FPM information after a recurrence or upon progression (Guillen et al., 2021).

Other limitations

The models and strategies used for FPM assays have other limitations. The “take rate” of some cancers as *ex vivo* or PDX models varies, so not every cancer can be modeled for FPM. Organoid models tend to be simplistic, excluding cells of the tumor microenvironment, which can be key mediators regulating drug responses (Wu and Dai, 2017). Tumors grown as PDXs develop a more realistic tumor microenvironment, but interactions with human microenvironmental components, such as immune cells, is lacking. Given the growing presence of immunotherapy as a mainstay in treatment of many cancers, models for FPM incorporating a functional immune microenvironment are paramount. New strategies for tumor-immune co-cultures (Boucherit et al., 2020) and new microfluidics engineering approaches (Shelton et al., 2021) are beginning to address this challenge (Shelton et al., 2021). Last, the cost of FPM assays is variable, and the greatest utility will come from strategies that allow cost-effective drug testing, perhaps through automation and miniaturization of assays.

EXAMPLES OF CLINICAL TRIALS AND FUNCTIONAL PRECISION ONCOLOGY TOOLS

Despite the challenges, several clinical trials are now incorporating FPM approaches. This is a challenge in itself; nearly all clinical trial designs are constructed to evaluate the performance of a therapeutic agent. There are few clinical trial structures to evaluate the performance of diagnostics like FPM assays. In fact, despite its widespread adoption, there still exist few prospective clinical trial data to support widespread use of genomics in individuals with advanced cancer.

Nevertheless, the utility of FPM approaches is being tested in clinical trials (Table 2). Allcyte, a company based in Vienna, uses an approach based on morphological changes in drug-treated cancer cells, which they call “pharmacoscopy” (Snijder et al., 2017). In the EXALT trial, a collaboration with the Medical University of Vienna, Allcyte tested the ability of this approach to assign therapy to 56 previously treated individuals with hematologic malignancies. Strikingly, they found that most individuals so treated demonstrated a progression-free survival time at least 1.3-fold longer than that of their prior therapy. Because pan-resistance almost universally tends to accrue with subsequent rounds of therapy in oncology, the ability to identify drugs that outperform prior therapies was reasonably

Table 2. FPM trials currently listed at [ClinicalTrials.gov](https://clinicaltrials.gov)

Identifier	Acronym	Title	Sponsor	Status	FPM platform	Target sample size
NCT03860376	none	Ex Vivo Drug Sensitivity Testing and Mutation Profiling	Florida International University	recruiting	ex vivo assays	22
NCT03133273	ONCOGRAM	Study of the Therapeutic Response and Survival of Patients with Metastatic Colorectal Cancer (Stage IV) and Treated According to the Guidelines of a Chemosensitivity Test, Oncogramme (ONCOGRAM)	University Hospital, Limoges	recruiting	ex vivo assays	256
NCT04470947	EXALT-2	Comprehensive Genomic Profiling and Next Generation Functional Drug Screening for Patients with Aggressive Haematological Malignancies	Medical University of Vienna	recruiting	ex vivo assays	150
NCT02927106	none	Beat AML Core Study	University of Florida	completed	ex vivo assays	22
NCT01190241	TSAP	Targeted Therapy Selection Based on Tumor Tissue Kinase Activity Profiles for Patients with Advanced Solid Malignancies, an Exploratory Study	Vanderbilt University	terminated	ex vivo assays	45
NCT04450706	FORESEE	Functional Precision Oncology for Metastatic Breast Cancer	University of Utah	recruiting	organoids	15
NCT03890614	none	Novel 3D Myeloma Organoid to Study Disease Biology and Chemosensitivity	Wake Forest	recruiting	organoids	70
NCT04561453	none	Feasibility Study of Multi-Platform Profiling of Resected Biliary Tract Cancer	University of Washington	recruiting	organoids	20
NCT03896958	PIONEER	The PIONEER Initiative: PrecisionInsights On N-of-1 Ex Vivo Effectiveness Research Based on Individual Tumor Ownership (Precision Oncology)	SpeciCare	recruiting	organoids	1,000
NCT04768270	none	The Culture of Ovarian Cancer Organoids and Drug Screening	Chongqing University	recruiting	organoids	30
NCT04755907	none	3D Bioprinted Models for Predicting Chemotherapy Response in Colorectal Cancer With/Without Liver Metastases	Peking Union Medical College Hospital	not yet recruiting	organoids	120
NCT04842006	SYNCOPE	Systemic Neoadjuvant and Adjuvant Control by Precision Medicine in Rectal Cancer	Helsinki University Central Hospital	not yet recruiting	organoids	93
NCT04826913	TUMOVASC	High Throughput Screening Device Based on 3D Nano-matrices and 3D Tumors with Functional Vascularization	University Hospital, Strasbourg, France	not yet recruiting	organoids	100
NCT03336931	PRISM	Precision Medicine for Children with Cancer	Sydney Children's Hospitals Network	recruiting	PDXs	400
NCT03219047	none	Patient-Derived Xenografts in Personalizing Treatment for Patients with Relapsed/Refractory Mantle Cell Lymphoma	MD Anderson Cancer Center	recruiting	PDXs	50
NCT04745975	none	Guided Treatment Based on Mini-PDX in Metastatic Triple Negative Breast Cancer	Fudan University	recruiting	PDX	100
NCT04373928	none	Personalized Precision Diagnosis and Treatment of Pancreatic Cancer	Changhai Hospital	recruiting	PDXs	100
NCT04602702	PDXovo	Hyper-Personalized Medicine Using Patient Derived Xenografts (PDXovo) for Renal Cell Carcinoma Patients	Sunnybrook Health Sciences Center	not yet recruiting	PDXs	50

(Continued on next page)

Table 2. Continued

Identifier	Acronym	Title	Sponsor	Status	FPM platform	Target sample size
NCT03878524	SMMART	Serial Measurements of Molecular and Architectural Responses to Therapy (SMMART) PRIME Trial	OHSU Knight Cancer Center	recruiting	multiple	40

interpreted as evidence of the utility of their function approach. It is notable that 40% of responders experienced exceptional responses lasting at least three times longer than expected (Kornauth et al., 2021; Wheeler et al., 2021). EXALT 2.0 is a follow-up study that randomizes individuals to one of three arms in which treatments are based on functional assays, genomic analysis, or physician choice. Another study evaluated response in 29 individuals with relapsed or refractory AML treated according to results from an *ex vivo* functional diagnostic, with most individuals receiving combination therapy (Malani et al., 2021). The investigators observed a 59% objective response rate, with 45% achieving complete remission of the leukemia (with or without complete hematologic recovery). The FORESEE study, mentioned above, is using a similar approach, with FPM drug response assays being used on tumor organoids to predict therapy response in individuals with metastatic breast cancer, also assessing the response ratio of the “informed” to the previous “uninformed” therapy. The TuPro study, being conducted at the University Hospital Zurich, is using a constellation of genomics, proteomics, single-cell analysis, and *ex vivo* drug sensitivity screening to predict treatment responses using the integrated information (Irmisch et al., 2021). Although data are still very limited to assess how well FPM performs in clinical trials, we are encouraged that there is an increasing number of active clinical trials, with results yet to be reported.

FPM can also function as a companion diagnostic for individual therapies. A rapid assay of drug-induced apoptotic signaling at the mitochondrion, called dynamic BH3 profiling (DBP), was performed in a study of lenalidomide plus mitoxantrone, etoposide, and cytarabine as an induction regimen for acute myelogenous leukemia (Garcia et al., 2020). Pretreatment blasts were exposed to lenalidomide *ex vivo* as part of the assay, performed blinded to outcome. A post hoc comparison of DBP results and clinical outcome showed that clinical response could be predicted nearly perfectly via this approach. DBP has also been shown to predict response to varied therapies in multiple murine models (Bhola et al., 2020; Montero et al., 2015; Townsend et al., 2016). Similar functional methods identified mitochondrial sensitivity to BCL-2 antagonism in CLL and AML (Del Gaizo Moore et al., 2007; Vo et al., 2012). This work led to clinical trial programs that yielded multiple regulatory approval for clinical use of the BCL-2 antagonist venetoclax for both diseases.

Assembling combinations

One of the advantages of functional methods is that they frequently identify several active drugs simultaneously. Although this bodes well for the use of FPM to design novel, personalized combinations, it also faces a significant regulatory challenge. Many of these combinations are likely to have never before

been tested in the clinic. Conventionally, a novel combination would require a phase 1 multi-drug dose escalation trial with at least a dozen affected individuals to identify a recommended phase 2 dose for each drug to be tested in combination. That approach would be impractical and inconsistent with the goal of identifying novel, individualized combination regimens. The time and resources to assign a single individual to a single novel combination regimen would be impossible to maintain. However, given the urgent need for effective combination regimens, especially in the advanced cancer setting, a solution must be found. This likely will arise as some form of intra-individual dose escalation, facilitated by a union of enthusiasm from affected individuals and their advocates to accept the increased risk for toxicity as well as an acknowledgment from institutional review boards (IRBs) and the U.S. Food and Drug Administration (FDA) that the potential benefit outweighs the risk.

If the goal is to assemble two-, three-, or even four-way combinations from FPM strategies, it will be challenging to test every possible combination *ex vivo* because this would be possible for only a very small panel of drugs before the number of conditions becomes unwieldy. Although much attention is focused on identification of mechanistic synergy in assembly of combinations, which requires simultaneous testing of more than one drug, it is not clear that synergy is required for highly effective combinations. For instance, perhaps no therapy has cured more cancer patients than the R-CHOP therapy used for B cell lymphomas, which has likely cured at least hundreds of thousands of individuals worldwide. The components of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) do not exhibit clinical synergy. Rather, each drug has an independent chance to kill a cancer cell (Palmer et al., 2019), so a cancer cell must be resistant to all drugs to survive. The same absence of synergy can be observed with other combination regimens (Palmer and Sorger, 2017). In practice, it is likely that more than one screening practice will be used. In some contexts, testing a limited number of combinations *ex vivo* will be a priority, whereas in others, testing a larger number of single agents will be a priority. FPM single-agent results can be combined with extrinsic information, including histology and other molecular annotations, to prioritize drugs to be included in higher-order combinations.

TO DO LIST FOR FPM

FPM has tremendous potential to provide a broadly applicable predictive biomarker for assigning therapy and for discovery. Here are four items that we feel will speed its adoption as a standard.

- *Limit pre-analytical variability.* The requirement in FPM for viable, unfixed tissue can introduce pre-analytical

variability into the downstream assays. At every step, including initial sample acquisition, sample storage, short-term viable freezing, shipping temperature and duration, sample dissociation, and *ex vivo* culture conditions and duration, there is an opportunity for variability to be introduced that will prevent reliable comparison among different approaches and with clinical outcome. Reducing variability will require improved reporting of standard operating details all the way from biopsy to performance of the assay. Improved sharing and communication among FPM practitioners will be necessary, perhaps under the aegis of a consortium or organization like the Society for Functional Precision Medicine.

- **Report on clinical correlation with FPM assays.** Although the utility of FPM is indubitably plausible, there is no substitute for rigorous comparisons of FPM results with clinical outcome. Fortunately, as recorded above, we have started to see such reports in the literature and are aware of many more that are in various stages of planning and execution (Table 2). As confidence grows in the utility of these assays to match individuals with drugs that provide benefits, a virtuous cycle of increased acquisition and banking of viable tumor samples followed by increased application of these samples and their assays to clinical trials is to be expected. Such a virtuous cycle should ultimately result in adoption of high-performing assays as standard clinical tools.
- **Increase uptake of FPM by pharmaceutical companies.** To a practitioner of FPM, the relative slowness of pharmaceutical companies to embrace FPM can seem puzzling because FPM seems to offer solutions to problems that chronically vex them. FPM has the potential to provide a companion diagnostic for nearly any cancer therapeutic agent. Thus, drugs without companion diagnostics that falter in clinical trials with borderline response rates could be rescued by FPM tools that might stratify individuals and dramatically improve response rates. Moreover, for agents that successfully target a protein or pathway but lack a clinical context, FPM can be used to survey a spectrum of cancer samples and identify those where biological activity is greatest. A few successes in this arena may enhance enthusiasm and establish FPM as an important clinical and pre-clinical stratification tool.
- **Share the data.** FPM data in models that represent real-world cancer cases are an invaluable resource for research and development of new, more effective therapies. The utility of FPM assays is limited by the number of drugs that are available for treatment, but many of the models generated for FPM assays can be grown indefinitely and utilized for new research to develop new therapeutic strategies. Of course, it is important to continue to document the ongoing fidelity of the long-term models for such an approach to be useful. We recommend that validated models, their genomic data, and their associated drug response data be made publicly available through resources like the NCI's PDX Network and Patient-Derived Models Repository. A specific "data commons" for FPM might be especially useful, allowing data deposition and sharing of knowledge within the scientific community.

Eventually, one could imagine utilizing a vast resource of genomics and FPM data to predict therapeutic responses for future affected individuals without actually having to grow their tumors for functional tests (Welm et al., 2021).

CONCLUSION

The full promise of precision medicine in oncology has yet to be realized, as more individuals may benefit from functional approaches. Genomics has low operational barriers (minimal tissue handling; sequencing is relatively cheap and ubiquitous) and high intellectual barriers (how to go from mutations to active drugs is often unclear), whereas FPM has high operational barriers (requirement of fresh tissue, survival of cells, credentialing of readouts) and seemingly low intellectual barriers (active drugs are identified directly). We predict that the next 5 years will see an increasing marriage of these two approaches in clinical trials whose completion will be necessary for establishment of FPM as a standard tool in clinical oncology. This union will be necessary for rational selection of active combination regimens that will ultimately be necessary for precision medicine in oncology to provide its greatest benefit to affected individuals.

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AUTHOR CONTRIBUTIONS

A.L., P.B., and A.L.W. wrote the paper.

DECLARATION OF INTERESTS

A.L.W. has received royalties from licenses of patient-derived xenograft or organoid models issued by the University of Utah. The University may issue new licenses in the future at its discretion, which may result in additional royalties. A.L. owns equity and serves on the scientific advisory boards of Zentalis Pharmaceuticals, Dialectic Therapeutics, Anji Onco, and Flash Therapeutics. A.L. has received royalties from licenses of BH3 profiling issued by the Dana-Faber Cancer Institute. The Institute may issue new licenses in the future at its discretion, which may result in additional royalties.

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