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Functional precision cancer medicine: drug sensitivity screening enabled by cell culture models

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Highlights

- Functional precision medicine are strategies to support clinical decisions on personalized treatment of cancer patients using functional tests on live patient cells, for example testing of cell viability after exposure to drugs.
- For hematological cancers, methods and test platforms have matured to a stage where drug sensitivity testing is being implemented in prospective clinical trials as a stratification tool, complementing genomic and transcriptomic information.
- For solid tumors, there is increasing evidence from co-clinical trials using a breath of test systems for assessing drug sensitivity of patient cells coupled to clinical outcome. This allows for validation of the tests.
- The field of functional testing in precision cancer medicine is thus moving towards its implementation as experimental diagnostics to provide more information to molecular tumor boards.

Abstract

Functional precision medicine is a new, emerging area that can guide cancer treatment by capturing information from direct perturbations of tumor-derived, living cells, such as by drug sensitivity screening. Precision cancer medicine as currently implemented in clinical practice has been driven by genomics, and today's molecular tumor boards rely extensively on genomic characterization to advise on therapeutic interventions. Genomic biomarkers can, however, guide treatment decisions only for a fraction of the patients. Herein we discuss the state-of-the-art for functional precision cancer medicine and the potential for new methods to stratify patients to different treatments beyond the current practice of mutational analysis.

1. Functional precision medicine

Functional precision medicine (see **Glossary**) is a rapidly advancing strategy to inform personalized treatment decisions for cancer patients based on functional readouts, such as direct **drug sensitivity testing** of the patient's cancer cells (**Box 1**) [1]. This approach was introduced more than four decades ago [2,3], but initial reports found functional assays to be too premature for clinical implementation, in part due to the low fraction of tumor samples that could successfully be cultivated and tested for drug sensitivity in the lab [4,5]. For solid tumors, studies have so far mostly been conducted retrospectively, and prospective evidence, key to fully adopting such technologies in clinical decision making, is still lacking. The field is more mature for liquid tumors, in which some prospective trials have already been published on the performance of functional assays for therapy response prediction [6,7] (**Tables 1 and 2**). This was preceded by retrospective studies also in hematological cancers, demonstrating that *ex vivo* drug sensitivity is associated with clinical responses to therapy [8-10]. While functional precision medicine has proven valuable for patient stratification to treatments in clinical trials and in some real-world case reports, considerable efforts are still required before it can be implemented in routine clinical practice. Now, improved cell culturing protocols have advanced functional precision medicine to a point where we must explore its potential to guide treatment decisions in clinical trials (**Table 1**) [1,11,12]. In this review, we discuss the current state of functional precision medicine, the advances for drug sensitivity screening enabled by cell culture models, and how artificial intelligence can be coupled to functional precision medicine to guide patient stratification.

2. The current standing of functional precision medicine

The evidence level for functional precision medicine is now being transformed from retrospective trials to prospective study designs, see Tables 1 and 2 for prospective trials in hematological malignancies, and retrospective trials in solid tumors, respectively. This is exemplified in a study by Malani *et al*, where a multidisciplinary functional precision medicine tumor board was created to guide clinical decisions for patients with acute myeloid leukemia (AML) (**Table 1**) [11]. The authors reported that actionable drugs were identified for 97% of the patients. Treatment recommendations were implemented for 37 individuals with a 59% objective response rate [11]. The EXALT trial guided treatment of 56 heavily treated patients with advanced hematologic malignancies based on drug testing (**Table 1**) [12]. Clinical benefit was defined as minimum 1.3-fold prolonged progression-free survival relative to that obtained on the previous line of therapy. Thirty patients (54%) achieved this at a median follow-up of 23.9 months [12]. The ongoing EXALT-2 trial (NCT04470947) is comparing treatment guided by functional drug screening, genomic profiling and physician's choice. The results from this study promise to add new insights into the strategies for next-generation clinical decision support.

3. Advances in functional precision medicine enabled by cell culture models

Recently, cell culturing models have been developed for a variety of different cancer types, which has enabled the field of functional precision medicine to move to combining the best features of each of these models through a series of advances. Suspension-based models, for example, were initially developed for cultivating primary cells from hematologic cancers in growth-supporting solutions, while tissue architecture-preserving models have been developed to mimic and study intact solid tumors and, optionally, parts of their microenvironment (**Figure**

1). Current efforts have focused on applying suspension-based techniques to solid tumor organoids, which can potentially advance throughput in drug screening by bypassing some of the cumbersome steps associated with solid substrates for organoid growth [13,14]. Conversely, co-culture methods, originally developed in solid tumor assays, are now implemented in suspension-based protocols [15,16], which is hoped to increase relevance of the readout to drug responses *in vivo*, since tumor cells are known to extensively communicate with a variety of host cells. In the following subsections, we will review recent advancements in cancer drug sensitivity screening technology models.

3.1 Suspension-based models are compatible with high throughput drug screens

Hematological cancers appear as single-cell suspensions when obtained from blood samples or bone marrow draws. This appearance makes them readily dispensable and highly compatible with existing high-throughput drug screening protocols, for characterization against larger compound libraries [17] [18]. One group recently carried out *ex vivo* drug sensitivity testing of 63 drugs on blood cancer samples from 246 patients. They showed that the malignancies, which could not be identified from genomic biomarkers based on target vulnerabilities, could be stratified into subgroups based on therapy responses. The *ex vivo* drug sensitivities in drug response-defined subgroups were associated with treatment outcome [17]. Another group identified treatment-induced changes in vulnerabilities, which could inform individualized combination regimens using single-cell, image-based sensitivity profiling (pharmacoscopy) of paired samples. In this group's study, the samples were collected before and during treatment with the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib, from chronic lymphocytic leukemia (CLL) patients [18]. However, the study was performed on non-proliferating cells without stimuli from the tumor microenvironment in the bone marrow and with only 18 h of drug exposure as it has remained a challenge to cultivate CLL cells for longer periods of time,

which could reduce the clinical relevance of drug sensitivity readouts. Since CLL cells undergo spontaneous cell death when they are cultured *ex vivo*, several culturing models have been developed to mimic the CLL tumor microenvironment. A key component for culture success is that of signals provided by T cells (CD40L, cytokines) and nurse-like cells (APRIL, BAFF), which are both found in the microenvironment of CLL [15,19]. Importantly, drug sensitivity testing on CLL cells that were pre-cultured with these microenvironmental factors over longer time periods (≥72 h, allowing for the relatively slow proliferation rate of CLL cells) has been used to guide personalized treatment of relapsed CLL (**Table 1**) [6,20]; and protocols have also been developed to sustain viability and proliferation of multiple myeloma (MM) cells for functional testing [21,22].

3.2 Suspension-based models can be studied by flow cytometry protocols

In addition to drug sensitivity, suspension-based high throughput drug screens can inform on single-cell protein profiles by flow cytometry protocols, which can then be applied in biomarker discovery pipelines [23-25]. As an example, Melvold *et al* studied mechanisms of disease-specific sensitivities to MEK inhibitors in CLL, MM, and mantle cell lymphoma (MCL) cell lines by flow cytometry-based protein profiling [26]. We found association between high expression levels of Myeloid cell leukemia sequence 1 (Mcl-1) or B-cell lymphoma - extra large (Bcl-xL) with low sensitivity to the MEK inhibitor trametinib when combined with the Bcl-2 antagonist, venetoclax. Interestingly, we demonstrated that the low sensitivity could be overcome by exposing the cells to an Mcl-1 or Bcl-xL inhibitor [26]. The same flow cytometry-based approach has also been used to map drug synergies at high resolutions [27]. Combined treatment of CLL cells with ibrutinib and venetoclax induced apoptosis in a synergistic manner at doses that are much lower than what is currently recommended in clinical practice. This suggest that it is possible to reduce established

treatment doses ensuring efficacy and decreased toxicity. Indeed, retrospective studies have shown that dose adjustments of CLL therapies did not compromise treatment outcomes [28-32].

3.3 Standardized solid cancer cell lines represent a compromise between suspension-based models and tissue-architecture preserving models

For solid tumors, standardized single-cell type and two-dimensional cell line models do not reflect the three-dimensional architecture or multi-cell constituents of the tumor of origin, and are therefore not considered good models to predict individual patient treatment responses [33]. Nonetheless, the use of cell lines may still provide valuable information. One obvious advantage with standardized cell lines is that access to material is not a limiting factor for larger experimental setups, as nicely demonstrated in a study on cell lines from breast, colon and pancreatic cancers, where the effects of 2025 drug combinations were analyzed [34]. A study of this type would likely not be possible using patient material, due to limited availability, but does provide important knowledge that can be used for characterizing novel drug combinations and linking drug responses to biomarkers for the tested cell lines. However, the loss of tissue architecture, and the evolutionary selection pressure set forth in the cultivation of planarly grown cells, are considered to negatively influence the relevance of cancer cell line observations with respect to predicting individual patient therapy responses. To address this limitation, diverse tissue architecture-preserving models have been developed to facilitate functional analyses of the three-dimensional tumor in its microenvironment (**Figure 1**).

3.4 Tissue architecture-preserving models for solid tumor assays

Three-dimensional models for solid tumors can retain tissue architecture and multi-cell composition of the cancer. Such lab systems span from simpler setups for studies of only the

pharmacodynamic properties of a drug in lab-cultivated primary cancer cells, to more sophisticated setups that can include host factors and allow the study of pharmacodynamic-pharmacokinetic properties [35]. However, with increasing complexity, throughput and clinical applicability generally decreases. This calls for assays that are as complex as necessary for their clinical relevance, and as simple as possible to deliver robust results in a clinically actionable time frame. In the following sections we will discuss protocols for cancer organoids and spheroids, xenografts and implantable drug reservoirs, that are all developed to predict drug responses in solid tumors.

3.5 Cultivation of tumor cells in xenograft models

Xenografted tumors represent a highly complex platform to probe therapy responses, which also enables the study of certain drug pharmacokinetic properties in the recipient animal host, including distribution, metabolism and elimination (**Figure 1**). However, since xenograft models do not contain the immune component of the tumor microenvironment, they cannot fully recapitulate the *in vivo* situation. Furthermore, success rates for PDX engraftments have been reported to be around 10-30% for major tumor types [36], indicating that such setups will only be informative for a fraction of all cancer patients. In addition, the time required to grow tumors for drug testing may be too long for the method to deliver results in a clinically actionable time frame. The animal host size is one important factor that determines time to grow testable tumors and assay throughput, and may range from patient-derived xenografts (PDX) in zebrafish embryos contained in 96-well plates for drug screening to PDX models in mice. Generally, smaller animal host size requires shorter time to grow tumors and allows higher throughput. It has been shown that successful engraftment in recipient animals is an independent negative prognostic factor for certain cancer diagnoses, suggesting that more aggressive tumor types are also those where information that can support clinical decision-

making is most likely obtained [37,38]. High costs, complex experimental conditions, and the time required before a conclusion can be made based on the findings, are factors that need to be considered for the implementation of these models in clinical diagnostics.

3.6 Cancer organoids

Cancer organoids (Figure 1) represent a compromise between two-dimensional models and xenografts, in which tissue architecture is retained to some extent by allowing isolated tumor cells to grow into three-dimensional structures. For many solid tumors, this will improve cultivation success and allow the representation of inter-cell signaling, and of physiologically relevant chemical gradients [39]. Organoid models also have the advantage that they allow use of patient-matched normal tissue to grow organoids, and these may serve as a control for toxicity to normal tissue. Similar to suspension-based models, organoid cultures enable extensive pharmacodynamic profiling, as demonstrated by Bruun *et al* in their characterization of drug responses to 40 drugs across 22 patients [40]. Cancer organoids have been studied as a **diagnostic test** for numerous solid tumor types, with the majority of trials performed co-clinically or retrospectively matched with a recorded clinical outcome, and with an emphasis on colorectal cancer (CRC), breast cancer and ovarian cancer [41]. The test sensitivity and specificity to predict therapy responses have been highest for CRC, pancreatic cancer, and head-and-neck cancer squamous cell carcinomas. While small numbers of patients preclude firm conclusions for individual cancer types, an overall cross-tumor type sensitivity of 0.81 (95% CI 0.69-0.89) and specificity of 0.74 (95% CI 0.64-0.82) clearly invigorates future research for solid tumor functional precision medicine (see **Table 2** for examples). Moreover, in another study it was shown, that cancer organoid setups perform better for chemotherapies than for other therapies [42]. Ooft *et al* found a good correlation between irinotecan-based therapy and organoid responses to its active metabolite SN-38 in colon cancer patients [42],

but no correlation between responses to targeted therapies in organoid cultures and patients [43], and these observations calls for additional investigations to cultivation, drug exposure protocols and experiment readouts to identify causes for the discrepancy.

One approach to improve cultivation protocols involves optimization to the solid substrate on which cells are grown in the lab. Common to all protocols for cultivation of tumor cells on solid supports is the use of protein-rich substrates that favor growth of cells that *in vivo* is surrounded by a proteinaceous gel and other cell types. One drawback with systems based on cultivation of cells in animal-derived extracellular matrix hydrogels, such as Matrigel, is that inter-batch variations influence the reproducibility. Additionally, scarcity of animal-derived matrix proteins further limits the scaling of most organoid-based methods. Together, these drawbacks argues for synthetic replacements to animal-derived matrix hydrogels [44].

Another approach to improve cultivation protocols for cancer organoids is the introduction of additional cell types found in the tumor microenvironment, such as fibroblasts and immune cells [45,46]. One limitation with many of the organoid-based assays is that tumor microenvironment components are isolated and artificially reintroduced to the generated microenvironment. Air-liquid organotypic models allow the characterization of cancer organoids with immune cells that are endogenously incorporated in to the stroma for studies of cancer immunotherapy responses [47,48], and could represent a way to preserve parts of the microenvironment.

In order to bypass experimentally controlled organoid cell type composition in the microenvironment, tumor specimens can be grown as intact micro-dissected tissues, where tissue components and cell-cell interactions are preserved (**Figure 1**) [49,50]. Such setups, often referred to as spheroid or explant cultures, retain a number of cell types in addition to the cancer cells, such as endothelial cells, immune cells, and cancer associated fibroblasts [51].

As new and effective targeted therapies continue to enter the clinic at an increasing rate (see Attwood *et al* [52] for a nice overview of the progress over the past 25 years), efforts must be directed at identifying assays, culture conditions and readouts that are informative for drug responses in patients. Cancer organoid-, explant- and spheroid-protocols are fairly manageable for expert users, provide readouts in a matter of a few weeks, and can be subjected to medium throughput drug screening, where at least a few dozen therapies can be tested for individual patients. These characteristics, coupled with the preliminary evidence from small-scale co-clinical trials (**Table 2**), render these setups tangible for next-generation companion diagnostics at tumor-boards. However, evidence from prospective clinical trials, already available for several functional precision medicine frameworks for hematological malignancies, are still missing for solid tumor assays.

3.7. Miniaturized, microfluidic assays for functional precision medicine

While suspension-based and solid tumor models have been extensively characterized with protocols developed and optimized for medium to high throughput drug screens, it still takes time to grow organoids and to have results from drug sensivity screening in a clinically actionable time frame. This calls for faster methods also to assess functional features.

With improved miniaturization technologies, microfluidic platforms (**Figure 1**) have been developed during the past decades, and have changed the paradigm for miniaturization of biological assays. In particular, droplet microfluidics supporting manipulation of miniature droplets such as merger, splitting, recombination, detection, incubation, sorting and other processes, can be combined to support workflows for developments towards diagnostics for individually tailored cancer therapy. Yet another approach may involve rapid miniaturized assays on cell suspensions directly from a solid tumor. This approach may maintain the epigenetic imprinting and “memory” of the three-dimensional context for some hours, allowing

functional screens to characterize cells; for example, by dosing drugs to individual or small groups of cells, and to bring cancer cells and immune cells from the tumor microenvironment together with or without therapeutic interventions [53-55]. Such emerging methods may deliver results from functional testing much faster and allow testing of drug combinations.

3.8 Implantable drug reservoirs can identify drug responses *in vivo*

Lastly, efforts to test multiple therapies in the patient to which the therapy can be described, are being developed. In order to test drug sensitivity *in vivo* in candidate patients, implantable drug reservoirs (**Figure 1**) are inserted in to the patient's tumor and can release drugs in a spatially and temporally controlled manner [56]. Two separate studies reported that up to 8 or 16 different drugs or drug combinations could be assessed simultaneously [57,58]. The local drug delivery may allow identification of optimal therapy prior to systemic exposure. In early models, a biopsy from the drug delivery site was needed to assess drug effects. To overcome this limitation, a more recent study demonstrated the development of a so-called "lab-in-a-tumor" implantable microdevice, which, in addition to the drug, delivers a fluorescent cell-death assay. This is then detected by an integrated fluorescence imaging probe, allowing for real-time drug response analysis [59].

While technology is now in place to model drug sensitivity in a variety of cancer types, a pressing challenge relates to handling of the large data-sets that are being produced, and how to optimally use the information to stratify patients for appropriate treatments.

4. Artificial intelligence-guided patient stratification

To develop next-generation **patient stratification** of high accuracy, the integration of both laboratory/clinical, genomic and functional data should jointly contribute towards new algorithms to identify multi-marker panels for prediction of treatment responses (**Figure 2**)

[60]. Such an approach is put to work in the ERA PerMed CLL-CLUE consortium[1]. The study aggregates available data-sets from CLL patients who have been treated with targeted therapies in clinical trials[1], and for which the treatment outcomes are known [61,62]. The aim is to test predictions in prospective clinical trials on CLL. Similarly, the ERA PerMed project ONCOLOGICS[2] benchmarks evolution-based machine learning algorithms for their predictive capacity for targeted drugs. The final algorithms are aimed at identifying responders and non-responders to anti-cancer drugs for patients that have received therapy at molecular tumor boards at Institut Curie or at Charité Comprehensive Cancer Centre. Functional precision medicine models provide additional insights into mechanisms that can explain prediction successes and failures beyond what is captured in genomic assays, and can include also data collected upon drug perturbations to optimize the machine learning algorithms.

Artificial intelligence-guided patient stratification is implemented in some prospective clinical trials, but is not yet the norm in clinical practice [63]. The PreVent-ACaLL trial (NCT03868722) employs the machine-learning model CLL-TIM to identify newly diagnosed CLL patients with high risk of severe infection and/or treatment within two years of diagnosis [64]. These patients are allocated to a combination treatment with acalabrutinib (BTK inhibitor) plus venetoclax [65], rather than watch-and-wait, which is the standard of care for these patients. The intention of the study is to reduce the risk of infection, which can lead to fatal outcomes in this patient group [66]. These models are developed based on patient characteristics collected before treatment initiation. For a more dynamic prediction of treatment outcome, an alternative is to take into consideration data collected over time. The **Continuous Individualized Risk Index (CIRI)** uses the same principle as "win probability" models in

[1] www.era-learn.eu/network-information/networks/era-permed/multidisciplinary-research-projects-on-personalised-medicine-2013-pre-clinical-research-big-data-and-ict-implementation-and-user2019s-perspective/tailoring-the-targeted-treatment-of-chronic-lymphocytic-leukemia

[2] https://www.era-learn.eu/network-information/networks/era-permed/multidisciplinary-research-projects-on-personalised-medicine-2013-pre-clinical-research-big-data-and-ict-implementation-and-user2019s-perspective/computational-modelling-and-functional-validation-platform-for-personalised-colorectal-cancer-clinical-therapy-decision-support

other fields, i.e. sports, by integrating risk assessments throughout the disease course [67]. The model was generated for breast and hematologic cancers, and resulted in improved outcome predictions compared to existing prediction algorithms [67]. This is not surprising, given that current models are based on the "average" patient.

Drug combinations are expected to improve cancer therapy responses [52], and is the focus of recent drug screening projects [34]. A challenge for machine learning algorithms that predict combination responses is the availability of training data required for such algorithms. In fact, the prediction of drug combinations encompassing three-way, four-way and higher order combinations cannot realistically be trained by data complexity similar to that being predicted, since the number of experiments increases exponentially with the number of single drugs available. For example, a drug panel of 150 drugs corresponds to over 10,000 pairwise combinations and over half a billion 5-way combinations, effectively prohibiting patient-specific observations to be generated due to scarcity of available material, and due to enormous experimental setups. Rather, efforts will have to focus on prediction of higher-order combinations from marginal spaces, i.e. from baseline states or potentially from single drug responses [68-70]. Recent advances have gone from effectively finding and describing drug synergies in data mathematically to predict synergies (and evade toxicities) using random forest algorithms and Bayesian models, the latter also including uncertainty estimates [71,72]. In parallel, precision pharmacovigilance is developed to assess drug safety for the individual patient [73]. A combination of computer-assisted individualized pre-selection of drug combinations and testing in functional assays can be one way of advancing multi-drug combinations to patients.

5. Concluding remarks and future perspective

Functional precision medicine has a proven value in clinical decision-making through multiple clinical trials, especially within hematological cancers, complementing genomic information. The technological developments now also provide solutions suited for solid tumors, as well as scalability with regard to number of drugs, manageable platforms and methodology for measurements. An increasing number of platforms and approaches are well suited to deliver complementary diagnostic approaches in addition to genomics and transcriptomics to guide treatment recommendation, but are still not applicable in routine diagnostics (see **Outstanding questions**).

The diagnostic infrastructure must allow for dynamic implementation of technological refinements to provide solutions securing optimal conditions for all cell types, and time-frames must align with clinical needs. Clinical decision-making utilizing information from functional assays should in the future be part of modern molecular tumor boards. Implementing and integrating novel and comprehensive diagnostic tests as decision support in tumor boards will be demanding. Traditionally, implementation of predictive biomarkers requires evidence provided by prospective clinical trials. Now, as precision medicine trials need an increasing range of tests to identify and/or stratify patients into treatment groups, the performance and validation must be assessed in novel ways. This will ensure a trans-disciplinary structure, where novel diagnostics accompanied by tailored interpretation tools provide a link to systems that can continuously integrate new knowledge generated by incoming data that is associated to clinical outcome information. Implementing the emerging functional analyses as prospective stratification tools for different cancers will be an important first step in this direction.

Before functional analyses can be fully utilized in a routine diagnostic setting, platform validation, compatibility with local infrastructure, standardization of measurements and validation of threshold for treatment recommendations must be performed. To test and validate the functional assay readouts, clinicians and researchers must conduct prospective clinical

trials, and compare the performance of functional precision medicine guided therapy choices to other decision support algorithms. For trials in which each patient has received a completely individualized therapy, and where no other patient has received the same therapy, trials can be designed to compare clinical benefit for algorithm A vs algorithm B. In contrast to randomized two-arm trials, the trial design in precision medicine studies offer the opportunity to feed back information about response and outcome to treatment decision support platforms, which can improve the treatment match and response predictions. Recording observed therapy responses in the clinic for the involved patients, and continuous monitoring by centralized computer algorithms can allow ineffective therapy suggestions to be quickly dismissed, and effective therapies to be quickly brought forward. In addition, feed-back of clinical observations will allow rapid identification of detrimental side-effects.

Drug toxicities from use on novel indications and in novel combinations can also be modelled by machine learning/AI. Machine learning approaches benefit significantly from large datasets. The generation of such datasets from clinical and translational studies comes with the responsibility to render the findings into improved patient management. A requirement for achieving this goal, is the application of interoperability measures such as the use of community-defined and adopted standards for phenotypic, genomic and drug response data, along with available infrastructure for FAIR (Findability, Accessibility, Interoperability, and Reuse) sharing, with consolidated ethical and legal frameworks, and with protocols for computations across datasets. The definition of data formats that can capture both perturbation data and baseline data in a standardized way will improve the use of such data as it becomes available to **molecular tumor boards**. However, the requirements for standardization and interoperability aspects are currently open challenges.

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Author contributions

S.S.S. and Å.F. wrote the manuscript. All authors reviewed, edited and approved the final version of the manuscript.

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Glossary

Artificial intelligence: The ability of a computer or machine to mimic the problem-solving and decision-making abilities otherwise typically requiring human intelligence.

Continuous Individualized Risk Index (CIRI): A Bayesian framework to dynamically determine outcome probabilities for individual patients over time.

Diagnostic test: A generic term for any approach used in clinical practice to identify the nature or severity of a condition or disease.

Drug sensitivity testing: Exposure of cells to drugs followed by assessment of cell viability.

Functional precision medicine: A strategy to inform personalized treatment decisions based on a cell-perturbation read-out such as drug sensitivity testing of the patient's cancer cells.

Machine learning: An application of artificial intelligence that allows computer algorithms to automatically improve by adapting to new data without human intervention

Microsatellite instability: A characteristic of certain DNA repair defects, where short sequences of repeated bases (microsattelites) are different in cancer cells compared to healthy cells in the same individual.

Organoid: A three-dimensional tissue culture generated from primary stem or cancer cells that is intended to mimic the properties of its organ of origin.

Patient stratification: The distribution of patients into subgroups based on similar characteristics.

Molecular tumor board: Multidisciplinary meeting to review diagnostics for referred patients, and recommend anti-cancer therapy based on advanced diagnostics. Board members typically include medical doctors, pathologists, molecular biologists, geneticists and other disciplines. Tumor boards are established at leading cancer centers worldwide.

Xenograft: The transplant of an organ, tissue, or cells to a recipient of a different species

Text boxes

Box 1: Functional precision medicine

Functional precision medicine is a diagnostic discipline that takes into account cell and tissue responses to perturbations. This is in contrast to traditional pathology diagnostics, which focuses on static conditions of cells and tissues at specific timepoints and locations of the disease [1,74]. Perturbations represent controlled modulation of culture conditions, and can include drug exposure, immune stimulation, temperature control, gene expression modulation etc. Readouts span all measurements that can reliably be collected from cultures, and can include viability or other measured states, such as proteomics, transcriptomics, and metabolomics [1]. Cells under study can include single cell types, e.g. cancer cells, or specified multiple cell types, e.g. cancer-immune cell co-cultures, or unspecified multi-cell cultures such as microtissue collections derived directly from tumors or other tissues of interest [35].

Box 2: Genomic precision medicine cannot advise on therapy for all patients

The Human Genome Project [75], which was declared completed in 2001, greatly accelerated both drug discovery targeting specific molecular aberrations in cancer, and the efforts to identify biomarkers that predict drug responses [76]. A number of single genetic-based biomarkers have since been identified and approved for clinical decision support. For example, BCR-ABL1 (breakpoint cluster region gene – Abelson proto-oncogene) fusion in chronic myelogenous leukemia (CML) is strongly linked to sensitivity to imatinib and its derivatives. For solid tumors, the BRAF (v-raf murine sarcoma viral oncogene homolog B1) V600E mutations in malignant melanoma and lung cancer, are prominent examples of biomarkers that can select patients for therapy with drugs inhibiting BRAF/MEK (mitogen-activated protein kinase kinase) signaling [77,78]. In general, overall therapy responses to a particular drug have

proven difficult to infer based on single genomic alterations. The basis for effective personalized treatment decisions is therefore shifting from single, ‘static’ genetic biomarkers to encompass a global assessment of the cancer omics data. Examples of more complex genetics-based biomarkers include genome-wide assessments of tumor mutation burden (TMB) and microsatellite instability (MSI).

With decreasing costs and higher throughput, transcriptomics-based readouts have been tested for their aptitude, not only in reporting static traits of tumor cells and tissues, but for capturing the phenotypic properties of a tumor sample. Gene-expression profiling has revealed subtypes within tumor types that do not reflect histologically recognized entities. The readouts of multiple gene transcripts can therefore yield “signatures” or “profiles” that correlate with clinical behavior and/or treatment responses beyond what can be predicted from histology-based diagnostics alone. For instance, several multi-transcript tests are now implemented in the diagnostics of breast cancer; as they provide prognostic information which can also be used for patient stratification. In the WINTHER trial [79], where transcriptomics were included in one arm as decision support for intervention, the percentage of patients that could be matched with a potentially effective therapy increased to about 35%, compared to 23% for the arm that solely used genome-informed stratification . While DNA-based markers can assume that the detected mutations come from cancer cell DNA, and not from other cell types, this is not generally true in transcriptome-based analyses. Gene expression readout represents an average across the tumor and its microenvironment; that is across cell cycle phases and cell types. A key challenge in assessing the transcriptome of multi cell-type specimens, such as biopsies, is to deconvolute the signal into the contributing individual cell types so that activities in cancer cells can be distinguished from activities in other cell types, such as immune cells or fibroblasts [80].

Tables

Table 1. Prospective studies in hematological cancers using functional assays to guide cancer therapy.

Reference	Cancer type	Patients included in the study	Functional approach	Clinical response to treatment
Kornauth *et al*, 2022 [12]	Hematologic cancers	143 patients; 56 (39%) patients received treatment	Image-based single-cell drug profiling	30 patients (54%) achieved more than 1.3-fold enhanced progression-free survival compared with their previous line of therapy
Malani *et al*, 2022 [11]	AML	186 patients; 37 patients (20%) received treatment	Drug sensitivity testing	Clinically meaningful complete or partial responses in 17 of 29 patients (59% objective response rate).
Leonard *et al*, 2016 [7]	Mediastinal germ cell tumor and AML	1 relapsed/refractory patient	Drug sensitivity testing	Stable disease (AML), relapse of metastatic germ cell tumor after 5 months of therapy
Skånland *et al*, 2022 [6]	CLL	1 relapsed/refractory patient	Drug sensitivity testing	Partial response
Yin *et al*, 2022 [20]	CLL	1 relapsed/refractory patient	Drug sensitivity testing	Partial response

AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia;

Table 2. Co-clinical trials in patients with solid tumors and matching functional testing with clinical outcome.

Reference	Cancer type	Patients included in the study	Functional approach	Clinical response to treatment
Ooft *et al*, 2019 [38]	CRC	32 patients; 12 received FOLFIRI, 10 received irinotecan, 10 received FOLFOX	Drug sensitivity testing	For predicting patient responses to FOLFIRI, the test had 100% specificity, 83% sensitivity. The test was not effective for predicting responses for the other tested chemotherapy regimens.
Wang *et al*, 2021 [81]	CRC	30 patients in pilot; 71 patients in blinded study	Drug sensitivity testing	63% sensitivity, 94% specificity
Chalabi *et al*, 2020 [82]	CRC	11 patients	Drug sensitivity testing	50% sensitivity; 100% specificity
Yao *et al*, 2020 [83]	Locally advanced rectal cancer	80 patients; all received chemoradiotherapy in a neoadjuvant setting, organoids were tested against 5-FU, irinotecan, radiation, or chemoradiation	Drug- and radiotherapy sensitivity testing	For chemoradiation 78% sensitivity and 92% specificity

Li *et al*, 2018 [84]	Esophageal adenocarcinomas	10 patients; patients received the ECX regimen (epirubicin, oxaliplastin, capecitabine), CF (cisplatin, 5-FU), or no chemotherapy	Drug sensitivity testing	For the organoid cultures that were considered insensitive to drugs prescribed to patients, the drug resistance matched the high tumor regression grades (TRG) found
Sachs *et al*, 2018 [85]	Breast cancer	12 patients with clinical follow-up data	Drug sensitivity screening	Tamoxifen was the only drug for which differential responses were recorded (1 sensitive, 1 insensitive, rest undetermined), and the organoid observed drug sensitivity matched clinical observations
Vlachogiannis *et al*, 2018 [86]	Gastrointestinal cancer	11 patients with CRC; 4 patients with gastroesophageal cancer	Drug sensitivity screening for a number of chemotherapies and targeted drugs, including paclitaxel, regorafenib, cetuximab, and investigational compounds	For the therapies administered to organoids and to patients, the organoid assays demonstrated a predictive performance of 100% sensitivity, 93% specificity, 88% positive predictive value and 100% negative predictive value, when

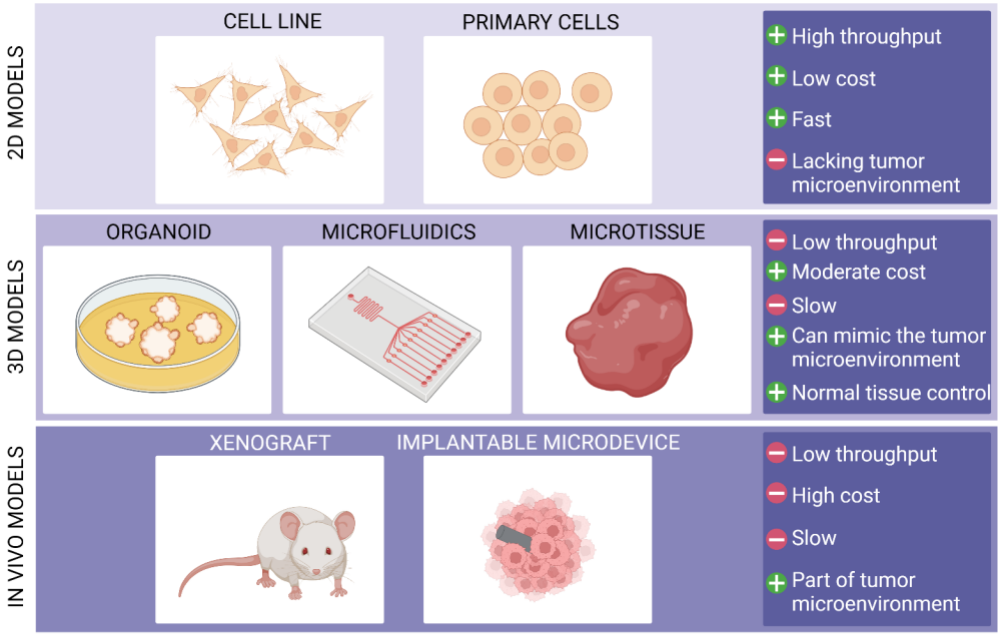
				compared to clinical response data
Driehus *et al*, 2019 [87]	Head and neck squamous cell carcinoma	7 patients for which radiotherapy testing was done and compared with clinical responses	Drug- and radiotherapy sensitivity screening	Correlation between relapses and therapy sensitivity reported; of four organoid least sensitive to therapy, three patients experienced a relapse, while for the three most sensitive organoids no patients experienced a relapse within the observed period
de Witte *et al*, 2020 [88]	Ovarian cancer	5 patients with drug sensitivity testing and clinical follow-up data.- For two patients, two organoids were derived.	Drug sensitivity testing	3 patients with organoids sensitive to therapy achieved stable disease. 2 patients with least sensitive organoids, had progressive disease
Grossman *et al*, 2022 [89]	Pancreatic cancer	11 patients with matched organoid and clinical outcome data	Drug sensitivity testing	Organoids from 4 patients were found to be insensitive to all tested drugs and patients from whom these were derived experienced progressive disease

				upon therapy with the same drug cocktail. For the organoids from 7 patients that were sensitive to at least one drug in the tested drug combination, all 7 patients experienced stable disease or better.
Kong *et al,* 2018 [90]	Locally advanced rectal cancer	17 patients with matched organoid and clinical outcome data	Functional immunotoxicity assay	All six patients who were classified as complete responders were correctly classified based on tumor-infiltrating lymphocyte scoring

CRC, colorectal cancer. Where available, test sensitivity and specificity are reported for the organoid platform's performance in predicting clinically observed patient responses.

Figure 1

a.



b.

	BASICS	STRENGTHS	LIMITATIONS
GENOMIC		• Patient benefit proven • Preserved and archival tissue can be examined • Low material need	• Static picture • "Noise" from passenger mutations, requires filtering and interpretations • Therapy response must be predicted (based on prospectively collected evidence)
FUNCTIONAL		• Dynamic • Direct therapy responses can be observed • Drug combinations can be assessed	• High material need • Need for live material • Cell selection/skewing may occur

Figure 2

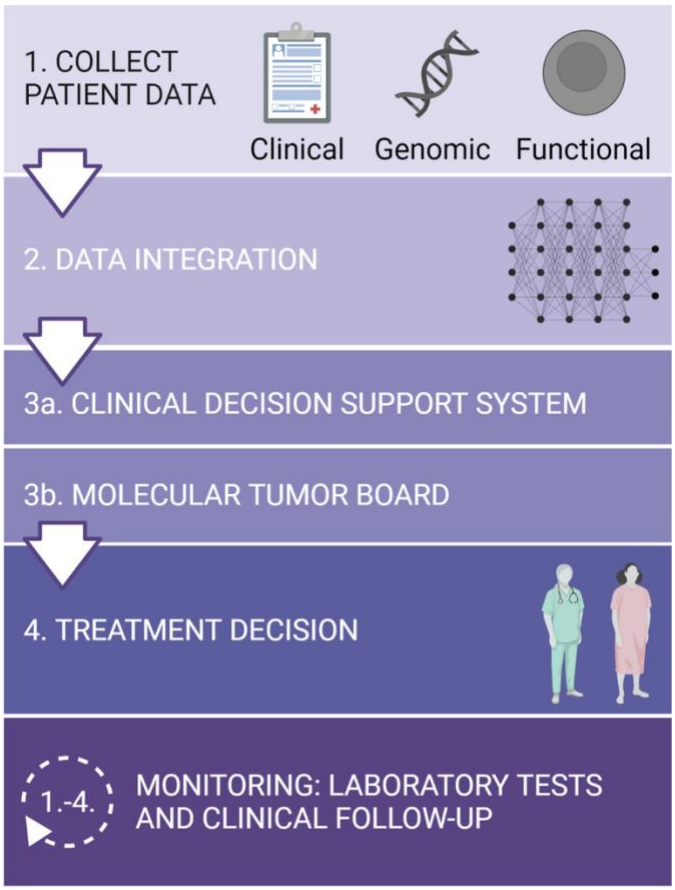

1. COLLECT PATIENT DATA
Clinical
Genomic
Functional
2. DATA INTEGRATION
3a. CLINICAL DECISION SUPPORT SYSTEM
3b. MOLECULAR TUMOR BOARD
4. TREATMENT DECISION
1.-4.
MONITORING: LABORATORY TESTS AND CLINICAL FOLLOW-UP

Figure legends

Figure 1. *Ex vivo* and *in vivo* models for functional precision medicine.

a) Available models for functional precision medicine include two-dimensional models (upper row; cell lines and primary cells), three-dimensional models (middle row; organoids, microfluidics and microtissue), and *in vivo* models (lower row; xenograft and implantable microdevice). The models have different strengths and weaknesses related to throughput, cost, speed and incorporation of tumor microenvironment (right column). The estimated time required to perform and analyze the experiments is indicated for each model (wks, weeks; mnts, months).

b) Strengths and limitations of genomic analysis and functional approaches for implementation of precision cancer medicine.

Figure 2. Model of a future dynamic precision medicine pipeline which integrates laboratory/clinical, genomic and functional data.

Different data-sets collected describing the patient's disease, including laboratory/clinical data, genomic data, and functional data. (1). Available data are integrated in machine learning models to identify multi-marker panels for prediction of treatment responses (2). The findings are considered by a clinical decision support system and a molecular tumor board (3) to guide treatment decisions for the individual patient (4). The disease is monitored continuously, and new data are fed back to the machine learning model to adapt the therapy during the course of the disease for optimal treatment.