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Precision cancer medicine: Concepts, current practice, and future developments

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[Content List](#) – This is an article from the symposium: “Cutting-edge implementation of precision medicine in Europe”.

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Precision cancer medicine is a multidisciplinary team effort that requires involvement and commitment of many stakeholders including the society at large. Building on the success of significant advances in precision therapy for oncological patients over the last two decades, future developments will be significantly shaped by improvements in scalable molecular diagnostics in which increasingly complex multilayered

datasets require transformation into clinically useful information guiding patient management at fast turnaround times. Adaptive profiling strategies involving tissue- and liquid-based testing that account for the immense plasticity of cancer during the patient’s journey and also include early detection approaches are already finding their way into clinical routine and will become paramount. A second major driver is the development of smart clinical trials and trial concepts which, complemented by real-world evidence, rapidly broaden the spectrum of therapeutic options. Tight coordination with regulatory agencies and health technology assessment bodies is crucial in this context. Multicentric networks operating nationally and

From the symposium: Cutting-edge implementation of precision medicine in Europe.

internationally are key in implementing precision oncology in clinical practice and support developing and improving the ecosystem and framework needed to turn invocation into benefits for patients. The review provides an overview of the diagnostic tools, innovative clinical studies, and collab-

orative efforts needed to realize precision cancer medicine.

Keywords: precision cancer medicine, personalized oncology, clinical trials, molecular diagnostics, precision medicine networks

Introduction

Precision oncology is a multifaceted endeavor, necessitating a rapidly increasing amount of diagnostic and treatment predictive information to inform decisions on treatment and enrolment in clinical trials. Various omics in addition to the clinically established genomic analyses have started to make their way into clinical laboratories, adding complexity and an increased need for processing of data with machine learning as a promising tool. To make use of the novel treatments and added information, innovative study designs are paramount.

In this review, (i) emerging trends in diagnostics, (ii) the need to understand disease complexity, and (iii) state-of-the-art as well as future directions for clinical studies in oncology are described. The review ends with (iv) examples of national precision medicine infrastructures that integrate these developments.

Diagnostically, the review covers methodological aspects and clinical implications of key approaches that extend beyond current DNA-based genotyping of tissue, well established in current clinical molecular pathology labs. The first is the use of information not only from individual variants but also patterns derived from larger datasets: mutational signatures, DNA methylation, or RNA expression profiles as well as proteomics and proteogenomics add new layers of information. DNA methylation and RNA expression profiles are already integrated in clinical routine for select malignancies and broad, early testing using comprehensive and multilayered profiling strategies (DNA, RNA epigenetics, proteins)—which support diagnostics (e.g., tumor typing) and therapy prediction at the same time—will likely become a standard in the near future. The new demands for information necessitate centralized structures that have both sufficient capacities and capabilities. Another approach to add clinically important information

is to apply techniques used for tissue analyses on other analytes. The most important example—the analysis of blood using liquid biopsies—is described in detail with examples of both circulating tumor DNA (ctDNA) and circulating tumor cell (CTC) applications. Liquid biopsies already play a certain complementary role in predictive testing, notably in non-small cell lung cancer (NSCLC) patients with stage IV disease, and it is quite conceivable that this field will expand to other cancer types and indications including the use of conventional chemotherapy and de-escalation strategies [1]. The third important direction of diagnostic development is the massive upscaling of data processing necessitated by the patterns of changes and the integration of multilayered data, offering a possibility to make better use of all analytical tools.

An example of where novel therapeutic approaches have both transformed patient outcomes and challenged the clinical testing is the field of immunoncology, enabled by a better understanding of the complex interaction between malignancy and host immune defense.

To develop new therapeutic strategies in the precision medicine context, clinical studies are crucial and must use established clinical routine tools as well as adding biomarkers with the potential to become the next generation of treatment predictive tools. Current state-of-the-art and novel clinical study designs adapted to the complexity generated by granular diagnostics and to regulatory needs are presented.

Finally, building the infrastructure needed to support the development of both diagnostics, treatment and clinical studies have emerged as an important challenge. To address this, precision medicine initiatives have been formed in most developed countries, either as a result of top-down decisions or as bottom-up initiatives. Challenges and possibilities of national efforts are illustrated by experiences from the French and Danish

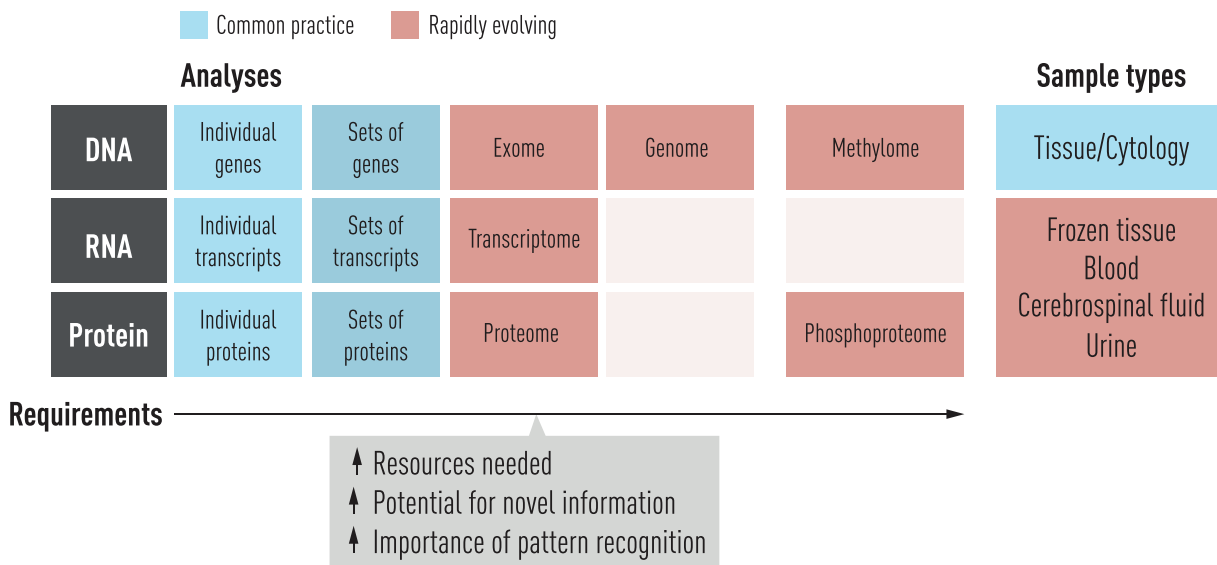


Fig. 1 Transition from single biomarker analyses to comprehensive multilayered diagnostic profiling in precision cancer medicine. High-throughput analyses enable scalable comprehensive characterization of cancer relevant biomarkers in increasing numbers of different sample types. Areas of routine clinical pathology are shown in green color, and areas still under development for broader clinical implementation are shown in red frames.

initiatives, partly shaped by differences between the countries in which they were formed.

Diagnostic tools

Cancer diagnostics is rapidly evolving from largely relying on morphology and assays assessing individual biomarkers to more comprehensive analysis approaches. The following examples delineate the current landscape and future developments in “omics”-analyses for precision oncology. Figures 1 and 2 summarize core considerations of these examples.

Mutational signatures as potential biomarkers for cancer prognosis and treatment prediction

Tumor genomes are shaped by mutational processes of exogenous (e.g., cigarette smoking and UV-light) or endogenous (such as defective DNA repair or 5-methylcytosine deamination occurring over time) nature that imprint alterations of different types and patterns in the tumor DNA. Mutational processes can act in parallel, sequentially, or during short periods of time in tumor evolution, and occur in either a clonal or a subclonal manner. This creates different patterns (like SBS mutations of a specific type and context) of DNA damage often referred to as “genetic scars,” in the tumor genome. Based on advancements in sequencing

technology combined with mathematical methods, many mutational processes have now been deconstructed into “mutational signatures” offering a mutational portrait of tumor evolution over time. Mutational signatures are currently divided into four main categories: SBS, double-base substitution, small insertion/deletion, and structural rearrangement (SV) signatures [2]. SBS signatures represent the most studied type, defined as a specific SBS pattern in a trinucleotide context (including the bases 5′ and 3′ of the mutated position), with yet 49 known signatures identified across cancers [3]. Over time, associations/correlations of several SBS signatures with specific mutational processes and clinical features have been proposed, including BRCA-deficiency (SBS3), mismatch repair deficiency (MMRd, SBS26 and SBS44), age at diagnosis (SBS1 caused by 5-methylcytosine deamination), smoking (SBS4), UV-light (SBS7), and apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) mutagenesis (SBS2 and SBS13) [4].

Although mutational signatures can reveal environmental and endogenous sources of mutagenesis in tumors, they have become interesting also for clinical use as a physiological readout of DNA damage caused by specific DNA repair deficiencies that have been shown to be predictive of response to

certain therapeutic regimens. Currently, the most clinically important applications are estimation of homologous recombination deficiency (HRD), relating to deficiency in repairing double-strand breaks, DSBs, through homologous recombination [HR] and MMRd. But in order for mutational signatures to be clinically useful, robust predictors are needed. Since the first reports of mutational signatures in 2012 [5], mutational signatures have been estimated through various technological platforms like whole-exome sequencing (WES), whole-genome sequencing (WGS), and targeted next-generation sequencing (NGS) panels fused with different mathematical tools (see e.g., Refs. [2, 6–8] for extensive reviews). For HRD detection, clinically validated assays exist (like myChoice CDx from Myriad Genetics and FoundationOne CDx from Foundation Medicine), and for MMRd, multiple algorithms suitable for NGS data have been reported (mSINGS, MSIsensor, MSIseq [9–11]). In addition, comprehensive classifiers fusing WGS data with machine learning have also been reported, like HRDetect [12] and CHORD [13] for HRD prediction, and MMRDetect for MMRd prediction [14], although these classifiers still lack full clinical validation.

As alluded to above, the currently most important clinical applications of mutational signatures lie in the prediction of MMRd and HRD in tumors. MMRd—caused by the inactivation of mainly the MSH2, MSH6, PMS2, or MLH1 genes (shown to be causal in experimental models [14])—has been shown to infer increased sensitivity to immunotherapies [15]. This increased sensitivity of MMRd tumors appears irrespective of tumor type, leading to the approval in 2017 by the US Food and Drug Administration (FDA) of pembrolizumab for tumor-agnostic use in any resectable or metastatic solid tumor with MMRd [16]. Compared to MMRd, HRD is more frequent, with particularly high frequencies in ovarian, breast, prostate, and pancreatic cancers [17]. Causes of HRD in these malignancies vary both regarding the gene(s) thought to be causative (typically DNA repair genes like BRCA1, BRCA2, PALB2, and others) and the mechanism of gene inactivation (e.g., somatic/germline variants and promoter hypermethylation). The main clinical indications of HRD today are increased sensitivity to poly(ADP)-ribose polymerase (PARP) inhibitors and DSB inducing chemotherapies like platinum-based agents (see Ref. [8] for extensive overview of clinical studies). HRD-positivity can be estimated/determined in

several ways by large-scale genomic aberrations (telomeric allelic imbalances, LOH, and large-scale transitions), presence of SBS3, presence of an indel signature with microhomology at deletion junctions, presence of two specific SV signatures, or by classifiers like HRDetect, CHORD, and SigMA that consider all or different sets of these readouts [2, 8, 18]. Based on HRD prediction, it has, for instance, been shown in clinical trials that HRD-positive ovarian cancers benefitted from PARP-inhibitors, even in the absence of BRCA1/2-mutations [19, 20], thus establishing HRD status as a treatment-predictive factor in ovarian cancer. In addition, in preclinical studies, WGS-based assays—such as HRDetect—can predict response to adjuvant chemotherapy in early-stage triple negative breast cancer [21], sensitivity to platinum treatment [2, 22], and PARP inhibitor sensitivity in breast cancer [23].

To further increase the feasibility of integrating mutational signatures in clinical decision-making, considering that WGS is not yet established as clinical routine in most diagnostic units, alternative sequencing approaches might be required (e.g., Refs. [24, 25]) or adapted targeted DNA panels appropriate also for formalin-fixed paraffin-embedded tissue. Another intriguing aspect is whether mutational signatures can be robustly detected in cell-free DNA (cfDNA), with early reports suggesting that it may be possible [24, 26]. If properly validated, cfDNA-based mutational signature analysis could provide a new way of monitoring responsiveness to directed DNA damage therapy in the future. Taken together, clinical usage of mutational signatures would bring us one step further to using even more aspects of the tumor genome to better inform individual patient treatment decisions.

Methylation arrays as diagnostic tools

Another molecular pattern that has proven highly clinically relevant for select clinical questions is that of DNA methylation. DNA methylation pattern of all cells relates to cell type and differentiation status. As a result, tumor cells exhibit a methylation pattern, which is typical for the lineage of origin. This connection is so strong that it has shown high suitability for tumor classification already demonstrated for human brain tumors and sarcomas [27–29]. The strong correlation of the overall methylation pattern in tumor DNA with cell lineage is only mildly affected by alterations due to tumor formation or progression.

Similar to gene expression, methylation patterns in tumor DNA can be assessed by different technical approaches. Most commonly used are array technology and sequencing upon bisulfite conversion as well as “nanopore” sequencing of native DNA. In principle, all three of these common approaches are suitable for providing data for methylation-based classification. There are pros and cons for every approach: Array technology is restricted to predefined CpG islands and entails dependency on single suppliers. Sequencing of bisulfite-converted DNA provides a broad spectrum and independency of technical platforms; however, it requires a higher level of data curation. “Nanopore” sequencing offers short preparation and analysis times; however, it currently exhibits a high rate of reading errors. For diagnostic use, array-based data acquisition is most frequently used for its standardized technology, technical simplicity, and the experience of very good results upon employing DNA from formalin-fixed and paraffin-embedded tumor specimens [29].

Diagnostic application requires standardized data generation protocols allowing interlaboratory comparisons and universally applied algorithms for data analysis. Currently, the most frequently used tools are so-called classifiers based on random forest methodology. These classifiers build on so-called tumor reference groups containing homogenous sets of the specimens most accurately diagnosed by the current standards, including histological, immunohistochemical, molecular, and clinical data. A set of several thousand CpG sites form the methylation arrays maximized for highest distinction between the given tumor reference sets is selected. Using this selection of CpG sites, approximately 10,000 decision trees are constructed. Each starts with a root node and contains a large number of terminal nodes, each of them linked to a specific diagnostic suggestion. Adding up, processing and analyzing the diagnostic suggestions provide a basis for the output of these classifiers, a diagnostic prediction (www.molecularneuropathology.org and www.molecularsarcomapathology.org).

The most mature methylation-based classifier is the brain tumor classifier first released in 2018 [29] and updated in 2021. The initial version recognized 82 different methylation groups, whereas the current version (v12.5) distinguishes 176 tumor methylation groups. This increase in tumor methylation groups is directly related to

the database, which in turn increases with use of the classifier by a growing international community. A basis of currently more than 100,000 datasets resulted in the identification of many novels and stratification of several established brain tumor types. Common to the novel tumor types is their overall rarity. Previous histology-based classification approaches failed to identify sufficient numbers of similar tumors for defining a novel tumor group. There is also a second type of group emerging characterized by histological pleomorphism preventing recognition on morphological grounds.

Array-based methylation data allow calculation of high-density chromosomal copy number profiles (CNP). Gene amplifications and homozygous deletions are immediately evident, and specific chromosomal gains and losses can be directly read from the CNP. Such data are useful for tumor entities with recurrent chromosomal alterations.

There is obvious interest in methylation-based classifiers; however, it must be kept in mind that most central classifiers are tools and not diagnosticians. A classifier cannot yet integrate the entire clinical presentation essential for a precise diagnosis in many settings. Methylation-based tumor classifiers—such as gene expression-based classifiers—currently only predict what they have been taught to recognize. They are not yet certified medical devices. On the other hand, classifiers identify methylation groups with a homogeneity hardly achievable by classical diagnostic approaches thus perfectly suited for study settings. They turn out predictions which are free of interindividual variations. They can also be trained to recognize extremely rare tumor groups based on reference sets arising from pools based on multi-institutional contributions.

In conclusion, methylation-based tumor classification has proven a powerful tool for many tumor types. Similar to the evolution of histology-based tumor classification, effort and input from an entire diagnostic community are needed. Will it be possible to establish tools and platforms receiving worldwide acceptance? This clearly calls for interinstitutional and international cooperation. Further, will there be successful integration of this tool in national health and insurance remuneration systems? Progress on this level demands input and coordination beyond scientific and diagnostic interests.

RNA sequencing for molecular classification of solid tumors

From a diagnostic perspective, RNA transcripts have also been proven to be a valuable tool for fusion gene detection, avoiding the problems of, for example, repetitive intronic DNA regions [30].

The use of unbiased RNA sequencing is, however, linked to gene expression patterns, a diagnostic tool sharing several key features described in the context of methylation arrays.

Initially using cDNA microarrays, differences in RNA expression patterns of breast cancer patients have been clarified [31, 32] and are used to identify clinically relevant molecular subtypes and to risk classify to inform treatment decisions [33–35].

As a diagnostic tool, RNA sequencing is currently mainly used to patients suffering from carcinomas of unknown primary (CUP). CUP is a histologically confirmed metastatic cancer for which the identification of the primary site has not been possible after an appropriate diagnostic approach that includes patient history, physical examination, imaging, pathology, and immunohistochemistry [36]. CUP accounts for less than 5% of all cancers and is associated with a poor prognosis. RNA sequencing is used to add diagnostic information but is also recommended to complement DNA sequencing for theranostic purposes, in order to identify druggable molecular alterations.

Various RNA-based techniques have been used over the last decade, aiming to identify the tissue of origin in CUP patients. These techniques include RT-qPCR [37], RNA microarrays [38], and—more recently—RNA sequencing [39–41]. RT-qPCR was introduced clinically as a robust, cost-effective way to assess expression levels for a limited number of transcripts identified through a more comprehensive gene expression analysis. cDNA microarrays filled this role but were limited to predefined transcripts. RNA sequencing has now largely replaced cDNA microarrays. It is dependent on high-quality RNA but has made it possible to analyze the entire transcriptome in one assay and to train algorithms using data from global analyses within large-scale cancer sequencing efforts such as The Cancer Genome Atlas (TCGA) or the International Cancer Genome Consortium [42]. All these techniques demonstrated their ability to accurately identify the tissue of origin in most cases. The clinical utility of these techniques has been evaluated in non-

randomized [39, 43] and randomized clinical trials comparing the outcome of patients treated according to the identified tissue of origin versus patients treated empirically, usually with platinum-doublet chemotherapy [44, 45]. Although non-randomized trials reported encouraging results with overall survivals exceeding 1 year, none of the randomized trials demonstrated an overall survival benefit when using RNA sequencing. These results might be explained by the fact that the reference treatment was relevant for a substantial number of patients of the experimental arm, therefore diluting the overall treatment effect.

Within the French Genomics Plan (Plan France Médecine Génomique 2025), a national CUP molecular tumor board (MTB) was set up in 2020. The TransCUPtomics RNA sequencing tool was developed and implemented as part of this MTB in 2022. TransCUPtomics not only uses RNA sequencing but also deep-learning approaches [40]. The tool has been trained to identify tissues of origin on datasets from 20,000+ samples, including 39 different tumor types from TCGA and 55 normal tissues from GTEx. The CUP MTB is composed of pathologists, molecular biologists, and medical oncologists who meet on a monthly basis, and the tissue of origin could be established in 64% of patients.

In conclusion, RNA sequencing is a powerful tool that can be used in the clinic to help subtype and risk stratify but also to aid in identifying the tissue of origin of CUP patients. As exemplified by the French CUP MTB, it can be successfully implemented in the clinic. Cross-border pooling of data, based on efforts such as the French CUP clinicogenomic database, will be important to further develop the algorithms used, and with added data, machine learning approaches will become increasingly important. From a clinical perspective, further data and work are also needed to assess the effects on patient outcomes.

Proteogenomics: a new dimension of information

The potential to gain clinically relevant data from analyses of the proteome is significant, and lots of effort is currently put in turning proteomics into a routine tool for cancer diagnostics [46–49]. Proteogenomics can elucidate genome–proteome connections including an analysis of sequence variants all the way to proteome impact of cancer genome alterations [50, 51].

Typically, a tumor has a myriad of genomic changes rewiring protein networks, driving cancer development. These genomic alterations are overlaid on each individual's germline genome. How these genome alterations synergistically alter proteome networks in cancer is impossible to predict with current knowledge. Transcript and protein level correlation are to a relatively low extent explained by the multiple regulatory levels impacting protein abundances, such as transcriptional, translational, protein turnover regulation [52]. Hence, efforts generating direct systems-level information on the proteome, representing an important layer of molecular phenotype, have been a rapidly growing field [53].

Methodologically, mass spectrometry (MS) analysis is the most used and versatile tool for generating proteome-wide information on clinical samples. Due to rapid development in the MS-field, today a comprehensive analysis of cell and tissue proteomes on tens and hundreds of clinical samples is feasible. Further, in-depth proteome analysis data can be used for the development of sensitive high-throughput analysis for a quantification of sets of proteins without need of antibody development [54]. Interesting developments have also taken place in affinity proteomics, especially in blood plasma proteomics—for example, by using antibodies in proximity extension assays [55, 56] or aptamers in plasma proteomics analysis [57]. Moreover, the entire field of increased multiplexing of immunohistochemistry analysis of tissues provides interesting spatial proteome information on a limited selection of proteins, providing valuable insights, for example, on immune infiltration phenotypes [58]. Finally single-cell proteomics is entering the scene by MS-analysis as well as using multiplex flow and mass cytometry analysis [59].

MS-based proteomics uses peptide databases to identify proteins in analyzed samples. These databases are usually constructed using reference genome of an organism. In cancer samples, such databases will miss all the altered proteins caused by cancer genome aberrations. Detection of cancer-specific proteins—so-called tumor-specific antigens or neoantigens caused by somatic genomic aberrations—is achieved by using tumor DNA and RNA sequence data to enrich the search databases by sampling specific sequencing data or, if not available, concatenated data from repositories.

Fast development in proteogenomics is demonstrated by many recent large studies on tumor cohorts with the discovery of distinct proteome subtypes, for example, of breast cancer [60, 61], lung cancer [62, 63], colon cancer [64], and many other cancer types. Common findings of these studies are discovery of prognostic proteomic subtypes that differ from transcriptomics and genomics subtyping, hence adding orthogonal information in cancer classification. Moreover, quantitative proteomics can refine information on how cancer-associated SNV, mutations, and copy number alterations impact protein levels in a cancer type-dependent manner and connect these to treatment possibilities [65].

Cancer immunotherapy targeting host-tumor interaction is an area in which proteomics and proteogenomics have a high likelihood of playing an important role to develop precision medicine. As an example, lung cancer proteogenomics analysis defined proteome subtypes associated with immune states and demonstrated a relation to cancer antigen burden. An interesting observation was that apart from tumor antigen burden, particular types of antigens impacted the proposed immune evasion mechanisms [62].

Both the affinity proteomics methods—currently vastly used in blood plasma analysis—and MS-methods used for body fluids, cells, and tissues provide valuable information for diagnostics and precision medicine. However, standardization and harmonization of analytical assays and platforms are needed to allow an analysis of large-scale clinical cohorts. This vast cohort analysis is needed to connect the proteome data output to clinical correlates and aid in defining clinical proteomics data cutoffs for various outcomes. The work that has started in several places to develop real-world prospective workflows at hospital settings needs to be scaled up to take next steps for proteome-driven biomarker trials. It is safe to predict that direct analysis of proteomes is going to be a crucial component for effective precision medicine. This is due to molecular information which is best studied at the protein level, such as complex regulation of cellular protein networks, protein's role in mediating cell-to-cell interactions, role of the extracellular matrix in cancer regulation, soluble peptides and protein mediators impacting tumor growth, microenvironment, and metastasis. However, major efforts are needed to convert biomarker discovery proteomics to predictive biomarker assays

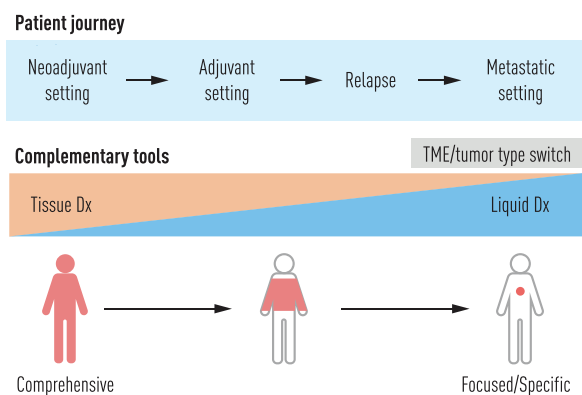


Fig. 2 Adaptive use of diagnostic tools during the course of disease. The analyses used throughout the patient's journey are adapted to clinical needs with comprehensive tissue-based testing at an early stage. Complementary tools, such as liquid biopsies, will be increasingly used during later disease stage to monitor disease and to identify resistance mechanisms. TME, Tumor microenvironment.

benefitting clinical decision-making. This will require new types of collaborative networks within the proteomics research community as well as true integration of proteomics in multidisciplinary collaborations to leverage lessons already learned in genomics, transcriptomics, and imaging-based precision medicine development.

Current clinical use of liquid biopsy in precision cancer medicine

The majority of cancer-related deaths are caused by the bloodstream-mediated metastatic spread of tumor cells from the initial lesion to distant regions. The identification and molecular characterization of CTCs in blood samples from patients with cancer has opened a new avenue to study blood-borne tumor cell dissemination.

Clinically, liquid biopsies (e.g., CTCs and ctDNA analyzed using noninvasive blood samples collected throughout the disease course) can be used for (i) early detection of cancer (using higher blood volumes), although screening is still challenging; (ii) tumor staging and monitoring of patients with localized cancer (to discriminate patients at low and high risk of recurrence); (iii) predicting metastatic progression in patients with advanced cancer; (iv) monitoring the efficacy of therapies and discriminating between early responders and

nonresponders; and (v) tracking tumor evolution with the identification of therapeutic targets and of resistance mechanisms.

Considering the current clinical use of liquid biopsies in precision cancer medicine, the first liquid biopsy test approved was for NSCLC [66]. Indeed, NSCLC is the model tumor to test liquid biopsy due to the many gene alterations (e.g., *EGFR*, *ROS1*, *ALK*, and *BRAF*) relevant to treatment. Patients with NSCLC harboring specific *EGFR* gene mutations are more likely to respond favorably to *EGFR* tyrosine kinase inhibitors. According to international recommendations, individuals with advanced NSCLC of non-squamous subtype should undergo molecular testing. However, it is often difficult to collect a lung tissue sample. Therefore, by using the ctDNA present in peripheral blood samples, liquid biopsies can be used to identify the patients who are candidates for *EGFR*-targeted therapy. Liquid biopsy testing is minimally invasive, is repeatable, and allows for the identification of gene alterations and their monitoring during the disease course. Many studies indicate that molecular testing results obtained using tissue and plasma samples are highly concordant, and the analysis of driver gene mutations in ctDNA has been implemented in clinical practice for patients with NSCLC following FDA and EMA approvals as well as ESO recommendations [67]. Although simple somatic mutations are quite easily detected, copy number changes and more complex biomarkers (e.g., HRD) can be challenging to identify. As with tissue-based panels, the detectability of breakpoints indicating gene fusions requires appropriate assay design. Importantly, tumor biology (high- vs. low-shedding tumors) significantly influences test parameters (e.g., sensitivity) of any assay as the number of DNA molecules per target locus needs to match the desired read depth per target locus. Other factors interfering with ctDNA quality and quantity and thus, for example, sensitivity and specificity of the liquid biopsy assay are pre-analytics and logistics. In other words, parameters unrelated to the power and performance of the sequencing machine in use very much shape the test performance of ctDNA-based analyses [68], a scenario that is sometimes overlooked in discussions around ctDNA testing. Another important aspect is the detection of variants related to clonal hematopoiesis [69, 70], which need to be accounted for in the testing approach and interpretation of variants identified in ctDNA molecules.

The *liquid biopsy* concept was introduced for the first time for CTCs in 2010 [71]. However, to obtain a comprehensive real-time view of cancer progression, we need to consider a broader definition of liquid biopsy that goes beyond CTCs and that includes also (i) other *tumor-derived circulating biomarkers*, such as cell-free ctDNA, circulating cell-free RNA (noncoding and messenger RNA), extracellular vesicles (oncosomes), tumor-educated platelets, and proteins; and (ii) *immune cells and circulating components of the immune system*, such as immune cells, cytokines, and interleukins. In addition, the liquid biopsy concept has been expanded to other physiological fluids: cerebrospinal fluid, urine, bone marrow, sputum, and saliva [72]. According to recent studies, the microbiome plays a significant role in cancer immunotherapy [73]. Thus, in 2023, the liquid biopsy definition must be expanded to the detection of the circulating microbiome in the blood coined *liquid microbiopsy*, which is the analysis of circulating cell-free microbial DNA in combination with a specific panel of proteins.

Clinical trials have also shown clinical utility for liquid biopsy testing in a growing number of malignancies. The trial CTC METABREAST (NCT01710605) demonstrated, for the first time, CTC clinical utility to decide whether a patient with metastatic breast cancer (MBC) should receive chemotherapy or hormone therapy [74]. In this study, patients with MBC were randomized into two arms concerning the choice of first-line treatment: clinician's choice and CTC count-based choice. In the CTC arm, patients with ≥ 5 CTC/7.5 mL received chemotherapy, whereas patients with < 5 CTC/7.5 mL received endocrine therapy [74]. The primary endpoint (i.e., progression-free survival) was met, showing that CTC count is a reliable biomarker for choosing between chemotherapy and endocrine therapy as first-line treatment in hormone receptor-positive, HER2-negative MBC.

CTCs have also been shown to predict treatment response to immunotherapy. In 2015, CTCs from patients with MBC were shown to express PD-L1 [75]. Thus, enumerating and also characterizing CTCs can be informative. Indeed, several groups have demonstrated that the PD-L1 expression profile of CTCs is clinically relevant in MBC [76] and also in metastatic NSCLC [77] and head-and-neck cancer [78]. As many new drugs and innovative therapies currently in development target protein

markers on tumor cells, CTCs—as a representative subset of more aggressive disseminating tumor cells—will undoubtedly become a key companion biomarker for cancer management in the near future. Moreover, the rare CTC lines that have been generated [79, 80] are metastasis-competent CTCs and are a crucial tool in the liquid biopsy field because they can be used to screen potential drug candidates and test their efficacy in this more aggressive subset of cancer cells *in vitro* before moving to animal studies and clinical trials.

To widely implement liquid biopsy in clinical practice, more interventional clinical trials are paramount. Importantly, the standardization of preanalytical and analytical methods is a prerequisite for its widespread clinical application.

Artificial intelligence to handle the vast amounts of data—challenges and possibilities

Even without a multilayered approach for cancer diagnostics in place, artificial intelligence (AI) has started to demonstrate its potential throughout medicine and pathology, in particular. With large amounts of image data in the form of histological slides that fill pathologists' workdays by requiring careful visual inspection for detecting pathological aberrations, machine learning raises high expectations about facilitating faster, standardized, and quantitative slide evaluations. A plethora of papers showing the capabilities of deep-learning approaches to analyze histological slides have led to claims that AI will soon revolutionize diagnostics and even replace pathologists. Although it can certainly be expected that AI will have a major impact on cancer diagnostics based on histopathology and beyond, current capabilities have to be carefully reviewed to manage expectations. Recently published studies [81] have in common that they show successful application of AI for relatively easy diagnostics tasks, such as detection of common cancers (breast, colon, and lung carcinoma or melanoma) against a background of normal tissues. Not only are these tumors morphologically relatively "simple," most of these studies also do not include difficult small biopsy samples, benign mimics of malignant tumors or borderline cases which require relevant experience. Although it would, in principle, be conceivable that AI could be trained beyond simple tasks, this would require substantially more data than currently available at any single institution or typical study consortium.

This *first challenge* of taking AI into clinical routine lies in the fact that the distribution of diseases is highly skewed and has a long tail: A few diseases are very frequent, whereas a vast number of diseases are rare, further aggravating the data availability problem. Particularly when it comes to applying for approval of such classification algorithms, the fact that not all possible diagnoses can be trained for will certainly pose a problem. It is therefore likely that current approaches will have to be complemented by novel machine-learning approaches that account for rare cases that may not be classifiable by the respective AI, but where the AI can be trained such that the unknown cases are labeled unknown and not attributed to the computationally closest, but incorrect, class.

The *second challenge* lies in the “black box” design of most current AI approaches that result in a lack of transparency of the decision process. Users have to “believe” the classification result and cannot verify it. This is a severe limitation, particularly in diagnostics in which physicians need to be able to at least make plausibility checks on the test results. Here, so-called explainable AI (ExAI) approaches offer a solution. Although they cannot truly causally “explain” the machine-learning result, they can trace back a result to the input data and identify the components of the input data that are most relevant for the prediction result. This can on one side help identify “Clever Hans” effects [82] in which the machine reaches a decision based on confounders but may on the other hand also facilitate the discovery of, for instance, novel biomarker signatures. For image data, the “explanations” are usually provided in the form of heatmaps [81, 83], but ExAI can help better understand any type of data, including high-dimensional omics profiles.

The *third challenge* relates to the generalizability of machine-learning models. Most current studies still use monocentric data for model training and performance estimation. However, it is becoming increasingly clear that this does not result in sufficiently robust models which generalize to real-world data from other sources. This poses a major limitation on the practical relevance of AI models and is gaining increasing attention [84]. Datasets have to be multicentric and balanced to account for, in the case of tumor histology, the full spectrum of morphological subtypes. Moreover, efforts have to be made to improve preprocessing and machine-learning techniques to become less sen-

sitive to staining variabilities and artifacts and to better capture relevant data properties and avoid overfitting.

Finally, although image analysis is a major focus of AI developments in pathology as described above, molecular “omics” profiling is another domain with high potential for AI to change the way we deal with such data. Although conventional NGS panels used nowadays in routine diagnostics to identify targetable mutations can be handled with classical data analysis strategies, increasingly common high-dimensional genomics and proteomics may benefit from machine-learning approaches by helping reduce the dimensionality and predict properties such as gene regulatory networks [85, 86] or DNA-methylation data [87, 88]. In this context, single-cell sequencing approaches are particularly well suited for AI-based analysis as they provide high-dimensional data for a high number of samples (tens of thousands of cells per experiment) [89] and, thus, allow training success beyond what is possible for bulk analyses in which numbers of samples are almost always smaller than the number of molecular measurements.

Although the abovementioned examples illustrate what AI can achieve already today and what limitations exist, whether AI will not only improve current diagnostics but also really revolutionize medicine hinges on its ability to integrate the different heterogeneous imaging, omics, and clinical data modalities and robustly predict patient outcome. To achieve this, both digitizations in medicine, including data standardization and structured reporting as well as machine-learning techniques, will have to be further advanced in the coming years.

Genomics in immuno-oncology—opportunities abound

The recent emergence of effective immunotherapies for cancer has transformed the landscape of systemic treatment for many solid and hematologic malignancies, as reflected by the approval of more than 50 new drugs by the FDA over the past decade [90]. Although the field was jump-started by the remarkable antitumor activity of antibodies blocking inhibitory receptors (termed immune checkpoints) and the majority of FDA approvals comprise anti-PD-1 based therapy, this class of agents has recently been complemented by other immunotherapeutic modalities including CAR T cells, bispecific antibodies, oncolytic

viral therapy, and antibodies directed against the novel immune checkpoint LAG-3 [91–93]. Despite these successes, primary resistance and/or secondary resistance to immunotherapy remains a substantial challenge for the field of cancer immunotherapy, and some of the most common cancers—including breast, colorectal cancer, prostate cancer, and pancreatic cancer—are largely unresponsive to the cancer immunotherapies available to date [94].

A clonally diverse and constantly evolving tumor is a highly complex ecosystem—as is the host's innate and adaptive immune system with its various immune cell types, cell surface receptors (mediating activation, inhibition, and antigen-specificity), and soluble factors (cytokines and chemokines). The interaction of these two systems over time (temporal), within the tumor and in different tumors present in various anatomic locations (spatial), has been conceptualized by the term “immune editing” [95]. Interrogation of these two systems, and importantly their intersection, has been greatly improved by the recent availability of NGS and single-cell sequencing tools. The ability to characterize immune cell populations, including their specificity, phenotypes, and function with much higher resolution, has already given unprecedented insights. For example, the dissection of human melanoma tumors using single-cell RNA and T-cell receptor (TCR) sequencing now allows for establishing a link between the antigen specificity and phenotype of tumor-reactive TCRs. Using this approach, tumor reactive CD8⁺ T cells were recently identified as exhibiting an exhausted phenotype and lack of memory cell properties, and for a substantial proportion of tumor reactive TCR, the recognized antigens could be identified as either neoantigens or melanoma-associated antigens [96].

Given their tumor specificity and lack of central tolerance as well as universal presence in tumor genomes, neoantigens encoded by genomic variants provide potentially formidable targets for cancer vaccines and adoptive T-cell therapy—the two main therapeutic approaches for neoantigen-specific therapy that have been tested in the clinic. Because of the universal presence and mostly private (non-shared) nature of the majority of genomic variations in cancer genomes, neoantigen-directed therapy is, in principle, applicable for most cancer patients; however, such approaches need to be customized to individual patients. The feasi-

bility, safety, and robust immunogenicity of personalized vaccines directed at neoantigens have already been demonstrated in patients with cancer. Early signals of antitumor activity have included objective tumor regression or decreased recurrence events in patients with melanoma, decreases of ctDNA associated with improved overall survival in patients with colorectal cancer, and pathologic tumor response as well as immunological surrogate of vaccine-induced tumor cell killing called epitope spreading, all associated with superior outcomes in patients with melanoma and other cancers [97, 98]. Adoptive therapy of tumor-infiltrating lymphocytes recognizing tumor neoantigens has demonstrated the ability to mediate durable regression of metastatic tumors in patients with solid tumors, including cholangiocarcinoma and breast cancer [99].

Further supporting neoantigens as key targets of antitumor immune responses, a correlation between high TMB (TMB-H) and improved outcomes after anti-PD-1-based therapy has been demonstrated in patients across a wide spectrum of tumor types [100]. As such, TMB in association with other markers—including expression of PD-L1 and T-cell inflammatory gene expression signatures—can help in predicting the clinical efficacy of these therapies. In fact, TMB-H (≥ 10 mutations/megabase) on its own was established as a reliable marker of response to PD-1 inhibition, leading to an accelerated FDA approval for pembrolizumab in patients with TMB-H tumor independent of the histology—a first in the field of immune-oncology.

Clinical trials

Similar to diagnostics, clinical trial designs reflecting the concept of precision medicine have rapidly evolved over the last 15 years. In the following section, we describe different trial concepts, distill challenges, delineate new approaches, and explore the role of academia in trial designs (Fig. 3).

A conceptual overview on current clinical trials designs

The design of clinical trials for precision medicine in cancer typically involves several key considerations but start with patient selection and biomarker testing.

- Patient selection: Most druggable genomic alterations are present in a small proportion of cancer patients. Although many driver

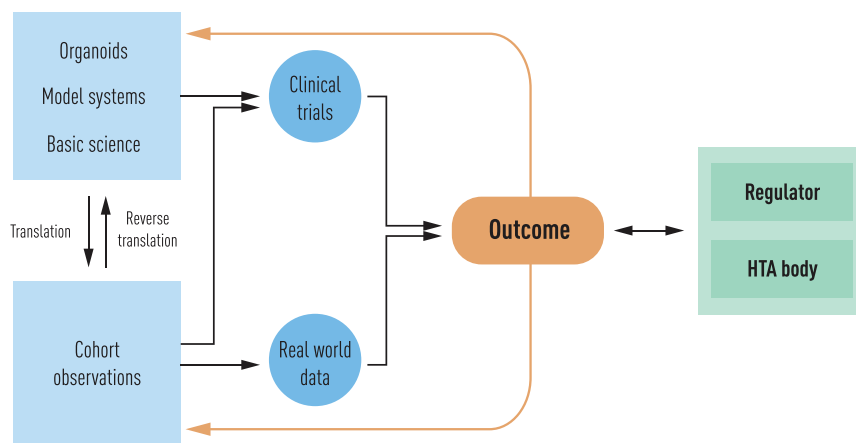


Fig. 3 Trial designs in precision cancer medicine. Adaptive trial designs involving real-world data (RWD) will become increasingly important. A dedicated ecosystem that allows translation (from bench to bedside) and reverse translation is required to generate new hypotheses and to design smart trials. A close interaction with regulators and health technology assessment (HTA)-bodies is paramount.

alterations are restricted to some tumor types, emerging targets for actionability in clinical trials might be present in many tumor types (pan-tumor) and lack distinctive clinicopathological enrichment criteria. Therefore, early and universal access to comprehensive genomic profiling is becoming a critical component for patient selection in precision medicine trials [101].

- Biomarker testing: Molecular heterogeneity and evolution of the tumor as well as the tumor microenvironment under treatment pressure may represent major challenges to precision cancer medicine. In addition, biomarkers in early stages of clinical development might lack assay technical standardization and thresholds. Very often there is a need to co-develop a biomarker and a drug (drug-target match) in precision medicine trials [101].

In addition to these two key concepts, the design of precision medicine trials has traditionally involved expansion cohorts of phase 1 trials in molecularly defined populations or single-arm phase 2 trials. However, in many contexts, there is a need to include control arms with standard randomization procedures or adjust the trial protocol in response to emerging efficacy data. Adaptive clinical trials in oncology are a type of clinical trial design that allows for modifications to be made to the trial protocol during the study based on interim results—including patient and biomarker selection criteria, drug dosage, combination therapies, and outcome measure. In precision medicine, adaptive trials help identify patient subgroups that may benefit or not from specific molecularly defined treatments with predefined utility/futility boundaries [101, 102].

The most common clinical trial designs used in precision oncology research are umbrella and basket trials. Umbrella trials test multiple treatments for the same type of cancer. The trial assigns patients with a particular type of cancer into subgroups based on specific biomarkers. Each subgroup is then tested with a different treatment or combination of therapies, frequently with a control arm receiving standard-of-care unmatched therapy. This design allows researchers to investigate multiple treatments simultaneously and identify which treatments are most effective for specific patient subgroups. However, umbrella trials can be very complex to design and to execute due to the need for multiple specific biomarkers and therapies, with hundreds of patients enrolled [103]. On the other hand, a basket trial is a clinical trial that tests a single treatment across multiple types of cancer sharing the same genomic or molecular alteration. Basket trials can be smaller and may be easier to design and execute. Major limitations of this approach include the assumption that the same molecular alteration has a similar impact regardless of histology (i.e., regardless of the tumor type; so-called tumor agnostic approaches), and the lack of a control arm that hinders the differentiation of predictive versus prognostic implications of an associated biomarker. Another important caveat is the possibility of insufficient representation of patients with certain tumor types that harbor the alteration of interest, leading to false-negative conclusions or limited generalizability [101]. Nevertheless, for rare genomic alterations with druggable drivers such as kinase inhibitors for fusions/rearrangements and immunotherapy for microsatellite instability, basket trials met the regulatory requirements and led to drug approvals in

a tumor-agnostic paradigm for precision oncology. Today, most precision cancer medicine trials have a simple basket design or have a basket component embedded in an adaptive platform trial [102].

Platform trials in oncology allow for the evaluation of multiple treatments or combinations of treatments within a single study with different biomarker selection criteria, with a combination of basket and umbrella designs. However, unlike traditional umbrella trials, platform trials are typically not restricted to a single tumor type, and patients can be reassigned to a different treatment arm based on their response to treatment and change in biomarker status. This allows for a real-time adaptation of the trial and can increase the efficiency by reducing the number of patients needed to test each treatment, accelerating the pace of drug development in cases where a signal-finding biomarker-drug match is critical [102].

As our understanding of cancer genomic complexity evolves, patient selection and biomarker testing standards must adapt together with clinical trial design. Most biomarker approaches used for drug matching in clinical trials neither consider the complete genomic landscape of a tumor nor the evolutionary plasticity of solid tumors. As detailed further below, joint efforts are needed to design future clinical trials that reflect these aspects.

Trials in precision cancer medicine: from principles to practice

Prospective observational registries and multi-arm cohort studies driven by academic centers and networks—such as Drug Rediscovery Protocol (DRUP), Molecularly Aided Stratification for Tumor Eradication Research (MASTER), Molecular Screening and Therapeutics, and Targeted Agent and Profiling Utilization Registry (TAPUR)—have proven valuable in recent years [104–109]. These programs are essential “signal-finding” endeavors that provide patients with access to otherwise unavailable treatments and generate an expanding range of hypotheses that prepare the ground for molecularly stratified interventional trials. In this latter field, basket studies have grown significantly in importance [110]. An early example was CREATE (Cross-tumoral Phase 2 With Crizotinib), conducted by the European Organization for Research and Treatment of Cancer (EORTC). CREATE was an international, biomarker-driven,

single-arm phase 2 study evaluating crizotinib in various soft-tissue sarcomas with constitutive activity of ALK or MET receptor tyrosine kinases [111]. The results demonstrated the efficacy of this compound in patients with *ALK*-rearranged inflammatory myofibroblastic tumor, thereby establishing a new therapeutic target based on insight into disease biology and effective multicenter collaboration across national borders [112, 113]. More recent examples, primarily driven by pharmaceutical industry, include basket studies of the small-molecule inhibitors larotrectinib and entrectinib, demonstrating that rearrangements of members of the NTRK receptor tyrosine kinase family are promising therapeutic targets in a wide range of entities [114, 115]. The frequency of these alterations is very low in most entities, except for, for example, infantile fibrosarcoma, where *NTRK* fusions, therefore, also have diagnostic value. This encouraging development has led to the designation of *NTRK*-altered soft-tissue sarcomas as an “emerging entity” in the new World Health Organization classification and to the development of recommendations for the diagnosis and clinical management of these tumors [116, 117].

Of particular importance to the continued progress in precision oncology is a feedback loop (reverse translation) from findings derived from registries and interventional studies to basic cancer research. Results from clinically informed basic research can in turn inform the next generation of molecular mechanism-guided therapies, which subsequently can be evaluated in clinical trials. The value of such interplay between clinical discovery and functional and mechanistic work in the laboratory can be illustrated by the critical question of whether the “druggability” of a genetic profile can be translated from one tissue context to another. For example, a multi-cohort basket trial showed that monotherapy with vemurafenib—an approved agent for the treatment of *BRAF* V600-mutant melanoma—was associated with an objective response in 13 of 26 cancer types other than melanoma [118]. Entities in which such monotherapy was ineffective included colorectal carcinoma, consistent with previous observations. This initially led to the conclusion that *BRAF* mutations are not a valid target in this disease. However, the understanding from basic research that resistance to *BRAF* blockade is due to EGFR-mediated reactivation of the RAS-RAF-MEK-ERK pathway [119] has led to the development of rational combination therapies associated with a significant

survival advantage over standard treatment [120]. This example illustrates two key challenges facing precision oncology as a very dynamic field—that is, the assessment of molecular alterations, response, and resistance in the context of current pathophysiological knowledge, and the identification of mechanism-based combination therapies.

Furthermore, the ever-increasing scope of molecular profiling permanently yields new insights into the vulnerabilities of individual cancers, which can prepare the ground for controlled clinical trials [121]. A recent example is the observation of a genome-wide footprint of impaired DNA repair via HR in patients with mesenchymal neoplasms [122, 123], made in the context of registry studies using WGS/WES. This led to the hypothesis that the spectrum of patients who might benefit from pharmacologic PARP inhibition may be broader than previously known, which is now being tested in a randomized phase 2 basket trial of olaparib in combination with cytotoxic chemotherapy versus treatment of physician's choice, using HR deficiency—determined by a WES/WGS-based multicomponent score—as the molecular eligibility criterion (NCT03127215, EudraCT: 2017-001755-31). Another example concerns fusions involving *NRG1*. The observation that patients with *NRG1* fusions—which occur, for example, in patients with *KRAS*-wild-type pancreatic cancer [124] and are prevalent in patients with invasive mucinous adenocarcinoma of the lung [125, 126]—can benefit from blockade of the ERBB pathway has prompted several controlled trials, and it seems likely that targeted drugs will be approved for the treatment of *NRG1*-rearranged cancers across different tumor types in the foreseeable future [127].

Given the relative rarity of molecular alterations compared to broader, histologically defined disease categories, precision oncology studies require the consistent collaboration of numerous institutions in coordinated national networks, such as the National Center for Tumor Diseases or the German Network for Personalized Medicine (DNPM) in Germany [128], or international alliances such as the EORTC and Cancer Core Europe [129]. In addition, increasing efforts are needed to develop precision oncology knowledge bases and dedicated tools to support MTBs to ensure seamless and ideally automated matching of molecular biomarkers, drugs, and clinical trials [130, 131].

Trials in precision cancer medicine: future perspectives

At present, precision medicine focuses mainly on the use of panel-based next-generation sequencing, which provides a fast and accurate way to simultaneously test for commonly altered genes [132]. However, it may limit the effectiveness of precision medicine by constraining the range of genetic variations that are being investigated, with the risk of missing rare molecular drivers.

The extension of profiling technologies beyond panel-based NGS to provide a larger coverage of the genome—as well as inclusion of other omics such as transcriptomic, epigenomic, and functional testing—may help identify additional molecular or immune targets that are druggable. One example is the German Cancer Consortium MASTER trial, which investigated the clinical value of WGS/WES and RNA sequencing in rare tumors [88]. Recommended therapies in the MASTER trial resulted in an objective response rate of 24% and a progression-free survival ratio of over 1.3 compared to previous therapies in 36% of patients. Furthermore, a recent report described an innovative technology that enables ex vivo image-based single-cell functional precision medicine (scFPM) drug testing, in a prospective, single-arm clinical trial (Extended Analysis for Leukemia and Lymphoma Treatment) [133]. It demonstrated the clinical feasibility and efficacy of scFPM-guided individualized treatments in patients with hematological malignancies, leading to an improvement in progression-free survival of more than 1.3-fold compared to previous therapies in 54% of tested patients. Lastly, tumor organoids have been utilized in *KRAS* and *BRAF* mutant colorectal cancer where this technology brought to light EGFR as a potential amplifier of oncogenic MAPK pathway utilizing a drug response assessment [134]. These initiatives indicate that expanding the horizon and utilizing other technologies beyond next-generation panel-based sequencing can maximize the benefit of precision medicine.

Beyond the identification of predictive biomarkers and signatures, it is equally relevant to expand the therapeutic armamentarium. In particular, leveraging new drug classes such as immune-oncology and antibody-drug conjugates would increase the likelihood of target-drug matching to advance precision medicine goals [135]. Most recently, trastuzumab deruxtecan—an antibody-drug conjugate composed of a humanized monoclonal

antibody (trastuzumab) linked to a topoisomerase I inhibitor (deruxtecan)—was granted FDA approval for advanced HER2-low breast cancer treated with prior chemotherapy, as it showed both progression-free survival and overall survival benefit over physician's choice of chemotherapy [136]. This approval raises the hope that precision medicine strategies can leverage standard biomarkers and technologies to direct patients to an expanded pipeline of novel agents entering clinic with favorable therapeutic indices. In addition to antibody drug conjugates, immune-oncology agents, adoptive cell therapies, T-cell engagers, and personalized cancer vaccines are a few emerging approaches that can be explored to enforce precision medicine. This concept is seen in a phase I trial of a personalized mRNA-based neoantigen-specific vaccine, given as adjuvant in combination with chemotherapy and anti-PD-L1 antibody atezolizumab, in patients with resected pancreatic adenocarcinoma. Clinical benefit in terms of longer recurrence-free survival was observed in 8 out of 16 patients who demonstrated a vaccine response by ELISPOT and T-cell clonal expansion, compared to vaccine nonresponders [137]. As these agents utilize biomarkers to drive patient selection—whether expressed as cell surface molecules or based on genomic alterations (e.g., neoantigens) in tumor cells—they hold the promise to broaden the realm of precision medicine.

Precision medicine has been focused on the treatment of patients with advanced progressive tumors. New emerging trends are shifting to other clinical scenarios and schemes; for instance, one aspiration is that this strategy would benefit patients who harbor substantial cancer risks but have not yet developed macroscopic disease [138]. Specifically, advances in the field of inherited cancer genomics with the increased use of hereditary gene panels may pave the way for precision prevention [139]. This concept can be demonstrated by using a combination of sulindac and erlotinib to prevent duodenal neoplasia in patients with familial adenomatous polyposis, an inherited disorder caused by germline alterations in the adenomatous polyposis coli gene. Furthermore, naproxen showed safety and efficacy when used as a chemopreventive agent in Lynch syndrome to prevent the development of microsatellite status instability (MSI-H)/mismatch repair deficient (dMMR) colorectal cancer [140]. Precision medicine may also be increasingly applied in the setting of molecular/minimal residual disease

detected using ultrasensitive technologies such as ctDNA or other liquid biopsy-based biomarkers [141]. In such scenarios, whereby the aim is cancer interception, the development of precision medicine would require a deep understanding of the molecular and immune landscapes in cancer cells and the tumor microenvironment to refine clinical decision-making and design the most tailored personalized therapeutic interventions to eradicate microscopic disease and increase cures.

Lastly, a current drawback to the advancement of precision medicine is the lack of dynamic pursuit, as most precision cancer medicine approaches in the clinical or research settings evaluate only one tumor sample collected remotely or just prior to molecular characterization. This kind of “static” or “cross-sectional” evaluation does not align with the dynamic changes in cancer cells and their tumor microenvironment. A tumor can exhibit a range of responses to treatment, from primary refractoriness to initial response followed by the development of acquired resistance. Therefore, future precision medicine trials must be dynamic, iterative, and preempt changes in patients' disease course over time.

New clinical indications for “old” drugs: the example of the DRUP trial

The DRUP (trial) is a Dutch multicenter, pan-cancer basket, and umbrella trial that has been enrolling patients since September 2016 [104]. The trial aims to test the efficacy and toxicity of commercially available targeted anticancer drugs in patients with advanced cancer. These patients have no remaining standard treatment options left, but their tumors may harbor potentially actionable variants identified by molecular diagnostics, for which the corresponding targeted therapy has not (yet) been approved. An analysis of 2520 Dutch cancer patients showed that in 13% of cancer patients, such actionable variants are present outside their labeled (approved) indication [142]. Therefore, theoretically, 13% of all Dutch cancer patients who exhausted all standard treatment options could be treated within DRUP. In this way, rather than developing new drugs, DRUP aims to identify new indications for existing targeted agents, providing a durable solution to the need for novel cancer treatments. The trial has a theoretically infinite number of possible parallel cohorts, defined by the patient's histological tumor type, targetable mutation and the targeted

therapy being used. Since its inception in 2016, DRUP has expanded continuously, with 35 participating hospitals across the Netherlands treating patients with a total of 35 drugs from 15 different pharmaceutical companies. Over 1300 patients have initiated treatment in one of the \approx 250 open cohorts, out of more than 2400 submitted patients reviewed by the central study team. All these patients had exhausted standard-of-care treatment options and therefore had a poor prognosis.

A recent interim analysis demonstrated that 33% of all included patients experienced clinical benefit, defined as at least stable disease for no less than 16 weeks [105]. Furthermore, a response rate of 13% and a complete response of 2% were observed (of all included patients), even though these are patients that were initially told that there are no treatment options left.

Once a cohort is completed and there is evidence of efficacy of the targeted therapy, it is expanded in consultation with the drug manufacturer and healthcare authorities [143].

An example of an expanded cohort within the DRUP study is the third stage tumor agnostic nivolumab cohort for patients with an MSI-H tumor. In this expanded third stage nivolumab cohort, the clinical benefit rate and response rate were 65% and 44%, respectively. These promising results were recently reviewed by the Dutch HealthCare Institute, which positively advised on the reimbursement of nivolumab within the Dutch healthcare system. As a result, patients with metastatic MSI-H cancers—about 1%–2% of all cancer patients—have access to nivolumab treatment from July 1 2022 regardless of tumor type. This success has received significant interest at all levels because it is an unprecedented example of an investigator-initiated study leading to acceptance and reimbursement of cancer medication.

Of note, DRUP has also served as a blueprint for the new DRUG Access Protocol (DAP), which enables patients to access approved on-label treatments, whereas discussions regarding pricing and data interpretation are ongoing [144]. This means that patients no longer have to wait years for novel treatments to be implemented within the healthcare system. Currently, more than 150 patients have been treated under the DAP.

Furthermore, data from the DRUP trial can lead to discoveries of novel disease (resistance) mechanisms and fuel basic research. An example of this is a recent published paper demonstrating the role of $\gamma\delta$ T cells in modulating the efficacy of cancer immunotherapy [145].

Thinking beyond the national DRUP trial in the Netherlands, DRUP is involved in setting up international collaborations between already ongoing “DRUP-like” studies across Europe (MEGALiT, Sweden; ProTarget, Denmark, IMPRESS, Norway; FINPROVE, Finland; DETERMINE, United Kingdom) as well as expanding and initiating DRUP-like trials across the European Union.

Precision medicine trials in pediatric oncology

Cancers occurring in children, adolescents, and young adults comprise more than 60 tumor types. They are characterized by low mutational rates and recurrent pathognomonic germline or somatic alterations, copy number variations, fusion transcripts, and hijacked enhancers [146, 147], exhibiting oncogenic activities in specific cell developmental stages. Due to multimodal therapies combining polychemotherapy regimens, surgery, and radiation therapy, the overall survival is around 85%. Nevertheless, outcomes for metastatic malignancies and certain central nervous system tumors remain poor and together with long-term sequels to standard treatments mandate novel therapy approaches with new mechanisms of action. The introduction of targeted agents two decades ago and the development of high-throughput sequencing technologies have significantly changed the way new anticancer therapies are developed. There are now multiple success stories on the specific genetic alteration–drug matches in pediatric cancers, starting from imatinib in *BCR/ABL* positive leukemia to the recently approved NTRK inhibitors. Several other key driver events—such as gene fusions that involve *ALK*, *ROS1* or *RET*, and *BRAFv600* or neurofibromatosis type 1 mutated tumors—have been associated with significant tumor responses and clinical benefit for the patients. However, worldwide precision cancer medicine efforts using high-throughput sequencing mostly performed in molecular profiling trials or registries—that is, NCI-pediatric MATCH (NCT03155620), MAPYACTS (NCT02613962), ZERO (NCT05504772), INFORM, iTHER, SM-PAEDS, and so forth—have

demonstrated that only a low number of patients exhibit such unique key driver events, representing approximately 5%–10% of alterations detected and patients explored [148–150]. Nevertheless, between 50% and 70% of patients have genetic alterations of known oncogenic events that are nowadays considered “actionable” or “targetable” either directly or indirectly through influencing their downstream pathways or effectors. Molecular profiling trials have further shown that only a proportion of patients subsequently receive a targeted therapy matching their genetic tumor profile. Furthermore, there is substantial evidence that matched targeted treatments can result in prolonged progression-free survival or interesting objective response rates for alteration-drug matches that are considered with high-level evidence or “ready for routine use” [148, 150], consistent with clinical trials on these agents (e.g., Refs. [151–153], etc.). Such alterations are now searched for in the diagnostic work up, and several of these targeting therapies are currently introduced in front-line strategies—that is, TRK inhibitors in *TRK* fusion positive infantile fibrosarcoma, *BRAFv600* in gliomas, *ALK* fusion in anaplastic large cell lymphoma. Moreover, 2%–10% of patients experience immediate clinical benefit through a revision of the clinical diagnosis, mostly through the detection of specific gene fusions, and their therapy can be adapted accordingly. The programs further confirmed the incidence of genetic predisposition syndromes in 8%–13% of pediatric patients.

In conclusion, the precision cancer medicine programs described here have brought a significant change and benefit to a small subpopulation of pediatric patients. However, most children and adolescents have tumors that harbor multiple genetic alterations that contribute to the biological behavior of the tumor and are involved in resistance mechanisms and clonal evolution. It is therefore not surprising that single-agent therapies do not result in clinical benefit for these patients, mandating therapeutic combination strategies. However, we are only at the beginning of developing innovative trial designs that aim to confront the underlying cancer complexity. Moreover, the trials themselves are confronted with their operational complexity. Most current early-phase trials are still not well adapted to the medical need of the precision cancer programs. There is a lack of clinical trials that allow for treating

patients according to their biological findings in a tumor-agnostic approach, independent from a pre-defined tumor histology. Conservative early clinical trial designs may result in frequent recruitment interruptions due to limited available slots during dose-finding parts, which is particularly difficult in children with rapidly progressing tumors that need to start treatment in a timely fashion. Pediatric trials may rather use trial designs that are based on Bayesian approaches, considering prior toxicity estimates. In contrast to adult-first in human trials, pediatric early-phase trials benefit from prior experience in adults—with descriptions of safety profiles, defined pharmacokinetics, treatment schedules, pharmacodynamic analyses having been explored in the adult population in addition to preclinical findings and can be extrapolated. Comparison of activity data in phase 2 can be difficult in the absence of data on specific molecular study populations. Furthermore, the number of patients is even more of a challenge in developing targeted agents in pediatric cancer, and trial designs as well as regulatory expectancies need to consider the incidence of the disease and genetic alterations.

Many clinical trials are guided by data from preclinical models. In addition to the long-term cultured cell lines, there are now a wide range of pediatric patient-derived xenograft models available that represent an invaluable tool for future research and can be explored professionally—for example, in the PIVOT and the ITCC-P4 consortium. However, despite preclinical data, we cannot neglect the need for biomarker-driven clinical proof-of-concept trials, such as the European platform trial AcSé-ESMART (NCT2813135). In the absence of a defined biomarker for response, the trial design should be hypothesis driven. An enrichment strategy for molecular alterations on the one hand may allow increasing the chance for signals of activity, whereas on the other hand, it may allow confronting responders versus non-responders in patients with or without genetic alterations. Additional retrospective comprehensive biomarker studies are indispensable for the successful development of targeted agents and necessitate access to sequencing raw data. Finally, yet importantly, a main challenge is the tolerability of combination strategies. They may mandate innovative scheduling or intermittent treatments, guided by target engagement and pharmacodynamics analyses.

Precision cancer medicine: national networks

Precision cancer medicine requires the expertise of multiple stakeholders. Networks play a crucial role in connecting experts and leveraging infrastructure, resources, and knowledge to build a coherent framework that enables precision cancer medicine within national healthcare. We here exemplify this approach by highlighting two major initiatives in Europe.

The French genomic medicine initiative

In 2016, the French genomic medicine initiative (Plan France Médecine Génomique 2025—PFMG2025) was launched in order to integrate genomic medicine into the French healthcare system within a care-research continuum. As a first step, PFMG2025 is focused on patients with cancers or rare diseases and will be expanded to additional disease entities—such as complex diseases—according to medical and scientific advances.

For cancer patients, this initiative leveraged a preexisting strong national framework that has been structured for many years to ensure equal access to precision oncology in France by providing access to both molecular profiling and innovative therapies targeting specific alterations. Two regional laboratory networks were structured to perform targeted molecular tumor and germline tests for all cancer patients in their region, mostly by panel-based sequencing. In parallel, the French National Cancer Institute (Institut National du Cancer—INCa) has created and financially supports a network of certified early-phase centers (CLIP²) that are distributed all over the country to improve patient access to unregistered molecular targeted agents. These centers design and conduct national and international early phase-clinical trials. Among the 16 CLIP², seven have a double label for adult and pediatric oncology. INCa has also developed the AcSé (Secured Access to Innovative Therapies) program that aims to provide access to targeted drugs outside of their planned or approved marketing indication [154]. These treatments are being studied in phase 2 clinical trials open to adult and pediatric cancer patients at treatment failure and with a malignant disease harboring an actionable genomic alteration. Five AcSé trials have been set up since 2013, and beginning now, AcSé-type research programs will open to multi-arm, multi-target, and multidrug trials [155, 156].

As the spectrum of molecular alterations that can be used to guide clinical management in cancer patients broadens continuously, attention was paid to connect comprehensive molecular profiling with the structuring of precision medicine already set up for cancer patients. Ten clinical indications in oncology have been selected by the French Health Technology Assessment agency (Haute Autorité de Santé—HAS) through successive calls for applications for healthcare professionals. They cover solid tumors, hematological malignancies, hereditary cancers, and pediatric cancers. Up to now, more than 3000 cancer patients have benefited from comprehensive molecular profiling (of which 38% of patients with solid tumors after first-line treatment failure, 23% of rare cancer patients, and 23% of pediatric cancer patients). The clinical benefit will be evaluated by the HAS for long-term reimbursement by the French health services. Beyond predicting response to a particular targeted therapy, comprehensive molecular profiling can contribute to refining the initial diagnosis, establishing familial risk and prognosis.

Two PFMG2025 clinical sequencing laboratories are operational and provide comprehensive molecular tests for all patients in France. For cancer patients, clinical grade WGS on tumor and normal pairs, as well as whole transcriptome sequencing and WES on tumor samples, is employed. MTBs have been structured for each clinical indication, whether at the national or regional levels, according to the expected number of patients. They are involved upstream and downstream of clinical sequencing, both to validate the clinical relevance of the prescription and to propose a therapeutic solution according to the actionable genomic alterations identified.

The central data analyzer (Collecteur Analyseur de Données—CAD) is the national infrastructure for PFMG2025 data sharing. Under implementation, the CAD will store clinical and genomic data that will be accessible both in clinical and research settings. Data reuse for research is intended to be as wide as possible, while ensuring their security and respecting a certain number of scientific and ethical criteria evaluated by a Scientific and Ethic Committee.

In addition, CAD is part of the European 1+ Million Genomes initiative [157]. In particular, CAD participates in the European Genomic Data Infrastructure project aimed at implementing the sharing

of genomic data on a European scale. Thus, the PFMG2025 genomic data will be made available on a European scale and French researchers will be able to access those produced in other countries.

The Danish genomic medicine initiative

In 2017, a national strategy for personalized medicine in Denmark was launched, including WGS, pooling of data and the use of material from biobanks. Meanwhile, many of the central aims of the strategy have been reached. First of all, the establishment of a National Genome Center (<https://eng.ngc.dk>) under the Danish Ministry of Health with responsibility for supporting the development of personalized medicine in Denmark and the development of a national infrastructure for personalized medicine will ensure that relevant patients obtain equal access to WGS. As part of the support of the infrastructure for personalized medicine, the regions and the universities have joined forces to establish certified regional data support centers for the secure exchange and use of data in research and clinical practice.

National multidisciplinary expert panels have defined the indications for clinical WGS. The majority of the patients are cancer patients, including those with childhood cancer, hematological cancer, inherited cancer, rare cancers, and advanced cancers. This requires substantial upscaling of already established research structures for patient referral, analyses, and interpretation, including MTB. The first MTB was initiated in 2013 as part of the CoPPO trial (Copenhagen Prospective Personalized Oncology), which now has included more than 3000 patients who undergo biopsy procedures followed by WGS or WES and RNA sequencing [158]. CoPPO was introduced as a part of the Phase 1 Unit at the National University Hospital, Rigshospitalet, with referral of patients from all over Denmark. This initiative has successfully attracted a number of early-phase cancer trials. For example, the Phase 1 Unit at Rigshospitalet was the first European site for the early trials of NTRK-inhibitors and also serves as a European hub for other trials. In 2018, the Phase 1 Unit launched a national virtual MTB that covers the entire country. The Danish National Molecular Tumor Board (DN-MTB) will serve as a basis for the increasing needs for molecular information required for both diagnostics and treatment decisions on an ever-expanding proportion of cancer





patients. This national, multidisciplinary collaboration is supported by oncologists, molecular biologists, bioinformaticians, pathologists, and clinical geneticists from eight centers across Denmark covering 5.7 million inhabitants. It provides an opportunity for multidisciplinary evaluation and discussion of each case with regard to actionable genomic alterations, strong genetic drivers, and potential resistance mutations, combined with the clinical history, histopathology, and patient status. The DN-MTB reviews approximately 1200 genomic profiles annually, mainly WGS/WES and large NGS panels. Thus, the DN-MTB ensures thorough and multidisciplinary prescreening of each candidate before inclusion in available trials.

The DN-MTB also plays a pivotal role in ProTarget, which is a Danish nationwide, interventional, multidrug, open-label, pan-cancer, non-randomized, prospective phase 2 basket trial investigating the efficacy and safety of targeted anticancer drugs when used off-label in patients with a malignant disease harboring actionable genomic alterations [159]. The trial aims to include 100 patients annually. At present time, 15 study drugs are available. ProTarget patient enrolment began in August 2020 and is ongoing. Last patient, last visit is undefined, and cohorts will open and reach completion successively depending on variant identification in individual patients (NCT04341181).

Patients are identified by local testing by any method in any type of tissue or blood sample for rapid, broad prescreening of potential candidates. However, if data are derived from small NGS panels or immunohistochemistry testing, treatment decisions may be made on potentially incomplete data. Furthermore, the tumor may have developed new oncogenic drivers or resistance mechanisms after the initial testing. To address these issues, fresh tumor biopsies are taken at baseline, analyzed by WGS, and presented at the DN-MTB to ensure that the genomic alteration is still present and relevant for targeted treatment.





The present trial design will result in a large number of cohorts consisting of rare combinations of genomic alterations and tumor types that will be difficult to complete. To accommodate this challenge and ensure that all cohorts will provide conclusive data, the protocol has been developed with a similar design as DRUP (NCT02925234), TAPUR (NCT02693535). Moreover, the Nordic Precision Cancer Medicine Trial

Table 1. Achievements and challenges in implementing precision cancer medicine.

	Achievements	Challenges
Diagnostics 	<ul style="list-style-type: none"> ■ Identification of biomarker/signatures ■ Development of high-throughput sequencing technologies ■ Decreasing of turn-around-time of sequencing ■ Integration of further omics (e.g., transcriptomics and proteomics) 	<ul style="list-style-type: none"> ■ Highly dynamic diagnostic needs and technologies ■ Increasing complexity ■ Development of AI tools ■ Integration of drug testing by model systems ■ Longitudinal tracking of disease (e.g., monitoring and resistance mechanisms) ■ Standardized EQA schemes for comprehensive genomic profiling
Therapy recommendation 	<ul style="list-style-type: none"> ■ Molecular tumor boards (MTB) ■ Evidence-based therapy recommendations ■ Harmonized evidence levels for variant interpretation 	<ul style="list-style-type: none"> ■ Integration of complex data and interaction ■ Further standardization and harmonization of variant interpretation and therapy recommendations
Drugs 	<ul style="list-style-type: none"> ■ Increasing drug portfolio for stratified treatment ■ Novel trial designs accelerating drug approval 	<ul style="list-style-type: none"> ■ Access to drugs governed by HTA bodies ■ Development of smart and fast clinical trials ■ Use of RWD/RWE in drug development
Financing 	<ul style="list-style-type: none"> ■ Long-term cost-reduction of sequencing ■ Basic funding by national networks 	<ul style="list-style-type: none"> ■ Sustainable funding/reimbursement for the entire diagnostics process including MTB ■ Short innovation cycles requiring continuous investments ■ Financing academia-industry-driven clinical trials ■ Financing a digital ecosystem allowing sharing, processing, analyzing data ■ Financing data collection (follow-up/monitoring) in registry-based studies

(Continued)

Table 1. (Continued)

	Achievements	Challenges
Data 	<ul style="list-style-type: none"> ■ First achievements in data harmonization ■ Setup of prospective registry trials 	<ul style="list-style-type: none"> ■ Big data/interpretation of data ■ Harmonization of data structures/establishing data infrastructure ■ Follow-up/response monitoring ■ Robust analysis of real-world data
Health professionals 	<ul style="list-style-type: none"> ■ High interdisciplinarity ■ Regional/national/international networks 	<ul style="list-style-type: none"> ■ Education and training of multidisciplinary teams ■ Lifelong learning in an innovative and highly dynamic environment
Legal and ethical aspects 	<ul style="list-style-type: none"> ■ Modular templates for patient information ■ Implementation of trust centers ■ Data use and access committees 	<ul style="list-style-type: none"> ■ Informed consent ■ Data sharing/use and privacy protection ■ Handling of incidental findings ■ Access to off-label treatments
Patient involvement 	<ul style="list-style-type: none"> ■ Involvement of patient representatives 	<ul style="list-style-type: none"> ■ More patient empowerment ■ Public visibility (e.g., access and data safety)

Note: Academia, healthcare, and the society at large have taken important steps in solving key issues, but despite these achievements, important challenges need to be met in all areas involved.

Network has been established [160], which connects DRUP and the Nordic trials: ProTarget, IMPRESS-Norway (NCT04817956), MEGALiT (Sweden, NCT04185831), and FINPROVE (Finland, NCT05159245) with the aim of merging data for specific cohorts. The network is focusing on further aligning objectives, endpoints, and eCRFs to facilitate data aggregation, which will be based on generally accepted principles and involve relevant pseudonymized data and clinical outcomes.

Conclusion

Precision cancer medicine has outgrown its infancy and demonstrated that the development of therapies based on a deeper understanding of specific disease mechanisms can improve patient outcomes and quality of life (Table 1). The next

wave of precision medicine in oncology requires comprehensive and adaptive molecular profiling approaches to cover the complexity and plasticity of the entire disease trajectory. Repeat liquid biopsies will play an increasing role and complement tissue-based testing. Such profiling approaches will be increasingly embedded in dedicated multidisciplinary programs and multicentric networks where significant clinico-molecular observations made in defined clinical cohorts can be translated reversely into basic research and inform the development of smart clinical trials. Collaborative efforts between pharmaceutical and the medtech industry, respectively, and academia each playing out their individual strengths are important in this context. Authorities involved in the approval process—such as health technology assessment bodies regulating access and reimbursement of

drugs and diagnostic assays—need to be integrated into the continuous development of precision cancer medicine. Similarly, adequate legal and ethical frameworks are required to successfully implement precision oncology programs on a national as well as supranational level.

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Conflict of interest statement

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