# Druggable genome and precision medicine in cancer: current challenges

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/FEBS.15788

Running title: Druggable genome and precision medicine

Keywords: Druggable genome, Precision medicine, Organoids, Drug resistance, Biobanking, Personalized therapeutics, Oncogenes

**Abstract**

The past decades have seen tremendous developments with respect to "specific" therapeutics that target key signaling molecules to conquer cancer. The key advancements with multiomics technologies, especially genomics, have allowed physicians and molecular oncologists to design "tailor-made" solutions to the specific oncogenes that are deregulated in individual patients, a strategy which has turned out to be successful though the patients quickly develop resistance. The swift integration of multidisciplinary approaches has led to the development of "next generation" therapeutics and, with synergistic therapeutic regimes combined with immune checkpoint inhibitors to reactivate the dampened immune response, have provided the much needed promise for cancer patients. Despite these advances, a large portion of the druggable genome remains understudied, and the role of druggable genome in the immune system needs further attention. Establishment of patient derived organoid models have fastened the preclinical validation of novel therapeutics for swift clinical translation. We summarized the current advances and challenges, and also stress the importance of biobanking and collection of longitudinal data sets with structured clinical information, as well as the critical role these "high content data sets" will play in designing new therapeutic regimes in a tailor made fashion.

**Abbreviations**

| | |
|---|---|
| **CARs** | chimeric antigen receptors |
| **CML** | chronic myeloid leukemia |
| **FDA** | Food and Drug Agency |
| **GPCR** | G protein-coupled receptor |
| **IFNα2** | interferon α2 |
| **IL-2** | interleukin-2 |
| **IMiD** | immunomodulatory imide drug |
| **MCSF** | macrophage-colony stimulating factor |
| **NSCLC** | non-small cell lung cancer |
| **PAP** | prostate acid phosphatase |
| **PBMCs** | peripheral blood mononuclear cells |
| **PD-1** | Programmed cell death protein 1 |
| **PDO** | patient-derived organoid |
| **PDX** | patient-derived xenograft |
| **PKI** | protein kinase inhibitor |
| **PROTAC** | proteolysis-targeting chimera |
| **TCRs** | T cell receptors |
| **TGF-β** | transforming growth factor-β |
| **TILs** | tumor-infiltrating lymphocytes |
| **Tregs** | regulatory T cells |

## Introduction

The first anticancer drugs available were mainly anti-neoplastic agents, also referred to as cytotoxic compounds, developed based on their ability to kill rapidly dividing cells regardless of their potential mechanism of action. The need for less toxic and cancer cell-selective drugs led to a different drug discovery strategy that aims to identify inhibitors of cancer-specific molecular targets and test their ability to affect cancer growth in preclinical studies, resulting in "targeted therapies" [1]. Though cytotoxic chemotherapy still plays an important role in cancer treatment, a shift occurred towards the development of targeted agents, driven by advances in genome sequencing techniques and molecular characterization of cancers [2]. The emerging multiplicity of therapeutic options also paved the way towards precision oncology, with an "a la carte" treatment strategy that can be adapted to individual patients.

Here we will provide an overview of the recent advances brought to cancer treatment by targeted therapies and precision oncology, as well as major challenges that arose with them, such as the inevitable emergence of drug resistance, or the limited predictivity of current pre-clinical models. We will also discuss some of the most promising therapeutic strategies, including immunotherapy and agents promoting target degradation, which bring novel exciting perspectives to the field of oncology.

## The druggable genome and the advent of targeted cancer therapy

Therapeutic target discovery has been focused on the druggable genome, as defined by Hopkins and Groom in 2002, i.e. the genes and gene products known or predicted to interact with orally bioavailable compounds [3]. Beyond the presence of a protein structure that can be potently bound by small molecules, good potential targets are proteins for which modulating the biological function might provide therapeutic benefit for the patient. In this perspective, a few protein families have been at the center of attention for cancer drug development, including protein kinases, G protein-coupled receptor (GPCR), and nuclear/hormone receptors [4]. Targeted therapy has proven to be a successful strategy in oncology, with the introduction of novel therapeutic agents, including monoclonal antibodies and small-molecule kinase inhibitors.

Monoclonal antibodies led the way, with rituximab, an anti-CD20 antibody approved for the treatment of low-grade B cell lymphoma in 1997, followed by a humanized anti-HER2 (ERB-B2

receptor tyrosine kinase) monoclonal antibody, trastuzumab, approved in 1998 for the treatment of HER2 overexpressing breast cancer. Overall, 28 monoclonal antibodies have been approved by the US Food and Drug Agency (FDA) for the treatment of various cancer types (Drugs@FDA, data in October 2020, Supplementary Table 1). Antibody-based therapies also include antibody-drug conjugates, which take advantage of the antibody specificity to deliver cytotoxic drugs to tumor cells, thus minimizing cytotoxicity while enhancing efficacy. The main limit of antibody-based strategies is the large size of antibodies, therefore restricting epitope accessibility to extracellular and membrane-associated targets.

Another classical strategy for targeted therapy is to block protein activity with small-molecule inhibitors. Notably, protein kinases are highly attractive targets for cancer therapeutics, because they are critical components of intracellular signal transduction pathways involved in various cellular functions deregulated in cancer, such as cell growth, proliferation, differentiation, apoptosis, cell survival, and angiogenesis. A breakthrough for small-molecule protein kinase inhibitors (PKIs) was the approval of imatinib in 2001, developed using rational drug design to target the fusion protein BCR-ABL driving chronic myeloid leukemia (CML). The promising results obtained with imatinib in patients with early stage CML raised expectations and paved the way for the development of numerous small-molecule kinase inhibitors (Table 1). For extensive reviews on kinase-targeted drug discovery in oncology see [5–7].

Most PKIs target the kinase domain, especially the first generation which are ATP-competitive inhibitors, classified as type I and type II inhibitors depending on whether they bind the ATP pocket of the kinase in an active or inactive conformation, respectively. These inhibitors require high target binding affinity, because they compete with ATP, which is present in high intracellular levels and binds the ATP pocket with extremely high affinity.

More recently, non-ATP-competitive inhibitors binding kinases outside of the ATP pocket were approved for cancer therapy: mTOR inhibitors temsirolimus and everolimus, in 2007 and 2009; followed by MEK inhibitors trametinib, cobimetinib, binimetinib, and selumetinib from 2013 to 2020. These allosteric inhibitors are generally more specific than ATP-competitive inhibitors, as they target protein domains bearing less homology within the kinases than the ATP pocket, resulting in less off-target effects [8]. In the last decade, covalent inhibitors have also reached the clinics, starting with the approval in 2013 of the BTK inhibitor ibrutinib; and of afatinib, which targets EGFR, HER2, HER4, and some EGFR mutants resistant to first generation EGFR inhibitors. This newer generation of small-molecule kinase inhibitors bind to their target with

covalent bonds, resulting in higher potency, prolonged pharmacodynamics, and fewer off-target effects [9]. The development of these last generation small-molecule inhibitors was the result of research efforts for solving major issues in cancer therapy: target specificity and drug resistance.

**Multitargeting therapy approaches to combat drug resistance**

A major and everlasting challenge in oncology is the acquired pharmacological resistance resulting from the selective pressure created by any drug treatment, whether they are kinase inhibitors or antibody-based therapies. Two types of resistance can be encountered: primary or intrinsic resistance, when the tumor does not respond to treatment; and secondary or acquired resistance, which occurs after an initial response to treatment and involves the selection of resistant cancer cells.

Resistance to protein kinase inhibitors might be due to mutation of key residues in the catalytic domain (e.g. gatekeeper residue), preventing compound binding to the target, or rewiring of the signaling network. One notorious example is imatinib: the initial enthusiasm for imatinib has been quickly dampened by the occurrence of cancer relapse caused by the emergence of drug resistance. The molecular causes of resistance to imatinib include mutations in the catalytic domain of ABL tyrosine kinase, thus impairing imatinib binding, or the activation of alternative signaling pathways, such as increased activation of Src family kinases [10]. Several alternative BCR-ABL inhibitors have since been developed, trying to overcome resistance to imatinib in CML: dasatinib, nilotinib, bosutinib, and ponatinib, which targets also the gatekeeper mutation T315I resistant to all other BCR-ABL inhibitors [11].

Developing selective small-molecule kinase inhibitors is challenging, due to the high conservation of sequence and structure shared among the ATP binding pockets of kinases. Indeed, numerous FDA-approved kinase inhibitors target multiple kinases [7]. The resulting lack of target specificity of these kinase inhibitors can lead to toxicity and side effects due to off-target inhibition. However, polypharmacological properties of drugs can sometimes be turned into a therapeutic advantage, for instance by targeting several kinases involved in cancer pathogenicity and progression with a single inhibitor [10]. Evidence suggests that it might not be sufficient to block one specific kinase to achieve a clinical benefit, whereas multi-targeted kinase inhibitors might be more promising in terms of therapeutic potency, and for preventing drug resistance [12].

One of the first multi-kinase inhibitor approved in oncology was sorafenib. Initially designed as a RAF inhibitor, sorafenib also targets several other receptor tyrosine kinases (VEGFR, PDGFR, c-

Kit and RET) [13]. This large range of cellular targets involved in tumorigenesis led to the approval of sorafenib for the treatment of several malignancies: advanced renal cell carcinoma, unresectable hepatocellular carcinoma, and differentiated thyroid cancer resistant to radioactive iodine treatment. The targeting of VEGFR and PDGFR also limits angiogenesis, thus countering tumor development on multiple fronts. Several other multi-kinase inhibitors were then approved for cancer treatment, for which inhibition of secondary targets contributes to their effectiveness. For example, imatinib primarily targets BCR-ABL in CML, but the identification of its additional targets c-KIT and PDGFR extended its use as first line therapy of c-KIT-positive gastrointestinal stromal tumors [7]. The discovery of novel targets for well-established anti-cancer drugs stresses the importance of rigorously investigating their molecular mechanism of action, in order to allow a rational use and combination of these drugs that could provide a better patient outcome.

**Combinatorial Treatments**

An alternative to multi-targeted kinase inhibitors is the combination of small-molecule kinase inhibitors with classical chemotherapy or with other targeted agents. Two strategies can be employed here: vertical pathway inhibition, targeting several effectors in the same signaling pathway; or horizontal inhibition, to prevent the overactivation of another pathway in response to target inhibition. One example of vertical pathway inhibition is the combination of the BRAF and MEK inhibitors, vemurafenib and cobimetinib, approved in 2015 for the treatment of advanced BRAF-mutated melanoma [14], although the success of this strategy is limited by the emergence of drug resistance. The horizontal inhibition strategy is still in its infancy and is currently being tested in clinical trials (e.g. BRAF or MEK inhibitor combined to PI3K inhibitor). However, there is so far no approved combination of two kinase inhibitors targeting different signaling pathways, mostly due to dose-limiting toxicity [15]. Horizontal pathway inhibition has nevertheless been successful, for example by combining the small-molecule PI3Kα inhibitor alpelisib to fulvestrant, an estrogen receptor antagonist, a combination approved in 2019 for some subtypes of breast cancer [16]. Additionally, the synergistic action of the HDAC inhibitor chidamide with EGFR inhibitors is being explored, with promising pre-clinical data for the treatment of non-small cell lung cancer (NSCLC) raising high hopes for this strategy [17].

In the development of synergistic treatment regimes, a rational combination of drugs targeting multiple hallmarks of cancer is of greatest importance (Figure 1). As described by Hanahan and Weinberg, there are 10 hallmarks of cancer ranging from “sustained proliferative signaling” to

"avoiding immune destruction" [18]. Thus, combining therapies which increase tumor immunogenicity and reverse tumor immunosuppression, have the benefit of killing the tumor cells while simultaneously promoting the activation of the immune system [19]. Another example of successful drug combinations are anti-angiogenesis agents such as the VEGF inhibitor bevacizumab, which was the first to be approved by the FDA in combination with an intravenous 5-fluorouracil-based chemotherapy regimen [20]. With a view to develop new combinatorial treatment strategies, other non-oncology drugs that have the potential to target cancer hallmarks and the related cancer biology might be considered as well. As only recently summarized by Zhang *et al*., there are multiple candidates, like metformin or indomethacin, that were originally approved for other indications but could also be considered for cancer treatment [21]. Metformin is an approved diabetes drug that has been shown to lower the risk of breast cancer in women with type 2 diabetes [22]. Another meta-analysis revealed a positive correlation of metformin and the overall survival of lung cancer patients [23]. In addition, an improved response to radiotherapy of patients with prostate cancer was demonstrated [24]. The underlying mechanism contributing to the anticancer activity of metformin is the negative regulation of mTOR, which is involved in tumor survival [25]. Moreover it has been demonstrated that metformin inhibits the secretory phenotype of senescent cells thus functioning as a senostatic [26]. Recently, senostatics as well as senolytics, which are drugs selectively killing senescent cells, are suggested to be beneficial as a secondary therapy after DNA-damaging therapies [27–29]. The rational reason behind this is that although therapy-induced senescence contributes to inhibition of tumor growth, it also opens up the possibility of relapse due to a resistant population. Tumor cells escaping from therapy-induced senescence have then the potential to be more tumorigenic and metastatic [30,31]. In addition, increased senescence in normal tissues due to DNA-damaging therapies is associated with long-term functional impairment, which is clinically expressed as an age-like phenotype. The administration of senostatics or senolytics could also compensate for this, which could in turn have a positive effect on the health of long-term cancer survivors [27].

The design of polypharmacological drugs and therapeutical combination strategy relies on a deep understanding of the pharmacology of each agent. Consistency and reliability of information on drug-target interaction, including secondary target or "off-target" information, is of utmost importance [32]. For this purpose, several online resources gathering detailed drug-target annotations have been developed, such as DrugBank [33] (www.drugbank.ca), DrugCentral [34] (drugcentral.org), the Drug Gene Interaction Database (DGIdb) [35] (dgidb.org), or the

ProteomicsDB database [36] (www.proteomicsdb.org). It is also crucial to precisely identify the signaling pathways driving a particular tumor type, and the key effectors to simultaneously target for achieving synergistic antitumor effects [37]. Furthermore, the combinatorial strategy is facing the challenge to achieve a good balance between survival gain and toxicity. Overall, rational drug combinations require a systematic investigation of the biological consequences of drug treatments, and the evaluation of the pharmacology of the agent beyond its primary mode of action.

**Druggable targets in the immune system**

Although targeted therapeutics surprised by their profound clinical response in genetically defined patient populations, fast developing drug resistance limited the excitement since clinical responses were often not durable. Therefore, other strategies have also been pursued to expand the repertoire of possible cancer therapies. A reasonable approach was the development of immunotherapeutics that support the anti-tumor immune response.

An efficient immune response depends on the complex interplay of a variety of signals and interactions and on the functionality of different immune cells [38]. However, cancer cells can adopt various immune suppressive mechanisms that lead to reduced immunogenicity and evasion of immune surveillance. Suppression of the anti-tumor immune response can be hereby mediated through the loss of target antigen expression [39], the recruitment of regulatory T cells (Tregs) [40], modulated dendritic cells (DCs), alternatively activated M2 macrophages [41], and myeloid-derived suppressor cells (MDSCs) [42]. Together with the cancer cells, these immune cells contribute to the creation of a suppressive tumor microenvironment by secreting inhibitory cytokines and expression of checkpoint inhibitors so that the functionality of tumor-reactive effector T cells (Teffs) is dampened [43,44]. Main strategies of immunotherapeutics to alleviate tumor immune evasion include immune checkpoint blockade, cytokine therapy and cellular therapy [43,44].

An immune suppressive tumor microenvironment is established through cytokines such as transforming growth factor-β (TGF-β) [45] and also through metabolites such as kynurenine. Consequently, several studies on how immunosuppressive factors could be targeted in the tumor microenvironment are being conducted to develop therapeutic strategies that support anti-tumor immune responses [46]. Among the targets that are evaluated for therapeutic purposes are the aforementioned TGF-β [47], the indoleamine 2,3-dioxygenase (IDO) family [48] which catabolizes tryptophan to kynurenine, the macrophage-colony stimulating factor (MCSF) [49] and

the vascular endothelial growth factor A (VEGFA), or their receptors [50]. Besides targeting immunosuppressive factors, the administration of pro-inflammatory cytokines to patients can potentially support the anti-tumor immune response by activating key immune effectors. In 1986, recombinant interferon α2 (IFNα2) was the first human immunotherapeutic approved by the FDA for cancer, and has since then been clinically assessed as monotherapy or in combination [51]. Today IFNα2 plays a rather subordinate clinical role since the effectiveness was exceeded by some other therapeutics [51]. Aldesleukin, a recombinant form of interleukin-2 (IL-2), represents another immunotherapeutic that was approved by the FDA for metastatic melanoma and renal cell cancer [52]. However, due to its severe toxicity and its tendency to amplify Tregs, IL-2 cannot be used widely for cancer therapy. Consequently, new strategies involving the generation of IL-2 mutants with varying binding affinities to the IL-2 receptor and of tumor-targeting IL-2 are now being developed to improve the efficacy, while reducing the toxicity of IL-2 [52,53].

Another meaningful cancer treatment strategy is the adoptive transfer of *ex vivo* expanded tumor-infiltrating lymphocytes (TILs) and of T cells engineered with chimeric antigen receptors (CARs) or recombinant T cell receptors (TCRs) [54]. As recently reviewed by Wolf *et al.,* clinical trials that investigated the effectiveness of TIL therapy focused mainly on patients with metastatic melanoma [55]. However, more and more clinical trials are now also conducted for the treatment of other cancers such as non-small cell lung cancer (e.g. NCT03215810) or ovarian cancer (e.g. NCT04072263). Due to the good response rate and the largely manageable treatment-related toxicities, the new focus is now to optimize TIL production, to include selection of T cell subsets, to coordinate the therapy with lymphodepletion and IL-2 application, and to expand the TIL therapy to combination therapy and other solid tumors [56]. Clinical trials evaluating the efficiency of TCR T cells have recruited patients with melanoma, colorectal cancer, advanced multiple myeloma and acute myeloid leukemia, among others [55]. So far, TCRs against melanoma-associated antigen (MAGE)-A3 and New York esophageal squamous cell carcinoma (NY-ESO)-1 demonstrated high response rates with rare durable and complete responses [55,57,58]. Due to a higher risk of autoreactivity and associated toxicities, a careful selection of targets is clearly required during the clinical development [55]. The benefit of CARs is that the MHC-independent antigen recognition enables them to recognize any molecule present on the surface of the tumor cells, and they recognize larger epitopes which reduces the risk of cross-reactivity [59]. The FDA has approved two anti-CD19 CAR T cell products in 2017 [55] and another one in 2020 [60] for the treatment of patients with certain types of B-cell malignancies.

However, solid tumors remain a challenge for CAR therapy due to the need of identifying specific tumor antigens [55].

Alternatively, T-cell mediated killing of cancer cells can be induced through therapeutic cancer vaccines. This approach is used, for example, by the FDA-approved therapy with Sipuleucel-T in prostate cancer. Here, a fusion protein of the prostatic acid phosphatase (PAP) and granulocyte-MCSF (GM-CSF) serves as a vaccine, which is incubated with autologous peripheral blood mononuclear cells (PBMCs). Due to GM-CSF, antigen-presenting cells within the PBMCs are activated, and after the infusion of the PBMCs into the patients, they are capable of inducing the replication of PAP-specific immune T cells that kill PAP-positive prostate cancer cells [61].

An immense breakthrough in the development of cancer therapeutics was achieved through immune checkpoint inhibitors that interfere with inhibitory signaling pathways of the immune system and enable tumor-reactive T cells to overcome regulatory mechanisms. The first FDA-approved immune checkpoint inhibitor, the monoclonal antibody ipilimumab, targets cytotoxic T-lymphocyte antigen-4 (CTLA-4), and has been initially approved in 2011 for the treatment of metastatic melanoma. In addition, combination therapy with ipilimumab and the immune checkpoint inhibitor nivolumab, which targets the programmed cell death protein 1 (PD-1), has been proven to be beneficial and is now approved for the treatment of metastatic melanoma regardless of BRAF mutation status, advanced renal cell carcinoma and metastatic NSCLC [62]. Especially, the immune checkpoint inhibitors targeting the PD-1/PD-L1 axis are considered very successful, as demonstrated by their wide area of application. For instance, the anti-PD-1 antibody pembrolizumab is, as summarized by Wei *et al.*, approved for the treatment of a multitude of cancers including metastatic melanoma, NSCLC, head and neck squamous cell cancer, classical Hodgkin lymphoma, urothelial carcinoma, and unresectable or metastatic microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors [62]. The landscape survey of Xin Yu *et al.* further emphasizes how strongly the field of PD-1/PD-L1 inhibitors is growing [63]. Between September 2017 and September 2019 alone, the FDA has approved 23 new indications for PD-1/PD-L1 monoclonal antibodies. Also, the list of clinical studies is expanding with more than 3000 clinical trials launched since 2006 [63]. Despite the success of immune checkpoint inhibitors that lead to long-term durable responses, only a fraction of the patients benefit from them. In addition, as reviewed by Draghi *et al*., tumor-intrinsic and tumor-extrinsic mechanisms are described, that can lead to tumor resistance and to disease progression after an initial response [64].

Further progress in the development of successful treatment regimens was achieved after understanding that the tumor microenvironment has a major impact on drug efficacy. This provided the rationale for combination therapies with immune checkpoint inhibitors, which have become the backbone of cancer therapy [46]. As already extensively reviewed, immune checkpoint inhibitors are currently being combined with numerous therapies and clinically tested to determine whether the tumors respond better to treatment [19,46,65,66]. Once again, toxicity might be an issue, and the synergistic benefits could be limited to a subset of patients [67]. As we discuss below, there are also other strategies besides combination therapies being explored involving the new therapeutic tools and biomarker-guided therapies [64].

## Targeting the "undruggable" genome using novel therapeutics

### *Drug design and efforts towards the discovery of novel inhibitors*

Two decades after the advent of targeted cancer therapy, a large portion of the druggable genome remains to be explored. Some research programs aim to highlight understudied areas of the druggable genome, such as the Illuminating the Druggable Genome initiative launched by the US National Institutes of Health in 2014.They resulted in a list of understudied protein kinases, GPCRs and ion channels that can serve as novel targets in drug discovery [68]. Worth noting is the small fraction of protein kinases that are currently targeted by FDA-approved agents [69]. To this date, the FDA approved 62 small-molecule PKI, including 55 indicated for cancer treatment (Table 1), which target only about 40 different kinases [70], while the human genome encodes more than 500 kinases.

Additionally, some major oncogenic drivers such as RAS, MYC, STAT3, Hippo/YAP or PAX3-FOXO remain "undruggable" in cancer, despite decades of research. Considerable efforts are deployed to develop novel generations of small-molecule inhibitors targeting "undruggable" proteins. One striking example is the recent discovery of $KRAS^{G12C}$-specific inhibitors. The RAS small GTPases have long been a conundrum for drug design, due to the lack of a druggable pocket besides the GTP-binding pocket, bound by its natural ligand with extremely high affinity. In the $KRAS^{G12C}$ mutant the cysteine residue was recognized as an opportunity for drug design by targeting it with covalent inhibitors, which led to the development of covalent $KRAS^{G12C}$ small-molecule inhibitors. In August 2018, AMG 510 was the first to enter clinical testing for treatment of NSCLC, closely followed by MRTX-849 in January 2019. Another future perspective is the

structure-based design of allosteric kinase inhibitors, which bind outside the ATP-binding site in druggable pockets potentially important for modulating the enzymatic activity, e.g. through changes in the kinase conformation or inhibition of effector binding [71–73]. One example is asciminib, a type IV allosteric BCR-ABL inhibitor currently in clinical trials for refractory CML. Its selective binding to the ABL myristoyl pocket induces a shift towards inactive kinase conformation [74]. Such strategies could enable the rational development of allosteric inhibitors with better target specificity while overcoming the limitations of active site occupancy.

***The rise of targeted proteolysis with PROTACs***

Rather than inhibiting enzymatic activity or effector binding, an alternative promising strategy is to induce the degradation of disease-related proteins using proteolysis-targeting chimeras (PROTACs). PROTACs are bi-functional molecules that possess a small-molecule binder, or warhead, which specifically targets the protein of interest and is chemically linked to an E3-ubiquitin ligase recruiting moiety. The induced proximity between the target and the E3-ubiquitin ligase leads to polyubiquitination of the protein of interest and its degradation by the proteasome. Several different strategies have been pursued to recruit ubiquitin ligases, including a VHL (Von Hippel-Lindau) peptide ligand to recruit the ubiquitin ligase $CRL2^{VHL}$, a ligand of the ubiquitin ligase MDM2, or IAP (Inhibitors of Apoptosis Protein) ligand modules. Many more options can still be explored for drug development using PROTACs, since there are more than 600 E3 ligases with different activity profiles and tissue distribution patterns [75].

More recently, the targeted-proteolysis strategy immensely benefited from the discovery of the "molecular glue" properties of the immunomodulatory imide drug (IMiD) thalidomide, and its derivatives lenalidomide and pomalidomide. Thalidomide received FDA approval for the treatment of multiple myeloma in 1998, and IMiDs are now widely used for the treatment of hematological malignancies. But only recently has the molecular mechanism underlying their anti-angiogenic and anti-neoplastic activity been unraveled, leading to a most exciting avenue for drug development. Cereblon (CRBN) was identified as the primary direct target of IMiDs responsible for their anti-cancer activity [76]. Binding of IMiDs to CRBN leads to recognition of neosubstrates by the CRBN-containing E3 ubiquitin ligase complex $CRL4^{CRBN}$, and their subsequent ubiquitination and degradation via the ubiquitin proteasome system.

PROTACs present significant advantages compared to conventional small-molecule inhibitors [75,77]. First, this strategy can be applied to proteins classically considered as "undruggable

targets" and scaffolding proteins, since PROTACs binding to their target is not restricted to the catalytic domain but can rather be through lower affinity and/or allosteric binding. Indeed, one PROTAC molecule can be "reused", thus reducing the number of drug molecules needed to achieve a given efficacy, a characteristic that could also have the benefit of reducing off-target effects. Additionally, since PROTACs induce proteolysis of the target, they are expected to present prolonged effects compared to classical inhibitors, and they could presumably limit the emergence of resistance mechanisms via paradoxical activation of signaling pathways. The design of PROTAC degraders requires careful assessment of cell permeability, target engagement through the formation of ternary complex, target ubiquitination, and target-specific degradation [78]. One particular challenge is that minor modifications in the linker position and length can strongly affect target selection and degradation in an unforeseeable way [75].

The first PROTACs to enter clinical trials were the degraders of androgen receptor ARV-110 and of estrogen receptor ARV-471, currently in phase 1 trial for the treatment of metastatic castration-resistant prostate cancer (ClinicalTrials.gov Identifier: NCT03888612) and of ER+/HER2- locally advanced or metastatic breast cancer (NCT04072952), respectively. PROTAC design is a prolific field, with the extensive development of degraders targeting proteins relevant for cancer therapy such as BRD4, BCL-$X_L$, BCR-ABL, or ALK [79]. Ongoing progress with other notoriously "undruggable" targets such as RAS [80] or STAT3 [81] gives hopes of expanding the druggable genome.

In the future, more PROTAC degraders and other targeted proteolysis therapeutics are expected to enter clinical development, which raises high expectations for the inhibition of "undruggable" targets.

**Bringing novel therapeutics to the clinics**

Despite the immense research efforts in drug discovery, only a few novel therapeutics are approved. This is in part due to the need to more accurately determine the driver mutations in a specific tumor, which is to be achieved by identifying new therapeutic targets through large scale tumor profiling projects. The failure of novel drugs in clinical trials can also be attributed to the limited predictivity of pre-clinical models in terms of efficacy, toxicity and drug resistance, which must be weighed against the ever-increasing costs of drug development and clinical trials.

***Identification of novel drug targets through tumor profiling projects***

A large number of tumors lack known and established driver mutations, which sometimes makes it challenging to distinguish amongst passenger mutations in tumors with high mutation burden. Many tools are now available, from genomics to in silico modelling, to analyse tumors and identify novel druggable targets.

Cost reductions in next-generation sequencing technologies enabled systematic large-scale cancer genome sequencing projects, such as The Cancer Genome Atlas [82], and the Sanger Cancer Genome Project, later integrated into the International Cancer Genome Consortium [83]. These comprehensive genomic, epigenomic and transcriptomic databases allow the research community to discover novel cancer-associated genetic aberrations, including non-coding mutations, oncogenic gene fusions, copy-number variants and drug-resistance mutations. Emergent complementary approaches include proteomic, phosphoproteomic and metabolomic analysis of tumors to precisely assess protein levels, enzymatic activity, and post-translational modifications. They constitute helpful resources to characterize tumor biomarkers, activated oncogenic kinases, novel fusion proteins or cancer-specific neoantigens for immunotherapeutics [84,85].

These large-scale tumor profiling data sets are therefore a precious tool for discovering clinically relevant biomarkers, and for stratification of tumor subtypes. They also provide the invaluable opportunity to expand the cancer targetome by identifying novel "actionable" mutations (Figure 2).

***Tumor biobanks to fulfill the need for high quality patient material***

Transcriptomic, proteomic, and metabolomic analyses of tumor samples require high quality material from pathology after surgery. Recent studies have inferred a role for post-operative ischemia (Cold Ischemia timings) that affect the activation of oncogenic pathways, thus impacting diagnosis [86,87]. However, high quality tumor tissue samples from patients are often not available for research once they have served their primary diagnostic purpose. This is due to multiple limitations, from insufficient size of the surgical sample to inadequate sample storage after surgery and the lack of proper patient consent. Often these Standard Operating Procedures are not standardized and accompanied with the collection of critical clinical data points. The comprehensive collection of clinical data sets with follow up data are pertinent to make informed clinical decisions on therapy design and management. A solution might be the constitution of comprehensive biobanks of high-quality cancer samples for research use obtained from referral

centers, with standardized collection procedures [88,89]. Establishing such biobanks requires a close collaboration between research facilities and participating hospitals. Availability of high quality tumor samples will be essential for research progress in understanding tumor mechanisms, analyzing enzymatic activity and activation of signaling pathway, as well as for discovery and validation of novel biomarkers for clinical use [90]. Integration of multiomics data sets with structured clinical information with Artificial Intelligence-assisted tools will be the norm very soon in treating cancer patients (Figure 2) [91].

***Improving pre-clinical models for an accurate validation of novel therapeutics***

Efficient pre-clinical models that maintain the tumor heterogeneity and microenvironment are essential for understanding cancer biology, disease development, drug efficacy, and response to treatments. Development of such models is a challenging errand. The majority of research is performed on patient-derived cancer cells cultured as two-dimensional (2D) monolayers and maintained in an in vitro setting. Cancer cell lines are quick to establish, cost-effective, and amenable to multiple experimental procedures including high throughput drug screens. However, the establishment of these cell lines requires phenotypic and genetic adaptation, and over time they acquire an undefined mutational background different from the original tumor. Moreover, cancer cell lines do not represent the cellular heterogeneity and architecture found in the original tissue [92,93].

Alternative and better models such as animal models and patient-derived xenografts (PDX), can maintain tumor heterogeneity and interactions between stroma, vasculature and immune components. PDXs are generated by direct implantation of human tumor tissue into immunocompromised mice. They have been used for biomarker identification and pre-clinical drug testing [94]. These models are compelling, but their establishment is time and resource consuming, very inefficient and varies among tumors. In addition, limited scalability makes high throughput analysis inefficient and expensive [95–97].

***Organoid models in cancer precision medicine***

The development of patient-derived organoid (PDO) culture systems provided a unique platform for personalized medicine. Three-dimensional structures termed “organoids” are generated from self-organizing stem cells that mimic key features and functionality of the original tissue. Organoids have unlimited expansion capacity, they are genetically stable, can be cryopreserved and are amenable to many techniques including genetic manipulation (reviewed in [98]).

Organoids can be developed from embryonic stem cells, induced pluripotent stem cells, or tissue-resident adult stem cells. Adult stem cells derived organoids can be derived from both healthy and tumor tissue [99].

To study cancer, organoids have been employed in two different ways; first healthy epithelial organoids have been genetically modified to unravel the roles of specific mutations in tumorigenesis. In colon healthy organoids, the consecutive introduction of the most commonly mutated colorectal cancer genes *APC*, *TP53*, *KRAS* and *SMAD4* gave rise to colorectal cancer organoids with characteristics of invasive carcinoma upon xenotransplantation into immunodeficient mice. Also, results showed that the loss of *APC* and *TP53* cause chromosome instability and aneuploidy, which are considered the initial hallmarks of cancer [100,101]. The second approach is the establishment of living biobanks constituted of patient-derived organoids from tumor and matched adjacent healthy tissue. Such biobanks have been generated for lung [102], breast [103], colorectum [104,105], colon [106], ovary [107,108], prostate [109], pancreas [110–112], esophagus [113], liver [114,115], and metastatic gastrointestinal cancers [116]. The organoids in these biobanks recapitulate the broad histopathological and molecular spectrum of the parental tumor and in many studies, engrafting PDOs into immunodeficient mice confirmed in vitro observations. Therefore, they provide a powerful platform to study and understand cancer, and useful tools for drug screening that facilitate the development of new drugs.

Colon organoid biobanks generated from cancer tissue and adjacent healthy tissue were sequenced to reveal all subtypes of colon carcinoma, showing that organoid culture is not restricted to specific subtypes. As a proof of principle, 83 approved drugs were screened using the biobank organoids and the results correlated with the known drug sensitivity based on known mutations [105,106]. Another study showed evidence that drug response measured in PDOs correlates to clinical outcomes. In this study, organoid lines were derived from metastatic gastrointestinal cancer patients who were enrolled in phase I to III clinical trials. The same drugs used in the clinical trials were also tested on PDOs whose responses mimicked the ones of the patients [116]. In the case of pancreatic cancer, Tiriac *et al.* tested chemotherapeutic agents in PDOs, with the specificity of PDO response reflected in the individual treatment response of the patient, including a longitudinal case study for an individual patient [117]. The identification of new therapeutic targets was described using liver tumor PDOs, out of 29 agents tested on PDOs, the ERK inhibitor SCH772984 was identified as a potentially effective agent for all subtypes of primary liver carcinoma. The efficacy was validated in vivo using xenotransplanted PDOs in mice [114].

Similar success was achieved in prostate cancer: 306 substances were tested in a high-throughput drug screen revealing the selective potency of the BCL-2 inhibitor, navitoclax, which is being tested in clinical trials of castration-resistant prostate cancer [118]. These are some examples confirming that PDOs can serve as useful preclinical models to assist precision medicine.

Unfortunately, organoid technology does not come without limitations. First, compared to standard 2D cultures the costs associated with this system are very high and organoids are labor intensive. Moreover, the success rate in generating tumor organoids varies greatly depending on the tumor type, with the added complication that healthy cells overgrow the others. Second, for high throughput drug screening and validation, automation is essential. Third, tumor organoids are purely epithelial and have no blood vessels, stroma, immune or nerve cells, which makes it impossible to study the influence of these cell types on the tumor. This problem is currently countered by coculture methods with immune cells, lymphocytes and the use of air-liquid interface systems [102,119,120]. In conclusion, PDOs have demonstrated their potential for both modeling cancer and drug screening for personalized medicine. It is expected that the scope and clinical impact of PDOs as a powerful tool will increase in the future.

**The input of precision medicine to oncology**

Over the last two decades, the advent of targeted cancer therapy has been accompanied by a shift towards precision oncology [121]. Precision oncology signifies the rational adaptation of a therapeutic strategy to individual patient-specific tumor biology and genetics. Indeed, extensive tumor profiling is essential for identifying reliable genetic biomarkers and actionable targets.

Before anything else, precision diagnostic is primordial to achieve patient-tailored treatment [91–93][122]. It is fundamental to determine the subpopulation of patients who would benefit from a given therapy, by identifying specific biomarkers that would help to predict the response to a treatment as well as the occurrence of adverse effects due to genetic predisposition.

With the implementation of precision oncology, the design of clinical trials has been adapted, resulting in two types of clinical trials based on precision diagnostics [121]. The so-called umbrella trials comprise multiple treatment arms, and patients with a given tumor type are assigned to different arms depending on tumor genetic alterations identified in each patient by genomic sequencing. Each patient therefore receives a therapy that specifically targets the driver mutation identified in their tumor. Another complementary approach are the basket trials, in which

patients are assigned treatments that are specific to oncogenic driver aberration, regardless of the cancer type.

Precision oncology also includes a close monitoring of the disease to assess the tumor's response to treatment and possible adaptations of therapeutic interventions in case of suspected drug resistance. Molecular testing can also serve after the start of the treatment to evaluate the occurrence of resistance mutation. The analysis of circulating tumor cell cDNA in plasma also represents a valuable advance as it provides a non-invasive method for monitoring the disease burden.

Combining the advances of precision diagnostics in oncology together with the newest targeted therapy strategies might be the path to improved cancer treatment.

**Concluding remarks**

The advancement in genomics have enabled to design patient specific therapies including cancer vaccines, which have not been discussed here, and clinical trials are already ongoing. Personalized therapeutics have gained momentum but the challenge remains in the collection, storage, processing of high quality biomaterials and collection of structured clinical information with longitudinal data sets. Acquiring patient follow up data has logistics issues including consenting and sharing of sensitive data for analytical platforms within and outside the institutions. AI assisted tools give the hope to digest enormous information to provide the options and snapshot of the underlying tumour drivers, for the tumour boards to make informed decisions. Yet, the druggable genome needs further attention and novel drugs are needed to cater to the unmet medical need in treating advanced cancers and drug resistance. While synergistic therapeutic regimes including immune modulators are becoming a norm, the role of the druggable genome in the immune system needs in depth characterization. The availability of public large-scale data sets have enormously helped cancer researchers, and in the future, the inclusion of data from different ethnicities will remain a key for a global outreach. Repurposing of drugs will be crucial, which will also cut the costs towards clinical development, further accelerating the approval process and clinical translation. Tumour boards across the hospitals are thankfully getting multidisciplinary, and experts already make their attempts to "understand" each other in the best interests of the patients, still waiting in the wards for the new hope.

**Acknowledgements**

We thank the members of the KR lab and Indivumed-IMCB Joint lab for discussions. KR's lab is supported by grants from CRC 1292, TP05, EKFS (SUNMAPK) and Deutsche Krebs Hilfe (DKH 70113042) and Forschungszentrum für Immuntherapie of the University Medical Center Mainz to KR. We thank Dr. Daniela Hoeller for critical reading of the manuscript. KR was supported through a Heisenberg professorship of the DFG (RA1739/4-1) and a GFK fellowship.

**Conflict of interest**

KR is a scientific advisory board member of Indivumed and Prof. Hartmut Juhl is the CEO of Indivumed group.

**Author Contributions**

CAD, KR,MP,HJ and KR contributed to the concept, design, writing and editing of the manuscript. CAD served as the lead and further prepared the figures with MP.

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**Tables**

**Table 1.** List of FDA-approved Protein Kinase Inhibitors for cancer treatment ([60] as of October 2020)

| Protein Kinase Inhibitor | Approval year | Indications | Primary targets |
|---|---|---|---|
| Abemaciclib | 2017 | Breast cancer | CDK4/6 |
| Acalabrutinib | 2017 | Lymphoma | BTK |
| Afatinib | 2013 | Lung cancer | EGFR; ERBB2; ERBB4 |
| Alectinib | 2015 | Lung cancer | ALK; RET |
| Avapritinib | 2020 | Gastrointestinal cancer | PDGFR; KIT |
| Axitinib | 2012 | Kidney cancer | VEGFR |
| Binimetinib | 2018 | Melanoma | MEK1/2 |
| Bosutinib | 2012 | Leukemia | BCR-ABL |
| Brigatinib | 2017 | Lung cancer | ALK; EGFR |
| Cabozantinib | 2012 | Thyroid cancer; Kidney cancer; Hepatocellular carcinoma | VEGFR2; MET; RET |
| Capmatinib hydrochloride | 2020 | Lung cancer | MET |
| Ceritinib | 2014 | Lung cancer | ALK |
| Cobimetinib | 2015 | Melanoma | MEK1/2 |
| Crizotinib | 2011 | Lung cancer | ALK; MET |
| Dabrafenib | 2013 | Melanoma; Lung cancer; Thyroid cancer | BRAF |
| Dacomitinib | 2018 | Lung cancer | EGFR |
| Dasatinib | 2006 | Leukemia | BCR-ABL |
| Encorafenib | 2018 | Melanoma; Colorectal cancer | BRAF |
| Entrectinib | 2019 | Lung cancer; Solid tumors | TRKA/B/C; ROS1 |
| Erdafitinib | 2019 | Urothelial carcinoma | FGFR |
| Erlotinib hydrochloride | 2004 | Pancreatic cancer; Lung cancer | EGFR |
| Everolimus | 2009 | Breast cancer; Kidney cancer; Neuroendocrine tumors | mTOR |
| Fedratinib | 2019 | Myelofibrosis | JAK2 |
| Gefitinib | 2003 | Lung cancer | EGFR |
| Gilteritinib | 2018 | Leukemia | FLT3 |
| Ibrutinib | 2013 | Lymphoma | BTK |
| Imatinib mesylate | 2001 | Leukemia; Gastrointestinal cancer | BCR-ABL; KIT; PDGFR |
| Lapatinib ditosylate | 2007 | Breast cancer | EGFR; ERBB2 |
| Larotrectinib | 2018 | Solid tumors | TRKA/B/C |
| Lenvatinib | 2015 | Thyroid cancer; Kidney cancer; Hepatocellular carcinoma; Endometrial carcinoma | VEGFR; FGFR; PDGFRA; RET; KIT |
| Lorlatinib | 2018 | Lung cancer | ALK |
| Midostaurin | 2017 | Leukemia | FLT3 |
| Neratinib | 2017 | Breast cancer | ERBB2 |
| Nilotinib | 2007 | Leukemia | BCR-ABL |
| Osimertinib | 2015 | Lung cancer | EGFR T790M |
| Palbociclib | 2015 | Breast cancer | CDK4/6 |
| Pazopanib hydrochloride | 2009 | Kidney cancer; Soft tissue sarcoma | VEGFR; PDGFRA/B; KIT |
| Pemigatinib | 2020 | Cholangiocarcinoma | FGFR |
| Pexidartinib | 2019 | Tenosynovial giant cell tumor | CSF1R; KIT; FLT3 |
| Ponatinib hydrochloride | 2012 | Leukemia | BCR-ABL |
| Pralsetinib | 2020 | Lung cancer | RET |

| | | | |
|---|---|---|---|
| Regorafenib | 2012 | Colorectal cancer; Gastrointestinal cancer; Hepatocellular carcinoma | VEGFR; RET; KIT; PDGFRA/B; FGFR1/2; RAF1; BRAF |
| Ribociclib | 2017 | Breast cancer | CDK4/6 |
| Ripretinib | 2020 | Gastrointestinal cancer | KIT; PDGFRA |
| Ruxolitinib phosphate | 2011 | Myeloproliferative disorder | JAK1/2 |
| Selpercatinib | 2020 | Lung cancer; Thyroid cancer | RET |
| Selumetinib sulfate | 2020 | Neurofibroma | MEK1/2 |
| Sorafenib tosylate | 2005 | Kidney cancer; Hepatocellular carcinoma; Thyroid cancer | BRAF; RAF1; VEGFR; PDGFRB; KIT; FLT3; RET |
| Sunitinib malate | 2006 | Gastrointestinal cancer; Kidney cancer; Pancreatic cancer | VEGFR; FLT3; RET; KIT; PDGFRA/B; CSF1R |
| Temsirolimus | 2007 | Kidney cancer | mTOR |
| Trametinib | 2013 | Melanoma; Lung cancer; Thyroid cancer | MEK1/2 |
| Tucatinib | 2020 | Breast cancer | ERBB2 |
| Vandetanib | 2011 | Thyroid cancer | EGFR; VEGFR2 |
| Vemurafenib | 2011 | Melanoma; Histiocytic sarcoma | BRAF |
| Zanubrutinib | 2019 | Lymphoma | BTK |

## Figure legends

**Figure 1.** Targeting the hallmarks of cancer. Figure modified from [123]. Also indicated are representative drugs that target various hallmarks of cancer.

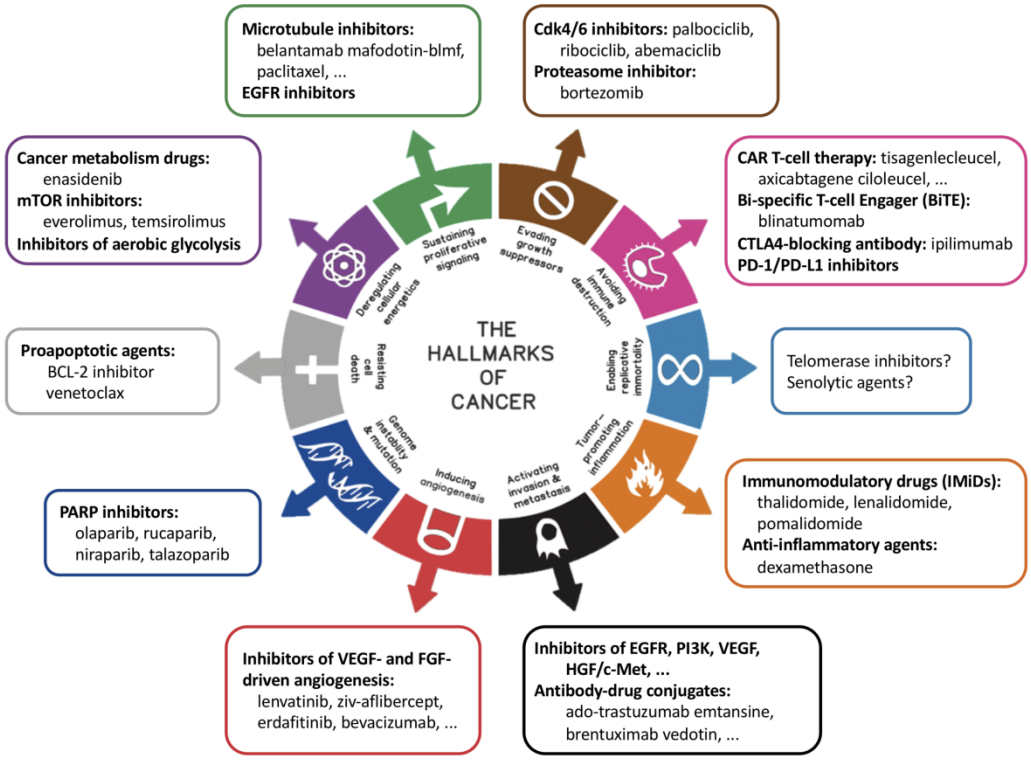
**Figure 2.** Overview of precision oncology: From the patient to the development of novel therapeutics. Shown in the illustration is the pipeline that starts with the collection of biological materials from patients for multiomics analysis, to drug development, validation and clinical outreach.

## Supporting Information

**Supplementary Table 1.** List of FDA-approved molecular entities and therapeutic biological products for cancer treatment ([60] as of October 2020)

ALL acute lymphoblastic leukemia; AML acute myeloid leukemia; APL acute promyelocytic leukemia; ASM aggressive systemic mastocytosis; ATC anaplastic thyroid cancer; cHL classical Hodgkin lymphoma; CLL chronic lymphocytic leukemia; CML chronic myelogenous leukemia;

CMML chronic myelomonocytic leukemia; CRC colorectal cancer; cSCC cutaneous squamous cell carcinoma; DLBCL diffuse large B-cell lymphoma; dMMR mismatch repair deficient; DTC differentiated thyroid cancer; ES-SCLC extensive-stage small cell lung cancer; FL follicular lymphoma; GEP-NET gastroenteropancreatic neuroendocrine tumor; GIST gastrointestinal stromal tumor; GM-CSF Granulocyte-macrophage colony-stimulating factor; HCC hepatocellular carcinoma; HNSCC head and neck squamous cell cancer; MCC merkel cell carcinoma; MCL mantle cell lymphoma; MDS myelodysplastic syndrom; MPD myeloproliferative disorder; MPM malignant pleural mesothelioma; MSI-H microsatellite instability-high; MTC medullary thyroid cancer; mTNBC metastatic triple negative breast cancer; MZL marginal zone lymphoma; NET neuroendocrine tumor; NHL non-Hodgkin lymphoma; NSCLC non-small cell lung cancer; Ph+ Philadelphia translocation-positive; PMBCL primary mediastinal large B-cell lymphoma; PTCL peripheral T-cell lymphoma; RCC renal cell cancer; sALCL systemic anaplastic large cell lymphoma; SLL small lymphocytic lymphoma; SM-AHN systemic mastocytosis with associated hematological neoplasm; STS soft tissue sarcoma; TMB-H tumor mutational burden-high; TNBC triple negative breast cancer; WM Waldenström's macroglobulinemia.

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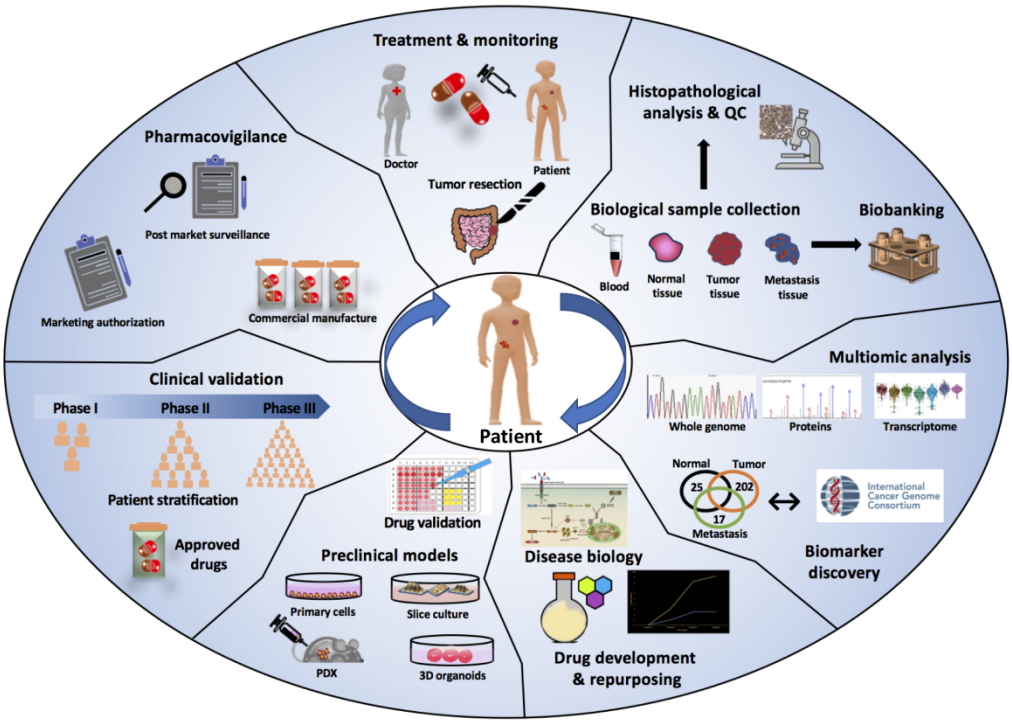


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