Drugging Undruggable Molecular Cancer Targets

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Abstract

Cancer, more than any other human disease, now has a surfeit of potential molecular targets poised for therapeutic exploitation. Currently, a number of attractive and validated cancer targets remain outside of the reach of pharmacological regulation. Some have been described as undruggable, at least by traditional strategies. In this article, we outline the basis for the undruggable moniker, propose a reclassification of these targets as undrugged, and highlight three general classes of this imposing group as exemplars with some attendant strategies currently being explored to reclassify them. Expanding the spectrum of disease-relevant targets to pharmacological manipulation is central to reducing cancer morbidity and mortality.

INTRODUCTION

> "There are known knowns. These are things we know that we know. There are known unknowns. That is to say, there are things that we know we don't know. But there are also unknown unknowns. There are things we don't know we don't know."
>
> Donald Rumsfeld

The earliest method used to identify drugs was to observe a phenotypic change, such as attenuated pain, reduced fever, lowered heart rate, or the death of bacteria on a Petri dish, after treatment with some agent. The pharmacological mechanism by which the substance acted was only subsequently uncovered after extensive investigations. Indeed, safety and efficacy, not mechanism of action, form the foundation of regulatory approval of drugs. Last year, for example, the FDA approved three new drugs with unknown mechanisms of action (1). Although phenotypic investigations are still integral components of the drug discovery process, strategies that focus on influencing the activity, localization, or interactions of specific molecular targets have quickly become de rigueur. Regardless of the discovery tactics, there are two broad classes of clinically approved drugs: small molecules, which typically contain <100 atoms with a composite molecular mass of <1,000 Da, and biologics, which include peptides, antibodies, modified nucleic acids, and vaccines. In part because of their size, small molecules generally traverse cellular membranes, effectively reaching intracellular macromolecules. Small molecules display a relatively limited overall surface area, seeking out solvent-accessible invaginations decorated with hydrophobic amino acids on proteins with considerable affinity (K_D < 1 μM) (2). Typically, these invaginations contain the catalytic site and represent no more than 2–5% of the total surface area of proteins. Biologics, in contrast, generally interact with a large surface area that contains multiple interaction sites. The larger size of the biologics frequently limits their delivery mode and makes altering intracellular targets more challenging. Nonetheless, small molecule– and biologic-based technological advances have significantly increased our available options for drugging intracellular and extracellular targets (see sidebar, General Advances That Have Eroded the Concept of Undruggability).

Since the completion of the Human Genome Project, there has been a continuous attempt to accurately assess the total number of potential drug targets (3). These discussions have been

GENERAL ADVANCES THAT HAVE ERODED THE CONCEPT OF UNDRUGGABILITY

The explosion of molecular oncology targets, from oncogenes and tumor suppressors to regulators of metastasis and immunological responses, has transformed cancer pharmacology. There continues to be an intense effort to identify new validated therapeutic targets. Large groups of candidate drug targets have been ignored, however, because they are perceived as inaccessible to pharmacological manipulation. Listed below are some of the advances that have countered this perception.

- Increased availability of protein NMR and crystal structures
- Enhanced chemical libraries with three-dimensional properties to simulate sites on protein targets
- Expanded availability of therapeutic antibodies and biologics
- Advanced computational methods to visualize dynamic aspects of molecular targets
- Evolving strategies to enable the selective degradation of specific proteins
- Emergence of delivery systems for nucleic acid–based compounds

Although issues remain with approaching some molecular oncology targets, there are encouraging signs that the landscape is changing.

primarily protein-centric in part because the relevant readout of the genomic information is protein-oriented. Moreover, there has been a strong emphasis on agonists and antagonists of receptors and enzymes, mainly because of their accessibility as drug targets. For the >1,400 unique clinically approved human drugs, there are an estimated >325 drug targets (3). Although a small subset of drugs have ambiguous targets (e.g., the gaseous anesthetics), lipid targets (e.g., the antiparasitic amphotericin B), free radical targets (e.g., the antioxidants), or no discrete targets (e.g., the osmotic diuretics), the vast majority of drugs function by interacting with proteins. Preexisting drugs or their analogs have had a puissant role in defining our current established drug targets (3). Naloxone was critical in identifying the first mu-type G protein–coupled receptor (GPCR), which binds to endogenous opioids important in pain (4), whereas [^{3}H]-U69,593 was used to demonstrate the κ-type receptor, which is important for the actions of the endogenous opioid peptide dynorphin (5). Similarly, the binding of [^{3}H]-PN200-110 and [^{3}H]-azidopine defined the dihydropyridine receptor of L-type Ca^{2+} channels (6), whereas the critical cellular sensor mechanistic target of rapamycin (mTOR) was identified using [^{3}H]-dihydro-FK506 and rapamycin (7, 8). The targets for the drugs in clinical trials were recently reviewed for all human diseases (9), and rhodopsin-like GPCRs remain the favorite pharmacological target, although they are uncommon among the cancer drugs. Excluding GPCRs and ion channels, however, proteins lacking an enzymatically active site are generally not considered pharmacologically vulnerable under the current inhibitor paradigm and are therefore labeled undruggable.

GPCR: G protein–coupled receptor

mTOR: mechanistic target of rapamycin

Oncology is now a major disease focus for new drug development. Last year, 8 of the 41 new molecular entities approved by the FDA were for cancer indications and were largely aimed at previously undrugged targets (1). Almost one-third of all current clinical trials are focused on cancer (9), and a substantial number of the novel oncology drugs are aimed at naive targets. This is in contrast to other disease areas in which only ~10% of all investigational drugs currently aim to alter previously unexploited targets (9).

WHAT ARE DRUGGABLE AND UNDRUGGABLE CANCER TARGETS?

Despite significant advancements in our fundamental understanding of its molecular basis, cancer remains largely a lethal disease. This lack of success feeds discussions about the number of bona fide as well as theoretical cancer drug targets. The early anticancer drugs were blunt instruments often aimed at DNA replication, mitotic machinery, or DNA integrity, and they had targets such as dihydrofolate reductase and topoisomerase (**Figure 1**). The past decade has seen an enormous evolution in our understanding of the molecular basis of cancer and the factors that drive its many forms. This has propelled efforts to seek drugs directed against growth factor receptors, enzymes, or other target classes with levels or activity that are elevated in cancer and thus thought to be drivers of the disease (**Figure 1**). However, the suitability of a particular molecular target for drugging has been heavily influenced by existing precedence.

In the cancer field, we have been blessed with a large number of possible targets, which implies we have multiple vantage points from which to launch a therapeutic campaign. Sequencing of human cancer genomes has revealed potentially thousands of mutations in each individual solid tumor, which could reflect a myriad of possible cancer-related gene targets (10, 11). Metabolomics and computational studies indicate that these numbers may be augmented by hundreds of nonmutational aberrations (12). However, it is vital to remember that the presence of a mutation, deletion, or translocation does not necessarily equate to critical functionality for cancer progression or maintenance. Many of the mutations or alterations could simply be coincident passengers associated with the intrinsic genetic instability of cancer and might not drive the malignant phenotype. Indeed, some computational methods suggest many tumors may require only three sequential

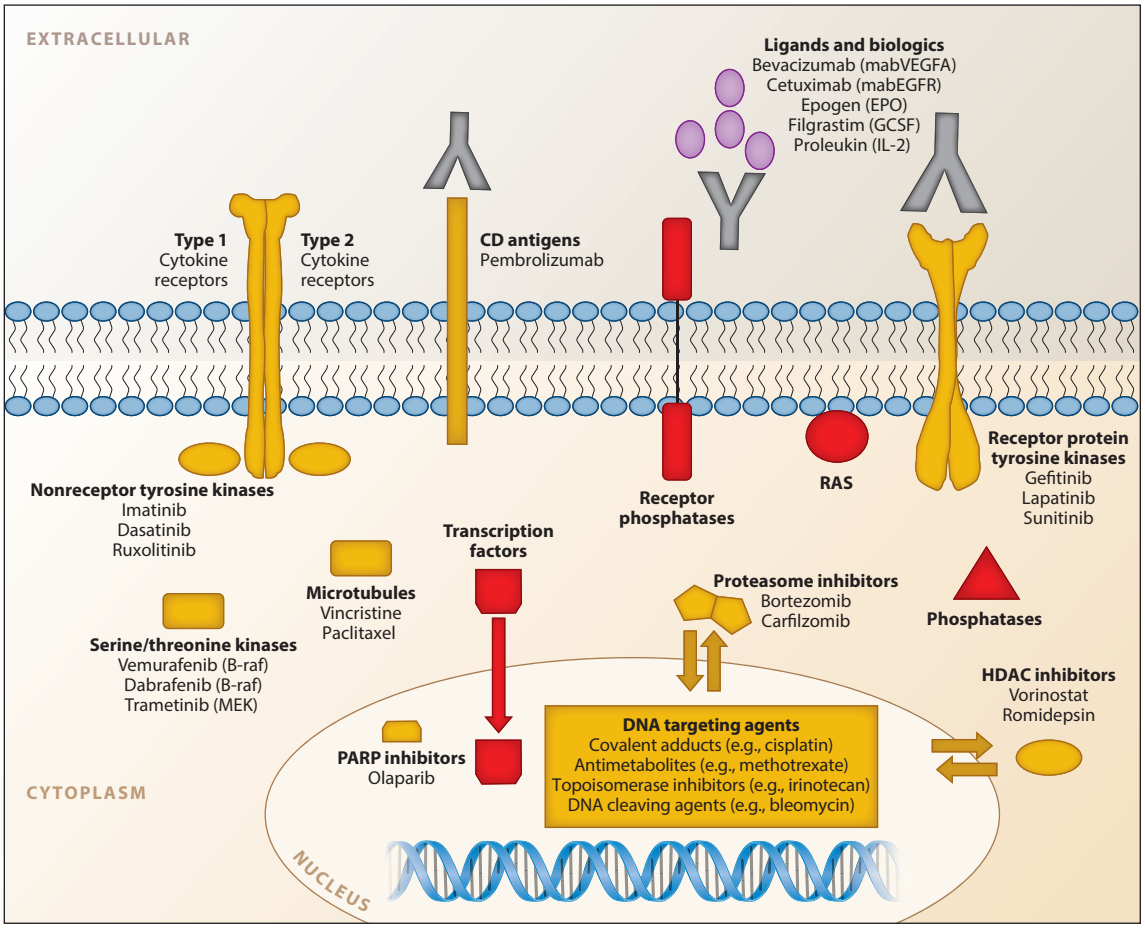

Figure 1

A schematic representation of contemporary molecular cancer drug target classes. A large number of extracellular and intracellular molecular cancer targets have drugs that disrupt their function and are generally considered druggable (some examples of which are shown in *yellow*). However, several target classes lag behind in this otherwise progressive drug-discovery climate. Phosphatases, transcription factors, and RAS family members (*red*) are three major classes that have been labeled undruggable. However, new therapeutic strategies are making these targets more accessible. Antibodies are denoted as gray Ys. Extracellular ovals indicate receptor ligands. Abbreviations: CD antigens, cell surface antigens; HDAC, histone deacetylase; PARP, poly(ADP-ribose) polymerase.

driver mutations (10). Because only ~2% of human proteins interact with the currently approved drugs for all diseases, this places a considerable burden on individuals responsible for selecting therapeutic targets among the potentially large number of oncogenic associated proteins. Some validated cancer drug targets fall into a class that is readily druggable: serine/threonine/tyrosine kinases, growth factor receptors, GPCRs, and receptor ligands. Other classes of cancer-relevant molecular targets, however, have been viewed as exceedingly difficult to address, at least by small molecules, and have been classified as undruggable because they are not an enzyme or are a loss-of-function target, e.g., the tumor suppressor p53 (**Figure 1**). However, small molecules directed against these traditionally undruggable targets are emerging (**Table 1**).

Table 1 Small molecules in clinical trials for traditionally undruggable targets[a]

Experimental therapeutic	Molecular target class	Cancer indication	Development phase
Phosphatases			
LB100	Protein phosphatase 2A inhibitor	Solid tumors	Phase I
RAS superfamily			
KD032 (Salirasib)	RAS antagonist; inhibits RAS methylation	Colorectal cancer	Phase I
GI-4000	Mutated RAS cancer vaccine	Resected pancreatic cancer, lung	Phase II
Transcription factors			
CPI-0610	BET inhibitor	Myelodysplastic syndromes	Phase I
TEN-010	BET inhibitor	Advanced solid tumors	Phase I
GSK525762	Bromodomain inhibitor	NUT gene midline carcinoma	Phase I
PRI-724	CBP/β-catenin	Acute myeloid leukemia, chronic myelogenous leukemia	Phase I/II
ARQ-761	E2F1 transcription factor stimulant	Solid tumors	Phase I
SAR405838	HDM2/p53 antagonist	Solid tumors	Phase I
APTO-253	KLA4 activator	Late-stage tumors	Phase I
DS-3032	MDM2	Lymphoma, solid tumors	Phase I
AMG232	MDM2-p53	Acute myeloid leukemia, chronic myelogenous leukemia, solid tumors	Phase I
MK-8242	MDM2	Solid tumors	Phase I
CGM097	p53/MDM2-interaction inhibitor	Late-stage tumors	Phase I
RG7112[b]	MDM2-p53	Leukemia, sarcoma	Phase I
HDM201	p53	Hematological malignancies	Phase I
ABT-RTA-408	Nrf2	Metastatic non-small-cell lung cancer, skin	Phase I

[a]Data from Reference 20.
[b]From ClinicalTrials.gov (completed Phase I studies) (March 4, 2015).

One strategy for addressing traditionally undruggable targets has been to avoid the use of small molecules and to seek alternative approaches. Thus, therapeutic tactics involving small interfering RNA (siRNA) or short hairpin RNA (shRNA), which are reviewed elsewhere in this volume (13, 14), have been explored. Silencing of protein expression by antisense or microRNA mimetics is being approached in some cancer clinical trials (**Table 2**). Selective protein degradation through small molecules holds the promise of exposing some cancer targets that heretofore have been viewed as not tractable (15). Trinucleotide DNA repeats, which clearly are responsible for some neurodegenerative diseases, are five times more prevalent in cancer-related human genes; this suggests that these are a new class of viable cancer targets (16). Strategies being developed for trinucleotide repeats in the neurodegenerative field have focused either on targeting abnormal mRNA with siRNA or shRNA or on accelerating protein clearance, and these may be transferable to cancer (17). The challenges for the genetic strategies are both the delivery methodology and the faithful expression in every tumor cell in the absence of some bystander effect; therefore, trinucleotide repeats remain in the generally accepted category of directly undruggable. Nonetheless, we believe the overall concept of druggable versus undruggable for cancer targets is rapidly becoming démodé. We would hope that recent advancements will help to promote the concept

Table 2 Biologic agents in clinical trials for traditionally undruggable cancer targets

Experimental therapeutic	Targeted mechanism of action	Cancer indication	Development phase
AZD9150	STAT3	Hematological malignancies	Phase I
Custirsen (OGX-111)	Antisense oligonucleotide (TRPM-2)	Non-small-cell lung cancer, prostate	Phase III
DCR-MYC	c-MYC	Hepatocellular carcinoma	Phase I
Imetelstat	Telomerase—oligonucleotide	Hematological malignancies	Phase I
ISIS-EIF4ERX	Antisense oligonucleotide [eukaryotic translation initiation factor 4E (eIF4E)]	Non-small-cell lung cancer, prostate	Phase II
MRX34	miR-34 mimic	Hematological malignancies	Phase I
NTO-1151	Ribonuclease inhibitor	Cervical cancer, vaginal cancer	Phase II
QBI-139	Variant of the human pancreatic ribonuclease 1	Solid tumor	Phase I
TKM-PLK1	RNA Polo-like kinase 1	Hepatocellular carcinoma	Phase II

of undrugged rather than undruggable cancer targets (see sidebar, General Advances That Have Eroded the Concept of Undruggability).

CONTEMPORARY EXAMPLES OF CHALLENGING MOLECULAR CANCER TARGETS AND STRATEGIES TO TARGET THEM

Phosphatases

Protein phosphatases counterbalance the enzymatic activity of protein kinases, and consequently, these two superfamilies have a central role in determining protein phosphorylation status, which can alter stability, macromolecular interactions, enzyme activity, subcellular localization, and ultimately protein function that controls normal homeostasis and disease processes, including cancer. Mathematical modeling suggests that the activity of the ~500 kinases encoded in the human genome primarily controls the amplitude of a given signal, whereas the ~100 phosphatases, which dephosphorylate Ser, Thr, or Tyr on protein substrates, appear to regulate the signal rate and duration, thus providing an orthogonal mode by which cellular processes can be managed (18). Genetic studies provide incontrovertible evidence that both kinases and phosphatases have a central role in determining cancer cell survival and response to drug treatment (19), but no two classes better illustrate drug surfeit and dearth. One of the most remarkable drug discovery achievements has been the identification and successful FDA approval of more than 35 protein kinase inhibitors, from the BCR/ABL inhibitor imatinib in 2001 for chronic myelogenous leukemia to the 2015 approval of lenvatinib, a multikinase inhibitor for thyroid cancer, and palbociclib, a cyclin-dependent kinase 4 and 6 inhibitor for breast cancer. Multiple clinical trials are underway to evaluate other protein kinase inhibitors for cancer. This is in contrast to only one compound, LB100, a PP2A inhibitor in active early phase clinical trials (20), and no FDA-approved cancer drugs that function as regulators of protein phosphatases. The foundation for this discrepancy has roots, at least in part, in the natural temporal lag that catabolic processes experience compared with anabolic processes. Also, it was once thought that phosphatases were merely constitutively expressed enzymes with little regulatory role in normal homeostasis or disease—a notion that has largely been debunked. Finally, many individuals classify kinases as intrinsically oncogenic and phosphatases as tumor suppressors, which clearly is not accurate. Nonetheless, this concept produced concerns

that a phosphatase inhibitor might block a tumor suppressor and thus cause rather than reduce malignancies. These preconceptions delayed phosphatase-directed discovery efforts.

The human genome encodes two major protein phosphatase families: Ser/Thr and Tyr phosphatases, of which there are ~38 and ~97 active enzymes, respectively. Excellent reviews of the composition of the two superfamilies are available (21–23). This seemingly small number of phosphatases compared with the >500 Ser/Thr and Tyr kinases encoded in the human genome generated theoretical concerns that any phosphatase inhibitor would be too promiscuous to be useful as a drug. Because of these theoretical issues and repeated failures to advance lead candidates for successful clinical trials, phosphatases have become one of the premier members of the undruggable caste (24, 25). Thus, it is useful to review some of the recent attempts to uncover drug-like molecules that alter phosphatase function and to explore what is arguably the most prototypical class of therapeutically inaccessible proteins.

Ser/Thr phosphatases. Early Ser/Thr phosphatase inhibitors were primarily isolated as natural products. Chemically complex molecules with multiple chiral sites, these natural products were quite promiscuous with respect to substrates, which makes them a challenge to develop for the treatment of cancer (25). More recently, notable advances have been made in the design of both inhibitors and activators of some cancer-relevant Ser/Thr phosphatases.

One of the better-studied, cancer-associated, Ser/Thr phosphatases is PP2Cδ, which is also known as PPM1D or Wip1. PP2Cδ was discovered as a DNA damage– and p53-induced transcriptional gene product (26). PP2Cδ dephosphorylates and inactivates p53 as well as several other stress-associated kinases that protect cells from apoptosis and senescence. Several human tumors have an amplified PP2Cδ locus and overexpress PP2Cδ. Mouse genetic studies have further validated this protein as an attractive target for inhibition. On the basis of the PP2Cδ p53 substrate, peptide inhibitors were designed and further refined, which resulted in a cyclic thioether peptide (F-pS-I-pY-DDC-amide) with an in vitro K_i of 110 nM against PP2Cδ (27) (**Table 3**). Although a useful tool compound, the two phosphoric acid moieties on the cyclic thioether peptide severely limit cell entry and PP2Cδ engagement. As an alternative to rational design, several groups have screened chemical libraries for inhibitors of PP2Cδ phosphatase activity (28–30) (**Table 3**). Most of these compounds lack the potency, target specificity, or bioavailability needed to encourage further pursuit. For example, CCT-007093 has an in vitro PP2Cδ IC_{50} value of only 8.4 μM and is a strong Michael acceptor, which would likely produce undesirable irreversible target inhibition (30). Yagi et al. (29) discovered a perhydrophenanthrene, SPL-001, with reasonable in vitro potency (IC_{50} = 480 nM) against PP2Cδ and some selectivity versus two other Ser/Thr phosphatases. SPL-001 might be a reasonable lead compound if found to have the appropriate in vivo specificity and pharmacokinetics (29). A particularly intriguing PP2Cδ inhibitor was reported recently by the GlaxoSmithKline group (28). They conducted two parallel screens: a standard high-throughput homogenous biochemical screen with a small-molecule substrate and a biophysical screen for high-affinity PP2Cδ binding. Initially, a series of capped amino acids were identified with an in vitro IC_{50} of 10–20 nM (28). The lead capped amino acid compounds were characterized as noncompetitive, allosteric PP2Cδ inhibitors binding to a conformationally flexible flap domain, which is involved in substrate engagement. This is a nonconserved sequence among homologous PP2C phosphatase family members, which is hypothesized to provide for PP2Cδ inhibitor selectivity. The peptide nature of these lead compounds results in poor cell permeability and has subsequently inspired a structure-activity relationship campaign that produced GSK2830371, which has an in vitro IC_{50} of 6 nM against PP2Cδ and in vivo activity against a B-cell lymphoma xenograft tumor model, albeit with a rather aggressive oral treatment schedule (150 mg/kg, thrice daily for 14 days) (28). Notably, GSK2830371 also rapidly decreases PP2Cδ

Table 3 Examples of preclinical inhibitors and activators of cancer-associated phosphatases

Phosphatase	Compound	Action	Reference(s)
PPM			
PP2Cδ (PPM1D or WIP1)	Peptide	Catalytic site inhibitor	27, 92
PP2Cδ (PPM1D or WIP1)	CCT007093	Catalytic site inhibitor	30
PP2Cδ (PPM1D or WIP1)	SPI-001	Catalytic site inhibitor	29
PP2Cδ (PPM1D or WIP1)	GSK2830371	Allosteric inhibitor	28
PPP			
PP2A/CIP2A	Rabdocoetsin B	Transcription inhibitor	93
PP2A/SET	FTY720	Activator by disruption of protein-protein interaction	94
PP2A/SET	OP449	Activator by disruption of protein-protein interaction	36
PP4	Fostriecin	Catalytic site inhibitor	95
PTP			
PTPN1 (PTP1B)	MSI-1436	Allosteric inhibitor	44
PTP-1D (SHP2)	Hydroxyindole carboxylic acid	Catalytic site inhibitor	96, 97
TC-PTP	Mitoxantrone	Allosteric activator	98
CDC25A	Quinones	Catalytic site inhibitor	25, 45
CDC25B	Aminoisoquinolinones	Catalytic site inhibitor	46
PTP4A3 (PRL)	Thienopyridone	Catalytic site inhibitor	99
PTP4A (PRL)	BR-1 and CG-707	Catalytic site inhibitor	100
PTP4A (PRL)	Antibody	Antibody	49, 50
R-PTPη	Peptide	Activator by disruption of protein-protein interaction	101
Eya2	MLS000544460	Allosteric inhibitor	80, 81

protein levels in treated tumor cells by a mechanism that is not fully described. Thus, GSK2830371 is an encouraging example of an unusual allosteric Ser/Thr phosphatase inhibitor.

Fostriecin (CI-920) is a natural product and is a potent catalytic inhibitor of four Ser/Thr protein phosphatases, including PP4C, the inhibition of which causes premature entry into mitosis and tumor cell death (31, 32). Fostriecin is one of the few phosphatase inhibitors to enter clinical trials, but the trials were closed before reaching a maximum tolerated dose because of limited drug supplies (25). Although no significant tumor responses were observed, interest in targeting this phosphatase remains (31). The development of fostriecin, however, may be challenging because it targets so many Ser/Thr phosphatases.

PP2A is a ubiquitous, multifunctional Ser/Thr phosphatase, which can function as a tumor suppressor but also as a facilitator of tumor cell survival after DNA damage (21). It is this latter function that inspires the use of the small-molecule PP2A inhibitor LB100, which sensitizes cancer cells to doxorubicin, temozolomide, and radiation, at least in part by causing the aberrant activation of cyclin-dependent kinase 1 (33) (**Table 1**). LB100 causes marked radiation sensitization in a human pancreatic cancer xenograft in the absence of overt toxicity to normal tissue (33). LB100 is unusual in that it is one of the only phosphatase inhibitors currently in clinical trials for cancer (**Table 1**). Acting as a tumor suppressor, PP2A also negatively regulates the oncoprotein c-MYC by dephosphorylating it at Ser62. In some cancers, the PP2A holoenzyme activity is inhibited by interactions with CIP2A and SET-binding proteins, and this has been targeted

for therapeutic intervention. A sphingosine-like molecule, fingolimod (FTY720), which is used to treat multiple sclerosis, activates PP2A by downregulating the PP2A inhibitor SET, dephosphorylating PP2A-C, and upregulating the activating PP2A subunits A and B55α, which results in increased endogenous ceramide levels and tumor cell death (34, 35) (**Table 3**). The natural product rabdocoetsin B activates PP2A by inhibiting CIP2A transcription, whereas a physiologically stable, cell-penetrating peptide, OP449, binds to SET and antagonizes SET's inhibition of PP2A (22, 36–38). OP449 increases Ser69 phosphorylation on c-MYC, which results in its inactivation, and OP449 has antitumor activity in preclinical models of breast cancer (36). OP449 also enhances the efficacy of Tyr kinase inhibitors in mouse myeloid leukemia models (39).

Tyr phosphatases. Because aberrant Tyr phosphorylation has a central role in cancer formation and progression, attention has focused on human Tyr phosphatases as potential targets for cancer treatment. Gene amplification or overexpression has been reported for 25 of the ~100 Tyr phosphatases in human cancer, which suggests a role in the etiology of the disease (40). As with the Ser/Thr phosphatases, some Tyr phosphatases are implicated as tumor suppressors, with 14 of the 25 potentially oncogenic phosphatases found to be downregulated, genetically deleted, mutated, or aberrantly spliced in some human tumors (40). Conceptually, of course, this complicates any use of therapeutics with at least the theoretical possibility that an inhibitor to any of these phosphatases might have on-target effects that could produce rather than reduce cancer. Such concerns, however, exist with many of the current cytotoxic chemotherapeutics. Efforts to identify compounds that selectively block oncogenic Tyr phosphatases with small molecules, silencing RNA, or even antibodies have largely failed to produce a strong preclinical candidate, thus fortifying the notion that Tyr phosphatases are not a druggable protein class. The specific challenges of targeting Tyr phosphatases for cancer have been reviewed previously (22, 24, 25, 40), and thus, in **Table 3**, we provide only a few examples of discovered tool compounds and discuss below some more recent developments.

There is continued interest in exploiting high-throughput screening of chemical libraries, although there is now an appreciation that the chemistry of Tyr dephosphorylation results in the frequent identification of nonselective oxidants, which react with the catalytic Cys (41). Intelligently designed analogs of known lead compounds continue to be pursued with the assistance of the increased availability of crystal and NMR structures for Tyr phosphatases and computational or in silico methods (41).

PTPN1 (PTP1B) has been extensively investigated as a drug target because of its proposed role in obesity and type 2 diabetes (42). The gene encoding PTPN1 is located on human chromosome 20q13, which is a cancer susceptibility locus (40). Mice that lack the *Ptpn1* gene have a significant delay in the onset of HER2-induced mammary tumorigenesis, and they fail to develop lung metastases, whereas tissue-specific PTPN1 overexpression leads to mammary tumorigenesis, which provides strong evidence for an oncogenic activity for this cytosolic, nonreceptor phosphatase (43). In HER2-transgenic mice, PTPN1 appears to function as an oncoprotein by regulating the RAS-mitogen-activated protein kinase and AKT signaling pathways (40, 44). Complicating PTPN1 as a molecular target is its potential role as a tumor suppressor, at least in lymphoma (40). Recently, a reversible, noncompetitive PTPN1 inhibitor, MSI-1436, was described with an in vitro K_i of ~600 nM and selectivity when tested against nine other phosphatases (44). MSI-1436 has a novel mechanism of action; it binds to two separate sites on PTPN1, including a region of 20 amino acids in the disordered C terminus of PTPN1, which results in the stabilization of an enzymatically inactive conformation. MSI-1436 has favorable preclinical properties in a HER2-dependent mouse xenograft tumor model and a transgenic mouse model of human breast cancer (44). Moreover, the primacy of PTPN1 as the molecular target of MSI-1436 has been established

by documenting a physical interaction between MSI-1436 and PTPN1 with a bead-immobilized compound and by using a mutant form of PTPN1 that is resistant to MSI-1436 (44). These results validate the targeting of allosteric sites on Tyr phosphatases as a viable strategy for identifying new therapeutic candidates for cancer. This strategy might be scalable to other phosphatase family members.

TFs: transcription factors

The oncogenic Cdc25 phosphatase family continues to be explored for selective catalytic inhibitors. Several potent naphthoquinones have been identified (25, 45) (**Table 3**), but these irreversible inhibitors are not likely to emerge as viable clinical candidates because of their propensity to generate reactive oxygen species, which are generally difficult to control, especially for therapeutic purposes. Focus is now directed toward reversible catalytic inhibitors, such as the aminoisoquinolinones (46), which do not have the chemical liabilities of the first generation of inhibitors (**Table 3**). Potent catalytic inhibitors of the PTP4A family have been reported that could form the basis of more pharmaceutically attractive candidates (47, 48). It is interesting that antibodies directed against PTP4A3, which generally is thought to be retained within the cytoplasm, have been observed to be effective inhibitors with preclinical activity (49, 50). This is an approach that deserves additional attention in the future.

As mentioned above, a number of Tyr phosphatases are tumor suppressors. Loss of PTEN (phosphatase and tensin homolog) is the second most frequently mutated tumor suppressor after the transcription factor p53. PTEN is both a protein and lipid phosphatase that controls cell survival and proliferation via the PI3K/AKT/mTOR pathway (51, 52). It is highly regulated through protein-protein interactions and through posttranslational modifications (53). One of the more promising strategies for augmenting or restoring the loss of PTEN function is by inhibiting casein kinase 2, which phosphorylates, destabilizes, and functionally inactivates PTEN. CX-4945 is an example of an orally available, ATP-competitive inhibitor of casein kinase 2α, which restores PTEN function and decreases the PI3K/AKT/mTOR signaling pathway (54). CX-4945 displays in vivo efficacy in a human T-cell acute lymphoblastic leukemia xenograft model (54) and in a chronic lymphocytic leukemia model (55). An alternative strategy being pursued is to find inhibitors of the E3 ligases that cause PTEN proteasomal degradation (53). Collectively, therefore, a wide assortment of complementary approaches to control the phosphatase family are being advanced, and this offers the hope that they can soon be evaluated in clinical trials.

Transcription Factors

Transcription factors (TFs) are convergence points in intracellular signaling processes that lead to gene expression. TFs are either directly or indirectly involved in a variety of cancer-associated aberrations of gene expression and transcription. Some of the earliest oncogenes identified were acting as TFs (c-MYC or STAT3) or as regulators of signaling pathways leading to proximal enhanced transcription. Thus, there has been considerable interest in developing strategies to modify these transcriptional alterations. TFs form protein complexes that are directed to specific sites on DNA. Preventing functional interactions between the nuclear TF protein and DNA or coregulatory proteins with small molecules has proven remarkably difficult until recently. The sites on TFs involved in these interactions are generally large, flat surface areas, in contrast to the deep, druggable binding pockets found on most enzymes or receptors. Currently, the best-developed strategy for altering oncogenic transcriptional events with small molecules prevents interactions between proteins rather than disrupts TF-DNA binding. We present several examples to illustrate successful contemporary approaches.

MYC-MAX. c-MYC is a TF belonging to the helix-loop-helix leucine zipper protein family that forms productive dimers with several proteins, including MAX, and is considered a master regulatory factor in normal cell proliferation, metabolism, differentiation, and apoptosis (56). c-MYC has long been considered the Higgs boson of anticancer drug targets because of its high expression levels and functional deregulation in many human cancers. Mitigating this enthusiasm has been the recognition that c-MYC is almost universally involved in normal physiological processes, which raised concerns that c-MYC inhibition would lead to unacceptable toxicities (57). Designers of disruptors have been thwarted because the MYC protein-protein interactions (e.g., MYC-MAX, MYC-TRRAP) occur on a relatively large, flat, and structurally indistinct interface (57). Nevertheless, early yeast two-hybrid screening studies revealed some interesting tool compounds, which suggest the feasibility of the concept (58). It is encouraging, therefore, that recent studies reveal additional small-molecule tool compounds (e.g., IIA6B17, NY2267, JY-3-094, and Mycro3) that interfere with c-MYC transcription or with c-MYC-MAX dimerization (56, 59). The challenges for the further development of these chemotypes, as with their predecessors, will be to increase their specificity and potency. Several provocative alternative approaches to regulating c-MYC are emerging. Omomyc, a MYC dominant negative peptide, sequesters c-MYC from its dimerization partners (e.g., MAX and MIZ-1), selectively preventing MYC-dependent transcriptional activation (56, 59–62). Omomyc displays potent antitumor activity in preclinical murine models of lung cancer and glioma (63, 64). Additional progress in the indirect targeting of MYC as an anticancer therapeutic strategy comes from studies with BET bromodomain small-molecule inhibitors, which potently suppress MYC gene expression by interfering with the acetyl-lysine recognition domains of MYC regulatory cofactors (65, 66). JQ1 represents a well-studied prototypic tool compound, which has helped credential bromodomains as viable cancer targets and has stimulated the identification and development of multiple BET bromodomain inhibitors that have progressed to Phase I clinical trials (**Table 1**). Interestingly, small-molecule stabilizers of the MAX homodimer also represent potential inhibitors of MYC signaling through interference with the heterodimerization of MYC-MAX (67). Additionally, a lipid nanoparticle formulation of c-MYC siRNA (DCR-MYC), when taken up by tumor cells, inhibits c-MYC translation and subsequent protein expression, which leads to a decrease in tumor cell growth (**Table 2**). This is yet another strategy to target MYC.

p53. TP53 is the most frequently mutated gene in human cancer. It encodes the tumor suppressing transcription factor p53, which is a key regulator of multiple processes that include DNA repair, metabolism, cell cycle arrest, apoptosis, and senescence (68). Thus, when inactivated by mutations or deletions, the loss of p53 disrupts a myriad of cellular processes and leads to malignancies. As a result, it has been one of the most sought after molecular cancer targets. There have been multiple strategies adopted to reconstitute p53 functionality. The small molecules PRIMA-1 and PRIMA-1MET, a methylated PRIMA-1 derivative, restore the active conformation of mutant p53 and produce sequence-specific DNA binding, which leads to cellular apoptosis (69). Both PRIMA-1 compounds inhibit the in vitro growth of mutant p53-expressing cells as well as xenograft tumor growth (69). The PRIMA-1 compounds increase p53-regulated gene targets, including p21, PUMA, and MDM2 (69). Interestingly, PRIMA-1 and PRIMA-1MET appear to be prodrugs with an active metabolite identified as methylene quinuclidinone, which alkylates p53 and restores an oxidative environment within the tumor cells. Highlighting an alternative mechanism of impact on p53 are a number of small molecules that inhibit the association of p53 with a negative regulatory binding protein MDM2 (also known as E3 ubiquitin-protein ligase MDM2), which blocks the transcriptional activity, promotes nuclear export, and stimulates the rapid degradation of p53 (68, 70, 71). Nutlins are the most well characterized small molecules that disrupt

this p53-MDM2 interaction. Nutlins bind to the N-terminal pocket of MDM2 and stabilize p53, which helps to retain its tumor suppressive functionality. There is extensive cell-based and in vivo evidence to demonstrate that nutlins increase p53 levels and apoptosis, and they decrease the tumorigenicity of p53-expressing cells. The nutlin derivative RG7112 has advanced to clinical trials for leukemia and sarcomas along with numerous small molecules with similar mechanisms of action that are currently in Phase I clinical trials (**Table 1**). These include AM-8553 (72), SAR405838 (73), CGM097, HDM201, DS-3032 (74), and MK-8242 (74). Thus, there is considerable clinical activity focused on inhibiting p53-protein interactions using small molecules.

KLF4: Krüppel-like factor 4

Nrf2: nuclear factor erythroid 2-related factor 2

Other transcription factors. The identification of novel chemical tool compounds is indicative of a pipeline of TF-targeted small molecules. Some of these tools have generated attractive clinical candidates that have advanced to early Phase I clinical trials (**Table 1**). This provides encouragement that TFs as a molecular target class can be placed under therapeutic control. For example, Krüppel-like factor 4 (KLF4) is a zinc finger DNA-binding protein that regulates cell proliferation, differentiation, apoptosis, and somatic cell reprogramming (63, 64). Suppression of KLF4 is a driver for a variety of hematological malignancies. The small molecule APTO-253, which is in Phase I clinical trials (**Table 1**), targets KLF4 via inhibition of human metal-regulatory transcription factor 1 (MTF-1), resulting in the promotion of KLF4 activity. Similarly, the small molecule ABT-RTA-408 activates nuclear factor erythroid 2-related factor 2 (Nrf2). Upon activation, Nrf2 induces the expression of a variety of cytoprotective proteins (i.e., antioxidants). ABT-RTA-408 is in development for metastatic non-small-cell lung carcinoma and skin cancer. PRI-724, which is in Phase I clinical trials for efficacy against hematological malignancies (**Table 2**), targets the recruitment of CBP (the binding protein of the cAMP response element–binding protein CREB) with β-catenin, an interaction that mediates the transcription Wnt/β-catenin signaling pathway proteins (63, 64). The E2F1 transcription factor is targeted by ARQ-761, a β-lapachone prodrug that is in Phase I clinical trials for solid tumors (**Table 2**) and promotes NQO1-mediated programmed cancer cell necrosis.

STAT3 is constitutively active in many human tumors and is responsible for uncontrolled proliferation, cell survival, and drug resistance (75). Several small-molecule STAT3 inhibitors have been recently uncovered by a phenotypic high-content screening campaign (76, 77), which could be refined for better cellular activity. The fungal metabolite galiellactone covalently binds to several of the cysteines on STAT3 and prevents DNA binding (78). Galiellactone inactivates cellular STAT3 signaling pathways and suppresses growth of hormone-refractory prostate cancer in preclinical mouse models (79).

The Eya family of TFs are overexpressed in many human tumors and have been linked to transformation, invasion, migration, and metastasis (80). The Eya TFs are the first to be identified with an intrinsic tyrosine phosphatase domain. The phosphatase activity is used in the DNA damage response after radiation and chemotherapy to dephosphorylate histone H2AX, which encourages cells to undergo DNA repair rather than apoptosis. MLS000544460, which was identified by a high-throughput screening, is an allosteric inhibitor of Eya phosphatase (81) (**Table 3**). Molecular docking, mutagenesis, and other biophysical methods document that this *N*-arylidenebenzohydrazide binds at a unique site on the opposite face from the active site to inhibit enzyme activity (81). Further, preclinical studies are required to determine if these mechanistically interesting lead compounds can be developed into potentially useful agents.

The chromosomal inversion inv (16) (p13q22) results in the fusion of the core-binding factor β (CBFβ) to smooth muscle myosin heavy chain (SMMHC) to create a driver mutation that ultimately leads to acute myeloid leukemia. In normal cells, CBFβ is an essential component of a heterodimeric complex with the TF RUNX1, which enables hematopoiesis (63, 64). The

CBFβ-SMMHC fusion protein has a higher affinity for RUNX1, and as a result, sequesters it from targeted genes. A bivalent small molecule, AI-10-49, inspired by a high-throughput screen, selectively binds and sequesters CBFβ-SMMHC, allowing endogenous CBFβ to bind RUNX1 and to restore normal gene expression (82). AI-10-49 displays favorable pharmacokinetics and delays leukemia progression in mice (82). This bivalent sequestration strategy may have general application to other malignancies that involve aberrant dimerized fusion proteins.

Gene silencing approaches also are being advanced. For example, ISIS-EIF4ERX is an antisense oligonucleotide that targets eukaryotic translation initiation factor 4E (eIF4E), which is involved in a wide range of cancers (63, 64). Essentially, the eIF4E-focused antisense oligonucleotides bind to target mRNA, which then signals its degradation via ribonuclease H (63, 64). Likewise, STAT3 is targeted using a similar antisense oligonucleotide-based strategy. AZD9150 is an antisense oligonucleotide that targets an untranslated 3′-region of STAT3, which ultimately inhibits STAT3 protein expression. AZD9150 is in a Phase I clinical trial for hematological malignancies (**Table 2**).

CBFβ: core-binding factor β

SMMHC: smooth muscle myosin heavy chain

eIF4E: eukaryotic translation initiation factor 4E

RAS

RAS was identified as the first human mutated cancer-causing gene, but >30 years of intense research has not yet yielded a clinically viable RAS inhibitor, which seeded the widely held perception that the RAS oncoprotein is not druggable (83). That RAS is a highly prized cancer drug target is undeniable, for there is incontrovertible evidence that RAS is mutated in up to 30% of all tumor samples, and most importantly, in three of the four most lethal human malignancies: colon, lung, and pancreatic (84). It has long been known that RNA silencing of RAS suppresses the in vitro and in vivo growth of RAS-mediated human cancer lines (85). Cox et al. (83) recently recorded the frustrating lack of results to identify pharmaceutically attractive inhibitors of the oncogenic effects of the three RAS genes: HRAS, NRAS, and KRAS. Like many other undrugged targets, RAS lacks any deep, drug-suitable hydrophobic pockets on the protein surface, which thwarts efforts to find direct high-affinity inhibitors. Some low-affinity inhibitors have been identified, but all suffer from lack of potency, metabolic liabilities, or untoward effects (83, 86). As a result, alternative strategies are being considered. There is continued interest in preventing RAS from adhering to the plasma membrane, which is a requirement for its oncogenic functionality. Early efforts targeted farnysltransferase, which posttranslationally modifies HRAS, but the clinical trials were disappointing despite the indisputable importance of the lipid modification in cancer (87). It may be necessary to block both farnysltransferase and geranylgeranyltransferase 1, but this generates concerns about possible off-target effects owing to the hundreds of other substrates for these enzymes (88). Preclinical studies that target both lipid posttranslation modifications with a RAS carboxyl terminal mimetic *S*-farnesylthiosalicyclic acid (salirasib) reveal antitumor activity with a subset of patient-derived pancreatic cancer xenografts (89). Moreover, limited toxicities are seen in a clinical trial with a limited number of patients with metastatic pancreatic adenocarcinoma (89). Consequently, it would seem useful to explore the question of whether to further target lipid modifications of RAS pharmacologically. It may be possible to direct U3 ubiquitin ligases with molecular glue strategies to accelerate the degradation of mutant forms of RAS and other historically undrugged targets (90, 91). Others are pursuing efforts to interfere with the multiple RAS effectors (83).

PERSPECTIVE

The past decade has seen the remarkable validation of molecular cancer targets that were previously perceived as challenging, if not undruggable. Most notable among these are the protein tyrosine kinases. This validation process has been greatly facilitated by the discovery and deployment of

novel small molecules and biologics. Peering into the future, although usually dangerous when discussing therapeutics, makes one think that other medicinal barriers will be broken soon. There is considerable excitement about the development of cancer targets that have been known for decades but have resisted effective therapeutic regulation, including c-MYC, RAS, and SRC. Advanced preclinical studies and even clinical trials provide encouragement that for a number of transcription factors, clinically useful small molecules may emerge. Essential oncogenic protein-protein interactions could also be faithfully and selectively disrupted or disabled. If history teaches us anything, it is that a successful prototype drug begets emulators.

SUMMARY POINTS

1. Cancer drug discovery and development has uncovered therapeutic approaches for several previously high-risk molecular targets with both small molecules and biologics.
2. Although many cancer-associated targets remain outside the reach of pharmacological intervention, it is more productive to think of them as undrugged rather than undruggable.
3. New structural information, knowledge about signaling pathways, advanced tool compounds, and sophisticated assays are producing attractive preclinical and clinical candidates.
4. There is a renewed interest in seeking clinical candidates for challenging cancer targets such as phosphatases, transcription factors, and RAS family members.

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