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Functional Interrogation and Therapeutic Targeting of Protein Tyrosine Phosphatases

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Abstract

Protein tyrosine phosphatases (PTPs) counteract the enzymatic activity of protein tyrosine kinases to modulate levels of both normal and disease-associated protein tyrosine phosphorylation. Aberrant activity of PTPs has been linked to the progression of a number of disease states, yet no PTP inhibitors are currently clinically available. PTPs are without a doubt a difficult drug target. Despite this, a number of selective, potent, and bioavailable PTP inhibitors have been described, suggesting PTPs should once again be looked at as viable therapeutic targets. Herein, we summarize recently discovered PTP inhibitors and their use in the functional interrogation of PTPs in disease states. In addition, an overview of the therapeutic targeting of PTPs is described using SHP2 as a representative target.

Introduction

Post-translational phosphorylation of tyrosine residues regulates a wide variety of cellular processes including cell survival, growth, and migration among other roles [1]. Tyrosine phosphorylation status is kept in check by the contrasting enzymatic activity of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) that append or remove phosphate, respectively. Considering the scope of cellular functions that PTKs and PTPs are associated with, it comes as no surprise that dysregulation of tyrosine phosphorylation is implicated in a number of disease states including cancers, diabetes, and immune disfunction [2,3]. While therapeutic agents targeting PTKs are already available in the clinic, no such compounds exist for PTPs. PTPs represent a promising class of therapeutic targets for a number of reasons. Treatment of patients using kinase inhibitors initially shows promise though these molecules are eventually rendered ineffective through a variety of resistance mechanisms.[4] Given the interwoven nature of PTKs and PTPs, it is tempting to envision PTP inhibition as a novel route to modify the same cellular pathways that may be targeted by PTK inhibitors. Furthermore, PTPs remain relatively unexplored in the context of disease treatment in humans and might represent a treasure trove of novel therapeutic opportunities.

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Among major challenges of targeting PTPs for drug discovery are the limited understanding of PTPs in disease biology and the general lack of PTP-specific small molecule probes for functional interrogation, target validation, and therapeutic development. Chemical probes can help address these issues by illuminating PTP druggability and providing the foundation for the development of novel therapeutics.

Genetic approaches have been widely employed to study the role of protein targets in disease state pathogenesis. The recent advent of CRISPR gene editing supplemented the already powerful genetic engineering tools such as RNA interference and gene knockout / overexpression techniques, providing scientists with a multitude of ways to quickly probe the role of proteins in a cellular environment [5]. However, these genetic approaches are limited in their ability to provide information on the temporal, spatial, and dynamic roles of enzymes in cellular signaling processes. Genetic manipulation of proteins may also result in confounding variables such as cellular compensation, resulting in unintended and unexpected cellular phenotypes. Furthermore, since these techniques usually result in complete ablation of proteins, the role of targets with multiple functions (e.g., enzymatic activity vs. protein-protein interactions) cannot be accurately delineated, limiting the applicability of this type of approach.

The availability of quality chemical probes is therefore of paramount importance [6]. In addition to complementing the variety of genetic techniques, small molecule probes are rapid and reversible, allowing for temporal interrogation of protein targets. In contrast to common genetic techniques, pharmacological probes must overcome potency and selectivity issues that are often time consuming and represent a significant hurdle in the development of quality probes.

Widely considered an “undruggable” class of enzymes, probe development for PTPs is hindered by two key factors [7]. First, residues composing the active site create a positively charged binding surface (Figure 1), and the high-affinity inhibitors that exploit this characteristic are thus negatively charged, reducing cell permeability. Second, the enzyme active site (pTyr pocket) is highly conserved among PTP enzymes, making selective PTP inhibitors difficult to develop. Despite these difficulties a number of potent and/or selective inhibitors have been developed for a variety of therapeutically relevant PTPs [8–10] including Phosphatase of regenerating liver (PRL), *Mycobacterium tuberculosis* PTP B (mPTPB), Low-molecular-weight protein tyrosine phosphatase (LMW-PTP), Mitogen-activated protein kinase phosphatase 5 (MKP5), and Src homology domain containing phosphatase 2 (SHP2) [11–17]. This minireview will focus on recently developed selective PTP inhibitors and their use in the functional interrogation of PTPs, as well as provide an understanding of the therapeutic value of PTP inhibitors using SHP2 as an example. While many quality inhibitors exist for PTPs, our goal is not to provide a comprehensive review of all inhibitors, but rather to showcase representative PTP inhibitors with various mechanisms of action (MOAs) including both allosteric and active site targeted inhibition (Table 1).

Functional interrogation of PTPs using small molecule inhibitors

Phosphatase of Regenerating Liver

Phosphatase of regenerating liver (PRL) enzymes consist of three homologous members, PRL 1, 2, and 3 [18–20]. Exogenous expression of PRLs promotes cell proliferation, and constitutive PRL expression increases the metastatic capabilities of cancer cells by activating the ERK1/2 and AKT pathways [21–23]. Furthermore, cells overexpressing PRL form highly metastatic tumors upon injection into mice, while knockdown of PRL reduces tumorigenesis *in vivo* [24]. In humans, elevated levels of PRL expression are observed in a number of cancers including colon [25], liver [26], ovarian [27], and others [20,28,29]. Additionally, PRL overexpression is strongly correlated to late-stage metastasis and poor clinical outcomes, providing further clinical significance. Taken together, these data implicate PRLs as oncogenes that are viable therapeutic targets [30].

PRLs can form homotrimers in both protein crystals and in solution, a property that is unique among phosphatases and has been shown to contribute to PRL-1 mediated cell growth and migration [31]. Leveraging this characteristic, it was hypothesized by Bai et al. that a selective inhibitor could be developed by binding to the interface of the PRL monomers, avoiding the drawbacks of active site inhibitors [11].

With three monomers forming the trimer complex in the PRL-1 crystal structure, it was rationalized that disrupting the interface between any one of these three monomers might preclude the formation of the trimer, thus reducing the PRL-1 mediated cell growth and migration. A structure-based virtual screen was carried out targeting the two different trimer interfaces on the PRL-1 monomer. Hits were validated biochemically, and the most effective detrimerizer of the hits was **Cmpd-43**. A structurally related molecule, **Analog 3**, was crystallographically confirmed to disrupt PRL-1 trimerization by binding to the interface between PRL-1 monomers. **Cmpd-43** was shown to have no effect on the phosphatase activity of a large panel of phosphatases including PRL-1 at 20 μM (Figure 2A). **Cmpd-43** was confirmed to attenuate ERK1/2 and AKT activity and reduce PRL-mediated cell proliferation and migration, while an inactive analog could not. Furthermore, **Cmpd-43** had no effect on a cell line transfected with the trimerization deficient PRL1/G97R loss of function mutant. Taken together, these data provided strong evidence that **Cmpd-43** selectively inhibits PRL-1 trimerization, resulting in an abrogation of PRL-1 mediated cellular signaling and a reduction in cell proliferation and migration. Furthermore, **Cmpd-43** was able to reduce tumor volume and weight in a mouse xenograft model in a manner consistent with the previously observed cellular results.

Protein-protein interactions (PPIs) are inherently difficult to target, characterized by large, flat, highly hydrophobic surfaces.[32,33] Thus, it is difficult to generate high-affinity small molecule inhibitors for these types of binding sites. However, with the crystal structure available for the PRL-1 detrimerizer, it is possible that structure-based design may be employed to optimize Cmpd-43 to overcome this issue of potency.

Since the discovery of these detrimerizing compounds, a number of PRL-1 active site inhibitors have also been disclosed, confirming the importance of this target.[34,35]

Collectively these observations provide strong evidence for the functional requirement for PRL trimerization and suggest that inhibition of PRL trimerization is a viable mechanism for developing novel therapeutics for PRL-associated diseases.

Mycobacterium tuberculosis protein tyrosine phosphatases

Mycobacterium tuberculosis (Mtb), the pathogenic cause of tuberculosis (Tb) infected approximately 10 million people in 2019 alone (WHO, 2020 tuberculosis report), causing 1.4 million deaths according to the World Health Organization (WHO). Tb is the leading cause of death worldwide from a single agent, and while substantial efforts have been made to reduce the morbidity and mortality of Tb, progress is hampered by a number of factors including drug resistance [36]. Thus, novel Tb targets are of great interest in the fight against Tb.

Tb encodes for a phosphatase, mPTPB, which is secreted into the cytoplasm of host macrophages, promoting bacterial survival by altering the host macrophage signaling [37]. Mechanistically, mPTPB has been shown to attenuate the innate immune responses by blocking the ERK1/2 and p38 kinase mediated IL-6 production and prevent cell death through activation of the AKT pathway in the macrophage [38]. Therefore, mPTPB represents a promising target for anti-Tb drug development.

A recently published potent and selective inhibitor of mPTPB was used to confirm previously observed cellular effects from pharmacological inhibition [12]. Briefly, compound **4t** increased the phosphorylation status of ERK1/2 and p38 and decreased AKT phosphorylation (Figure 2B). These observations are similar to those invoked by structurally unrelated small molecule mPTPB inhibitors [39,40], indicating that the noted cellular effects of **4t** in macrophages are indeed from specific mPTPB inhibition. With corroborating results between distinct probe series and genetic approaches, it is clear that pharmacological inhibition of mPTPB represents a viable route towards the development of novel Tb therapeutic agents, and other groups are continuing drug discovery efforts targeting this protein, denoting the importance of this target for the development of anti-Tb agents.[41] Existence of these inhibitors also supports the concept that selective, potent, and drug-like active site inhibitors are possible to develop.

Low molecular weight protein tyrosine phosphatase

Low-molecular-weight protein tyrosine phosphatase (LMW-PTP) is a ubiquitously expressed PTP whose function is not fully understood and appears to be disease state dependent [13]. The 18 kDa LMW-PTP is found in the cytosol of both prokaryotes and eukaryotes and has been shown to regulate a wide variety of signaling cascades and receptors, including the insulin receptor (IR) [42]. As such, LMW-PTP has been proposed as a therapeutically relevant target for type 2 diabetes (T2D). Studies have shown that decreased LMW-PTP activity is protective against increased blood sugar levels in both diabetic and nondiabetic groups [43] and genetic manipulation of LMW-PTP levels decreases insulin resistance in mice and increases IR phosphorylation in murine liver and fat cells [44], suggesting that LMW-PTP is directly responsible for IR phosphorylation status through its

phosphatase activity [42,45]. Taken together, these data suggest that LMW-PTP represents a promising therapeutic target for modulating T2D and insulin resistance.

In a recent study by Stanford et al. a global knockout (KO) murine model was used to demonstrate that global deletion of LMW-PTP reduced diabetes in LMW-PTP KO mice that were fed a high-fat diet compared to the wildtype (WT) control group [14]. LMW-PTP is not tissue specific, so the authors sought to determine if results from the global KO mouse model may be attributed to certain tissues. Indeed, the phenotypic results from the global LMW-PTP KO model were recapitulated only when LMW-PTP was deleted in the liver. Further investigation provided evidence that IR tyrosine phosphorylation was increased in the LMW-PTP KO mice compared to the control group, with increased phosphorylation levels of AKT and ERK, downstream proteins of the IR pathway.

To identify novel LMW-PTP inhibitors without highly charged functional groups, a high-throughput screen (HTS) was carried out to find inhibitors that did not display a competitive mode of inhibition, yielding a number of hits after a robust series of counter-screening and validation [14]. Structure-activity relationship (SAR) experiments for one hit resulted in the uncompetitive inhibitor **18**, with a half-maximal inhibitor concentration (IC_{50}) value of 0.46 μM and significant selectivity towards both isoforms of LMW-PTP. Compound **18** was determined to form a ternary complex with LMW-PTP and vanadate via crystallography, corroborating the uncompetitive mechanism of inhibition. The selectivity of **18** was explained by the structural information; compared to other PTPs, **18** is able to prevent hydrolysis of only the LMW-PTP phsophocysteine. As an example, the authors point to PTP1B, where the active site could accommodate both **18** and water molecules simultaneously, reducing the ability of **18** to prevent phosphocysteine hydrolysis.

With this novel and selective probe series in hand, the functional interrogation of LMW-PTP's role in IR phosphorylation and diabetes was carried out [14]. Treatment of human hepatocytes with the orally bioavailable and selective analog **23** resulted in increased IR phosphorylation, matching the observed effect of the LMW-PTP KO model (Figure 2C) [14]. Compound **23** was well tolerated by diabetes-induced obese mice that were fed a high-fat diet, with no change in body weight being observed. Additionally, both glucose tolerance and decreased fasting insulin levels were improved. Similar to the observed results in the cellular experiments, treatment of mice with **23** for two weeks resulted in an increased level of IR tyrosine phosphorylation, reducing phosphorylation of downstream AKT and ERK; these results were not observed in a liver-specific knockout mouse model treated with **23**, suggesting that the phenotypic effects of **23** are specifically from LMW-PTP inhibition. Taken together, these results provide critical evidence supporting the role of LMW-PTP as a novel therapeutic target in diabetes that is worthy of further exploration.

Mitogen-activated protein kinase phosphatase 5

Mitogen-activated protein kinases (MAPKs) are regulated by MAPK phosphatases (MKPs), a family of dual-specificity phosphatases (DUSPs) [46]. MAPKs are involved in critical signal transduction pathways, and abnormal activity of MAPKs is integral to a number of diseases including cancer [47], diabetes [48], and others [49]. MKPs are able to regulate the MAPKs by removing phosphate groups from threonine and tyrosine side chain hydroxyls on

MAPK activation loops [50]. The specificity of the MPKs is attributed to a kinase interaction motif that binds to specific MAPK substrates [51,52].

MKP5 has emerged as a potential therapeutic target for Duchenne muscular dystrophy (DMD) [53], a genetic disorder that prevents the ability of skeletal muscle to regenerate. This results in the replacement of skeletal muscle with fibrotic tissue, which is inevitably fatal [54–56]. The transforming growth factor-β1 (TGF- β1) pathway is partially responsible for the development of fibrosis, and as such represents a strategic route for the treatment of DMD [57]. Interestingly, mice that lack MKP5 expression show an enhanced ability to regenerate muscle tissue, and a MKP5 KO mouse model reduces the DMD phenotype [53]. This suggests a function for MKP5 in DMD, though the mechanistic role was not clear.

To this end, Gannam et al. developed an HTS biased to identify allosteric inhibitors using a phosphopeptide instead of a small molecule substrate [15]. After retesting and triaging compounds, the resulting 27 molecules were tested for selectivity against a panel of DUSPs. The most interesting molecule, compound **1**, displayed an IC_{50} value of 3.9 μM and displayed a 51-fold and 25-fold selectivity window against STEP-46 and PTP1B. Kinetic characterization of compound **1** revealed a mixed mechanism of inhibition by slowing down the rate of enzymatic catalysis. Co-crystallization of MKP5 and compound **1** provided insight into the results from the kinetic experiments. Compound **1** was determined to bind to a previously unrecognized allosteric site on MKP5. Interestingly, binding of compound **1** results in a conformational shift in the backbone of residues that form the active site, reducing the volume of the active site by 18%. Mutational analysis of the allosteric binding pocket revealed that the amino acids responsible for the interactions between compound **1** and MKP5 are required for catalysis and are not conserved across MKPs, explaining the selectivity of compound **1** for MKP5.

Discovery of compound **1** provided the ability to explore the role of MKP5 in disease states [15]. Corroborating previously observed results, treatment of mouse myoblasts (C2C12 cells) with compound **1** resulted in a dose-dependent increase in p38α and pJNK while having no effect on pERK1/2. Compound **1** was able to phenocopy MKP5-deficient mice and significantly increase myoblast differentiation, suggesting that selective inhibition of MKP5 by compound **1** increases levels of pJNK and pMAPK (Figure 2D). Hypothesizing that MKP5 is involved in the TGF-β1 driven progression of fibrosis, the authors investigated how a MKP5 knockout mouse model effects enzymes downstream of the TGF-β1 pathway. Mice lacking MKP5 ($MKP5^{-/-}$) had reduced Smad2 phosphorylation levels compared to wild-type control mice. Smad2 phosphorylation was significantly decreased in the TGF-β1 $MKP5^{-/-}$ embryonic fibroblasts compared to the $MKP5^{+/+}$ control cells while pJNK and pp38MAPK levels were increased with no change in pERK1/2. Furthermore, treatment of $MKP5^{-/-}$ cells with compound **1** displayed no additional effects on Smad2 phosphorylation levels or on pJNK, pMAPK, and pERK1/2, suggesting that compound **1** selectively targets MKP5 to counter TGF-β1 signaling in DMD. While more work needs to be done to further the understanding of MKP5's role in muscular dystrophy, these results clearly indicate that MKP5 is a viable therapeutic target for the treatment of DMD. Furthermore, the authors suggest that allosteric inhibition may be applicable to target other members of the MKP family, opening an avenue for the development of additional therapeutic agents.

Therapeutic targeting of SHP2

As one of the most highly studied PTPs, Src homology domain containing phosphatase 2 (SHP2) is considered a *bona fide* oncogene [7]. An in-depth analysis of all SHP2 inhibitors is beyond the scope of this mini review, thus we will focus on the recent use of SHP2 inhibitors and the therapeutic targeting of SHP2 through the use of these molecules. Reviews covering the variety of different SHP2 inhibitors are available elsewhere [58–61].

SHP2 resides upstream of RAS and functions as a shared signaling node [62–65]. In its basal state, SHP2 is autoinhibited by interactions between its N-SH2 and PTP domains, blocking the catalytic site from binding its substrate [66]. As such, the phosphatase activity of full length wild-type SHP2 is 5% of the catalytic domain alone [67]. However, upon binding to specific pTyr motifs on receptor PTKs, SHP2 is concomitantly localized to its intracellular substrates and brought into an active conformation [68]. The N-SH2 and C-SH2 domains interact with the pTyr motifs, weakening the intramolecular N-SH2/PTP interaction and revealing the active site, a requirement for the full activation of the RAS and ERK1/2 pathway [7]. Inhibition of SHP2 by both orthosteric and allosteric mechanisms phenocopy genetic depletion, resulting in decreased phosphatase activity and decreased growth of metastatic breast cancer cells in 3D models (Figure 2E) [69].

In 2016, scientists from Novartis revolutionized the PTP inhibitor field with their discovery of the first allosteric SHP2 inhibitors [70]. Assay design was key to this discovery; it was reasoned that screening compounds against SHP2 in an equilibrium between the open and closed conformation would bias results towards allosteric rather than orthosteric hits. The resulting molecule was determined to inhibit the full length SHP2 construct with greater efficacy than towards the PTP domain alone. Indeed, as was their intention, the inhibitor was shown to bind at the interface between all three domains, stabilizing the inactive conformation of SHP2. Structure-based optimization resulted in the first generation allosteric SHP2 inhibitor **SHP099** (Figure 3), with IC_{50} values of 0.07 μM and 0.62 μM against SHP2 and pERK respectively. In an attempt to optimize **SHP099**, Bagdanoff et al. employed scaffold hopping in combination with structural information from both previous and novel SHP2 allosteric inhibitors [71]. This resulted in **SHP389** (Figure 3), an improved analog of **SHP099** whose development was ultimately terminated due to sub-optimal drug-like properties. However, this study provided critical information about the key structural features required for binding to the allosteric site of SHP2. Eventually, Lamarche et al. described the development of **TNO155** (Figure 3), an orally available inhibitor of SHP2 (IC_{50} = 0.011 μM) that is currently in phase 1 clinical trials [72]. Inspired by this series, a number of SHP2 allosteric inhibitors were described by other groups; an in-depth review of these molecules, their SAR, and efficacies is described by Yuan et al. and will not be discussed here [58]. Of these SHP2 allosteric inhibitors, multiple are currently being tested in clinical trials as either a monotherapy or in combination with other approved anticancer therapies (Table 2) [73].

All of these trials are in progress at the time of this writing, so very little information regarding efficacy, safety, and tolerability is available. However, preliminary data regarding the efficacy of **RMC-4630** (Structure unavailable) in patients with KRAS-mutant non-small

cell lung cancer has been published. In this study, 56 patients are being treated with **RMC-4630**, and 19/23 patients diagnosed with non-small cell lung cancer (NSCLC) harbor a KRAS mutation [74]. The disease control rate for patients with $KRAS^{G12C}$ mutations is 71%, and tumor volume was reduced in three of these patients, suggesting preliminary efficacy. Additionally, one patient with a $KRAS^{G12D}$ oncogenic mutation displayed antitumor activity when treated with **RMC-4630**. Evaluation of blood cells and tumor biopsies suggested on-target efficacy according to pERK levels.

Though information regarding the efficacy of SHP2 inhibitors in clinical trials is sparce, the preclinical observations appear promising. Two groups independently identified SHP2 as a target for KRAS-mutant NSCLC cells, with synergism occurring when both MEK and SHP2 were targeted concomitantly using selumetinib or trametinib and **SHP099**, supporting the concept of the MEK/SHP2 combination therapies in clinical trials [75,76]. Another group used **RMC-4550** (Figure 3) to show the reliance of BRAF, NF1, and KRAS oncogenic mutants on the catalytic activity of SHP2 [77]. These RAS pathway oncogenes are often observed in NSCLC and, until very recently were not treatable with pharmacological intervention[78], suggesting SHP2 may function as a novel therapeutic target for patients with cancers reliant on these proteins [77]. An additional study from Wong et. al. provided further support for the combinatorial targeting of MEK and SHP2, where it was determined that pharmacological inhibition of SHP2 proved an effective therapeutic option for cancers where MEK inhibitors were no longer viable due to KRAS amplification [79]. Further support for the clinical evaluation of SHP2 inhibitors was detailed by Ahmed et al., where SHP2 was determined to reduce the efficacy of BRAF and MEK inhibitors in ERK-dependent tumors [80]. The combined inhibition of MEK and SHP2 was efficacious against a variety of cancer cell lines, though the success of this approach was dependent on a variety of factors and may not be broadly applicable [80]. Very recently, Liu et al. provided yet further supporting evidence for the utility of SHP2 inhibitor combination therapies. Inhibition of SHP2 using **TNO155** was able to overcome resistance to EGFR inhibitors osimertinib and nazartinib by preventing RTK-mediated MAPK pathway reactivation [81]. Additionally, because of RAS-MAPK feedback pathway regulation, combination of **TNO155** with MEK inhibitors was also shown to be synergistic, though this approach may be limited due to insufficient tolerability. The most effective combination during this study was seen when using both **TNO155** and $KRAS^{G12C}$ inhibitors, a promising result that is supportive of the potential success for clinical trials using this approach [81]. Taken together, it is apparent that there is abundant preclinical data to support the successful outcomes of these SHP2 mono and combination therapy based clinical trials.

Conclusion

Despite the obvious importance of PTPs in disease state pathogenesis, many have given up on this class of targets, describing them as “undruggable”. From a drug discovery standpoint, the difficulty of this class stems from the high sequence homology and highly charged active site. Many of the high-affinity inhibitors that were a result of early drug discovery campaigns often had multiple negative charges resulting in reduced bioavailability and cell permeability. However, recent developments have shown that not only is it possible to develop potent and selective PTP inhibitors, but these inhibitors can also be very

drug-like. Moreover, allosteric PTP inhibitors that modulate unique regulatory mechanisms represent another productive avenue for probe and drug development. Whether or not the clinical trials for the allosteric SHP2 inhibitors are successful is yet to be determined. However, it is clear that the discovery of these inhibitors has reinvigorated the field and PTPs should be once again considered highly valuable therapeutic targets.

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Perspective

- Protein tyrosine phosphatases represent a heavily underleveraged family of enzymes for the development of novel therapeutic agents. However, novel probes are required to bolster the confidence of the community that these compounds are druggable, enabling studies to validate specific PTPs in a disease-dependent context.
- Widely considered “undruggable” and given up on as viable drug targets, it is apparent that PTPs are high-value targets that may be targeted with drug-like small molecules by targeting allosteric binding sites in addition to the active site. Novel mechanisms of action have been utilized to develop PTP inhibitors that are effective and drug-like.
- Additional efforts are necessary to expand the PTP inhibitor toolbox. We hypothesize that new and re-emerging techniques such as proteolysis targeting chimeras and targeted covalent inhibitors will be utilized in the development of novel PTP probes, reinvigorating the PTP inhibitor field.

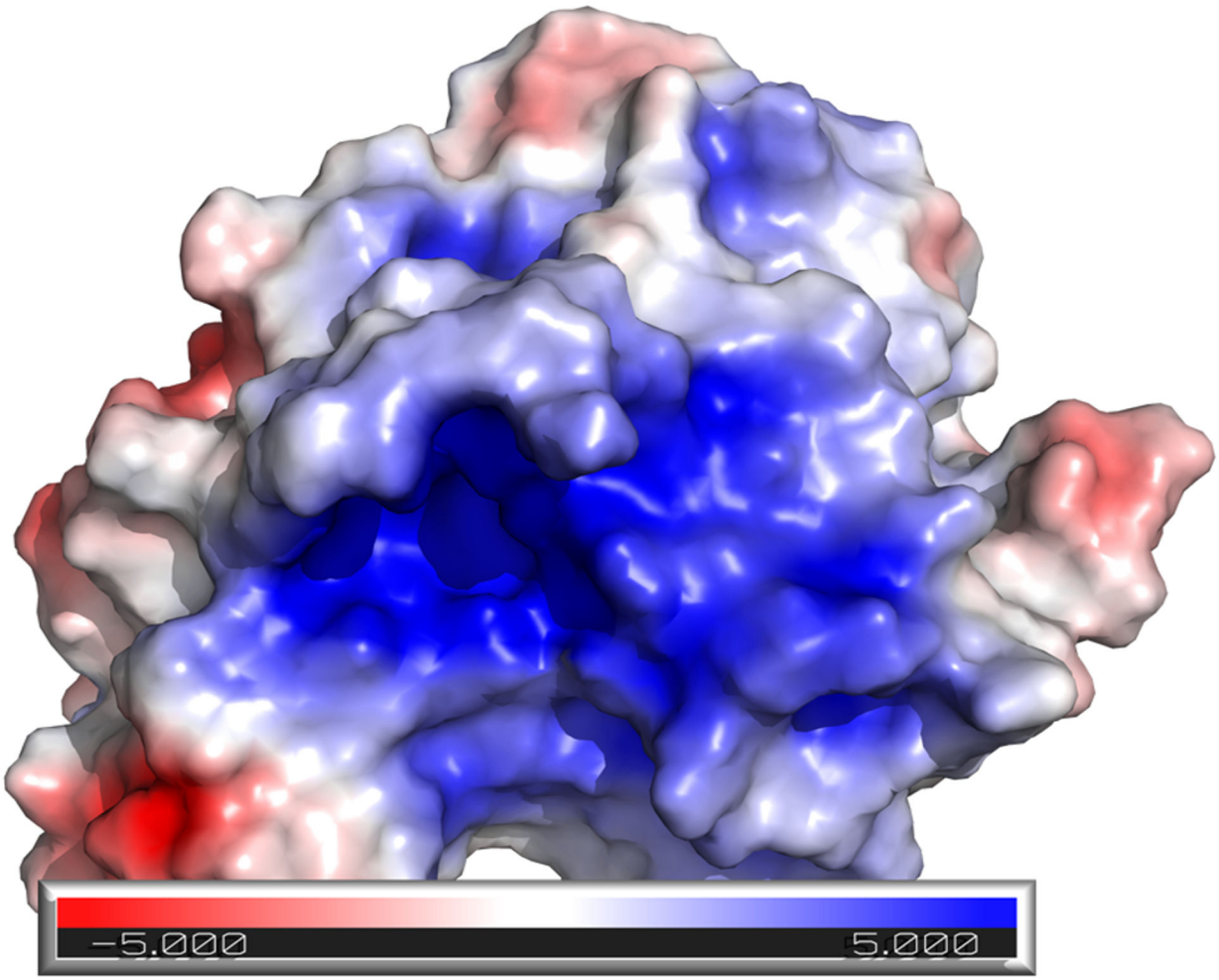


Figure 1.
The active site of Src homology domain containing phosphatase 2 (SHP2) (PDB ID 3ZM0) is positively charged (blue surface), a characteristic that is common among many protein tyrosine phosphatases.

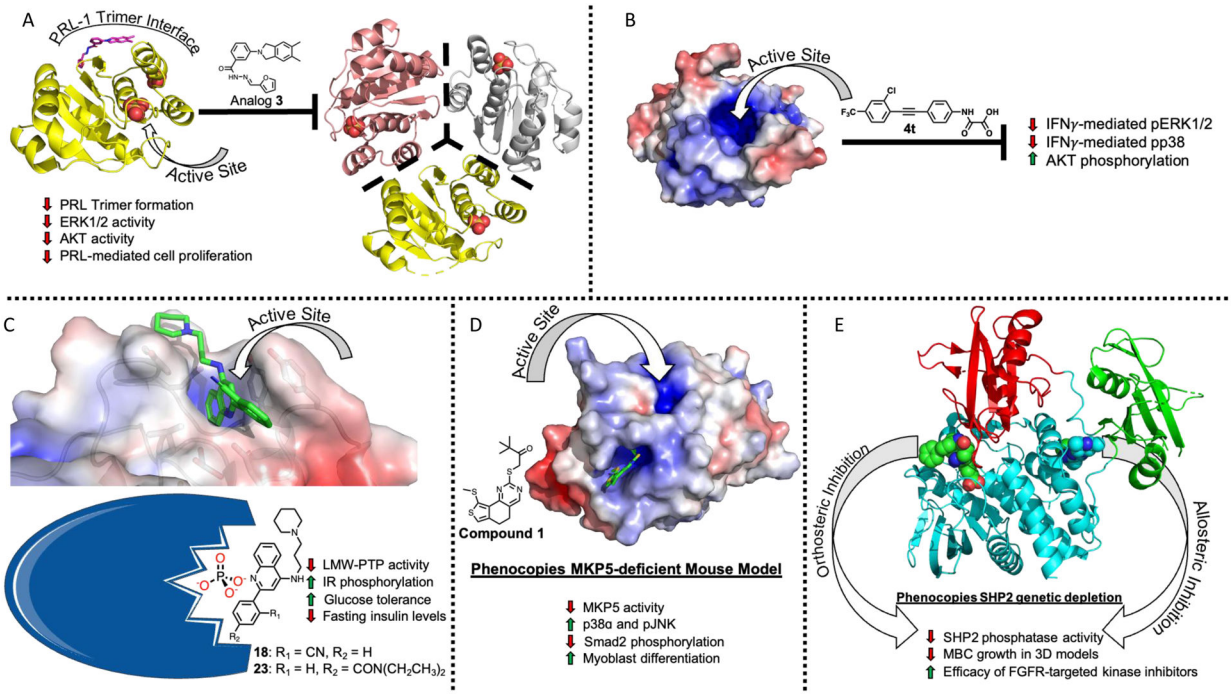


Figure 2.

A) **Analog 3** (magenta sticks) (PDB ID 5BX1) and **Cmpd 43** (not shown) both prevent PRL-1 (yellow) from forming a homotrimer, resulting in decreased ERK1/2 activity, decreased AKT activity, and decreased cell proliferation. B) Inhibition of mPTPB by compound **4t** prevents the interferon gamma (IFNγ) mediated phosphorylation of ERK1/2 and p38 and increase AKT phosphorylation. C) Uncompetitive inhibitor **18** (green sticks) reduces phosphatase activity of LMW-PTP (Cartoon depiction adapted from Servier Medical Art under the Creative Commons Attribution 3.0), resulting in increased insulin receptor (IR) phosphorylation, increased glucose tolerance, and decreased fasting insulin levels, linking LMW-PTP activity to diabetes. D) Allosteric inhibition of MPK5 by **compound 1** (green sticks) results in decreased MPK5 activity and increased levels of p38α and pJNK, leading to decreased Smad2 phosphorylation levels and increased myoblast differentiation. E) Active site inhibitors (green spheres) and allosteric inhibition (blue spheres) phenocopy genetic depletion of SHP2, decreasing growth of metastatic breast cancer tumors in 3D models and increasing efficacy of FGFR-targeted kinase inhibitors.

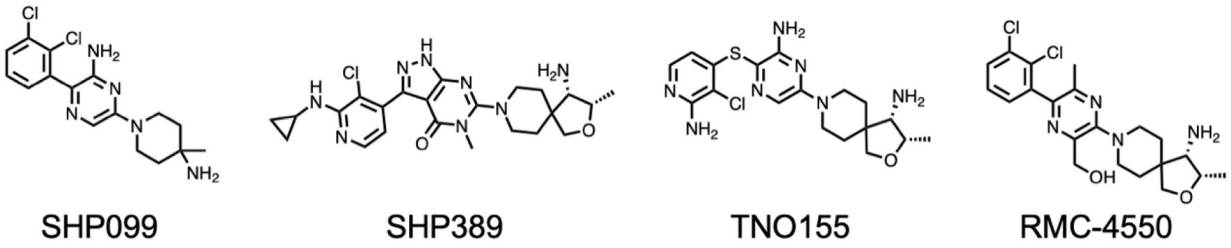


Figure 3.
Structures of select SHP2 allosteric inhibitors mentioned in this text.

Table 1.

PTP inhibitors described in this review

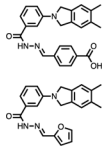
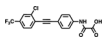
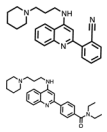
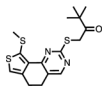
Target (Inhibitor)	Chemical Structure	Method of Discovery	Binding Site	Supporting Evidence
PRL-1 (**Cmpd 43** and **Analog 3**)		Virtual HTS	Allosteric site - PRL-1 trimer interface	Crystal structure and biochemical data
mPTPB (**4t**)		Fragment based and rational design	Active site	Docking and biochemical data
LMW-PTP (**18** and **23**)		HTS	Active site	Crystal structure and biochemical data
MKP5 (**Compound 1**)		HTS	Allosteric site	Crystal structure and biochemical data
SHP2 (See Figure 3)	See Figure 3	HTS, then rational design	Allosteric site	Crystal structure and biochemical data

Table 2.

Allosteric SHP2 inhibitors in clinical trials

Drug	Company	Status	Indication	Combination	NCT ID
JAB-3068	Jacobio Pharmaceuticals	Phase I (Ongoing)	• Non-small cell cancer • Head and neck cancer • Esophageal cancer • Other metastatic solid tumors	Monotherapy	NCT03518554
JAB-3068	Jacobio Pharmaceuticals	Phase I/II (Ongoing)	• Non-small cell cancer • Head and neck cancer • Esophageal cancer • Other metastatic solid tumors	Monotherapy	NCT03565003
JAB-3312	Jacobio Pharmaceuticals	Phase I (Ongoing)	• Non-small Cell Lung Cancer • Colorectal Cancer • Pancreatic Ductal Carcinoma • Esophageal Squamous Cell Carcinoma • Head and Neck Squamous Cell Carcinoma • Breast Cancer • Other Solid Tumors	Monotherapy	NCT04121286
JAB3312	Jacobio Pharmaceuticals	Phase I/II (Ongoing)	• Solid Tumors	Combination therapy with Pembrolizumab or Binimetinib	NCT04720976
JAB-3312	Jacobio Pharmaceuticals	Phase I (Ongoing)	• Non-small Cell Lung Cancer • Colorectal Cancer • Pancreatic Ductal Carcinoma • Esophageal Squamous Cell Carcinoma • Head and Neck Squamous Cell Carcinoma • Breast Cancer • Other Solid Tumors	Monotherapy	NCT04045496
TNO155	Novartis Pharmaceuticals	Phase I (Ongoing)	• Advanced EGFR mutant Non-small Cell Lung Cancer (NSCLC) • KRAS G12-mutant NSCLC • Esophageal Squamous Cell Cancer (SCC) • Head/Neck SCC • Melanoma	Mono therapy or combined with EGF816 (Nazartinib)	NCT03114319
TNO155	Novartis Pharmaceuticals	Phase I (Ongoing)	• Non-small Cell Lung Carcinoma	Combination therapy with Ribociclib or Spartalizumab	NCT04000529

Drug	Company	Status	Indication	Combination	NCT ID
			• Head and Neck Squamous Cell Carcinoma • Esophageal SCC • Gastrointestinal Stromal Tumors • Colorectal Cancer		
TNO155	Mirati Therapeutics Inc. and Novartis Pharmaceuticals	Phase I/II (Ongoing)	• Advanced Cancer • Metastatic Cancer • Malignant Neoplastic Disease	Combination therapy of MRTX849 with TNO155	NCT04330664
TNO155	Novartis Pharmaceuticals	Phase I (Ongoing)	• BRAF V600 Colorectal Cancer	Multiple of Dabrafenib with other drugs, including TNO155	NCT04294160
RMC-4630	Revolution Medicines, Inc. and Sanofi	Phase I (Ongoing)	• Solid Tumors	Monotherapy	NCT03634982
RMC-4630	Revolution Medicines, Inc. and Sanofi	Phase I (Ongoing)	• Solid Tumors	Combination of RMC-4630 with Cobimetinib or Osimertinib	
RLY-1971	Relay Therapeutics, Inc.	Phase I (Ongoing)	• Solid Tumors	Monotherapy	NCT04252339
BBP-398	Navire Pharmaceuticals	Phase I (Ongoing)	• Solid Tumors	Monotherapy	NCT04528836
ERAS-601	Erasca, Inc.	Phase I (Ongoing)	• Advanced or Metastatic Tumors	Monotherapy or in combination with undescribed MEK inhibitor	NCT04670679
N/A	Amgen	Phase I (Ongoing)	• Advanced Solid Tumors • Kirsten Rat Sarcoma (KRAS) pG12C Mutation	Sotorasib in combination with other therapies, including undescribed SHP2 inhibitor.	NCT04185883