PIM1 kinase as a target for cancer therapy

Anna Lena Merkel, Eric Meggers & Matthias Ocker
Philips University Marburg, Institute for Surgical Research, Marburg, Germany

Introduction: Inhibition of protein kinases has become a standard of modern clinical oncology. PIM1 belongs to a novel class of serine/threonine kinases with distinct molecular and biochemical features regulating various oncogenic pathways, for example hypoxia response, cell cycle progression and apoptosis resistance. PIM1 is overexpressed in human cancer diseases and has been associated with metastasis and overall treatment response; in experimental models, inhibition of PIM1 suppressed cell proliferation and migration, induced apoptotic cell death and synergized with other chemotherapeutic agents.

Areas covered: A PubMed literature search was performed to review the currently available data on PIM1 expression, regulation and targets; its implication in different types of cancer and its impact on prognosis are described. We present ATP-competitive PIM1 inhibitors and the state of the art of PIM1 inhibitor design. Finally, we highlight the development of the unusual class of highly selective and potent organometallic PIM1 inhibitors.

Expert opinion: As PIM1 possesses oncogenic functions and is overexpressed in various kinds of cancer diseases, its inhibition provides a new option in cancer therapy. Based on the ability of highly selective organometallic PIM1 inhibitors, promising in vivo applicability is expected.

Keywords: cancer, inhibitors, metal complexes, PIM1, serine/threonine kinase

Expert Opin. Investig. Drugs [Early Online]

1. Introduction

Malignant tumors continue to represent a global medical burden with more than 12 million new cases estimated per year and more than 7 million cancer-related deaths overall [1]. In Western countries, while cardiovascular diseases show a strong decline as a cause of death, cancer death rates remained stable over the past decades and represent the most common cause of death at the age of 40 – 79 [2]. The increasing identification and understanding of molecular pathways altered in cancer diseases has recently paved the way for the development of more specific anticancer agents, also known as targeted therapies [3,4]. Especially, inhibitors of growth factors and their receptor signaling have been investigated intensively and led to the approval of monoclonal antibodies (e.g., bevacizumab, cetuximab or panitumumab) or small molecule kinase inhibitors (e.g., erlotinib, sunitinib or sorafenib) [5]. This latter class of compounds can effectively target intracellular tyrosine kinases [6]. Yet, the specificity of these molecules is typically quite low due to the high homology of tyrosine kinase families [7] and the redundancy of these pathways allows cancer cells to either bypass the inhibited kinase or to develop resistance against a specific inhibitor. Recently, the serine/threonine kinase family has entered the focus of research. Small molecule inhibitors have been developed and tested in clinical trials for some members of this family, for example, the mTOR (mammalian target of rapamycin) complexes [8-10], polo-like kinases [11,12] or aurora kinases [13-15]. However, the overall efficacy of these inhibitors still
remains unsatisfying, with only low response rates and rather marginal improvements concerning overall survival in clinical trials [16-20].

Besides these well-established serine/threonine kinases, members of the PIM kinase family have recently been described to possess oncogenic and survival promoting properties [21,22]. We will here review the biological functions and medical implications of PIM1 kinase expression in cancer cells and highlight novel means of therapeutic intervention of PIM1 activity including organometallic complexes to specifically inhibit PIM1 activity.

2. Molecular biology and oncogenic functions of PIM1

2.1 Expression of PIM1 and its regulation

In 1984, PIM1 was identified as a common integration site for murine leukemia virus [23]; subsequently, its serine/threonine kinase activity was described [24]. PIM1 is expressed in two isoforms: a 34 kDa short variant and a 44 kDa long form; these two isoforms are synthesized by alternative transcription initiation (Figure 1) [25]. As it is a stress–response kinase, its expression can be induced by various cytokines, mitogens and hormones such as GM-CSF, G-CSF, IL-2, IL-3, IL-5, IL-7, IL-9, IL-12, IL-15, erythropoietin, Con A, PMA, interferon γ, steel cell factor and prolactin [26]. The most important pathways in the activation of PIM1 expression are Jak-STAT [27] and NF-κB [28]. Another transcription factor that can induce the expression of PIM1 is ERG, which plays a role in the initial stages of prostate carcinogenesis [29]. In solid tumors, the expression of PIM1 in response to hypoxia plays a crucial role and is HIF-1α dependent [28]. Furthermore, PIM1 is a target of different miRNAs, such as miR1 [30], miR33a [31] and miR328 [32], which reduce the translation of PIM1-mRNA or induce its degradation.

In contrast to other kinases, PIM1 is constitutively active [33]. Its activity is regulated at the level of expression and stability. In normal peripheral blood lymphocytes, the half-life of PIM1 is about 5 min, while it is prolonged in the chronic myelogenous leukemia cell line K562 (20 min) [34]. PIM1 is able to autophosphorylate [35], although the functional consequences of this phosphorylation still need to be investigated. One study reports that PIM1 can also be phosphorylated at Tyr218 by ETK, which enhances PIM1 activity [36]. PIM1 is degraded via the ubiquitin-proteasome pathway and can be protected from proteasomal degradation by the binding of heat shock protein Hsp90 [37]. In contrast, Hsp70 binds only to ubiquitinated PIM1 and promotes its degradation [37]. Additionally, the binding of the phosphatase PP2A mediates PIM1 dephosphorylation, ubiquitination and proteasomal degradation and is therefore a negative regulator [38].

2.2 Targets and functions of PIM1

Several proteins and pathways with oncogenic properties, for example cell cycle regulation and apoptosis control, have been identified as targets of PIM1 kinase activity.

PIM1 has been shown to stimulate cell cycle progression at various stages (Figure 2A). While phosphorylation of Cdc25A leads to enhanced G1/S transition [39], phosphorylation of Cdc25C induces increased G2/M transition [40]. A different way of how PIM1 regulates Cdc25C activity is the phosphorylation of C-TAK1, which induces its nuclear export and enables Cdc25C to be active [41]. Another cell-cycle-related target of PIM1 is the CDK inhibitor p21cip1/waf1. Here, phosphorylation at Thr145 leads to the nuclear export and inactivation of p21cip1/waf1 [42]. The phosphorylation of the CDK inhibitor p27kip1 at Thr157 and Thr198 induces its proteasomal degradation and cell cycle progression. Additionally, the transcription of p27kip1 is reduced due to the phosphorylation and inactivation of the transcription factors FoxO1a and FoxO3a [43]. Another mechanism of p27kip1 regulation is the phosphorylation of its ubiquitin ligase Skp2 at Thr417 by PIM1 which leads to Skp2 stabilization and p27kip1 degradation [44,45]. Moreover, PIM1 plays a role in promoting mitosis as it interacts with NuMA, dynemin/dynactin and HP1β. Here, PIM1 bridges the kinetochores via HP1β to the spindle assembly complex [46].

In addition to controlling cell growth pathways, PIM1 is able to block apoptotic cell death and thus can act as an oncogenic survival factor (Figure 2B). Phosphorylation of the pro-apoptotic protein Bad at Ser112 leads to proteasomal degradation and shifts the apoptosis threshold towards survival [47]. The inactivation of ASK1 through phosphorylation at Ser83 leads to decreased kinase activity and subsequently less phosphorylation of the stress kinases JNK and p38, which is associated with less caspase 3 activation and reduced cell death [48]. Apoptosis can also be blocked by PIM1 via the phosphorylation of PRAS40 at Thr246, which leads to the dissociation of PRAS40 from the mTOR complex and increases mTOR kinase activity and with increased phosphorylation of the mTOR downstream targets 4EBP1 and p70S6Kinase [49]. Interestingly, activation of mTOR has
been shown to interfere also with autophagy pathways, which indicate additional roles of PIM1 in energy metabolism and stress response [50,51].

Moreover, PIM1 influences the activity of different transcriptional regulators, for example NFATc1, p100, RUNX, SOCS1, RelA/p65, c-Myb and c-Myc (reviewed in [52]). The phosphorylation of c-Myc at Ser62 and Ser329 increases protein stability and thereby the transcriptional activity. c-Myc recruits PIM1 to the E boxes of its target genes, in which PIM1-dependent phosphorylation of histone H3 on Ser10 (H3S10) contributes to the activation of target genes and cellular transformation [53]. Elevated PIM1 levels seem to induce the p53 pathway in different cancer cell lines and murine embryonic fibroblasts. Here, PIM1 associates with and phosphorylates Mdm2 at Ser166 and Ser186 leading to stabilization of Mdm2 and p53. PIM1 also induces endogenous ARF, p53, Mdm2 and p21<sup>cip1/waf1</sup> in primary murine embryonic fibroblasts and stimulates oncogene-induced senescence [54]. It has also been described that the overexpression of PIM1 can inhibit cell and tumor growth by inducing senescence and DNA damage in a p53-dependent manner in human prostate cancer cells [55]. Moreover, PIM1 can mediate chemoresistance through the phosphorylation of the efflux transporter and putative stem cell marker BCRP/ABCG2 at Thr362 and the knockdown of PIM1 resensitized prostate cancer cells to chemotherapeutic drugs [56]. Additionally, PIM1 plays a role in hypoxia-induced chemoresistance [57] and tumor formation [51]. In line with this, PIM1 has also been shown to be involved in regulating homing processes of hematopoietic cells by interacting with the CXCR4-CXCL12 system [58], which is also an important regulator of solid tumor metastasis formation and thus indicates an additional oncogenic function for PIM1, which needs to be elucidated further.

### 3. PIM1 expression in human cancer diseases

Besides its association with murine leukemia [59,60], alterations in PIM1 signaling have been found in various
human tumor diseases too [52,61]. In hematologic malignancies, PIM1 expression correlated with poor prognosis in various leukemias [62], mantle cell lymphoma [63] and diffuse large B-cell lymphoma [64]. In solid tumors, overexpression of PIM1 has been detected in bladder cancer (with higher levels in invasive than in non-invasive cancers) [65] and in prostate cancer specimens, where it was also correlated with poor prognosis and therapy response [66-70]. Similar findings were obtained for esophageal [71] and gastric cancer [72,73], where PIM1 was upregulated after H. pylori infection in a cell culture model [74] and in gastric epithelia from patients [75]. In head and neck cancer, PIM1 was highly

---

**Figure 2. Oncogenic signaling pathways of PIM1.**

A. Cell cycle control by PIM1. Phosphorylation of target molecules leads to progression of the cell cycle. PIM1-mediated phosphorylation of cell cycle inhibitors activates cyclin/Cdk complexes, stimulates cell cycle checkpoint transitions and facilitates the kinetochore assembly as a prerequisite for mitotic division. Additionally, the transcription of c-Myc target genes is activated. B. Inhibition of apoptosis by PIM1 is mediated via phosphorylation of target molecules like Bad, ASK1 or PRAS40 which inhibit pro-apoptotic functions (e.g., the JNK/p38-caspase 3 axis) or stimulate anti-apoptotic (Bcl2) and pro-survival (mTOR) pathways.
expressed in more advanced stages and associated with poor prognosis and lymph node metastasis [76,77]. Here, PIM1 expression was also shown to be predictive for response to radiation therapy [78]. Interestingly, PIM1 was also found as a tumor-associated antigen in blood from colorectal cancer patients and has been proposed as a sensitive novel biomarker for this disease [79]. In contrast to these findings, a significant downregulation of PIM1 mRNA was found in primary non-small-cell lung cancer and PIM1 was even further suppressed in lymph node metastases compared with nodal negative tumors [80]. Elevated expression of PIM1 has also been described for patients with pancreatic cancer. Yet, high levels of PIM1 mRNA were associated with a prolonged median survival of 23.4 months compared with 13.8 months in patients with low PIM1 mRNA [81]. This is surprising as PIM1 has been shown to be directly correlated to oncogenic K-ras mutations and hypoxia also in pancreatic cancer models [51,82]. PIM1 was also reported to contribute to radio- and chemotherapy resistance in pancreatic [83], prostate [84], and lung cancer [85], which confirms the above described oncogenic properties in modulating hypoxia response or apoptosis signaling in tumor cells [57].

The change in PIM1 expression cannot be explained by gene rearrangements or amplification [62,86], although somatic hypermutations have been described in different lymphomas [87,88]; also this cannot be the reason for increased PIM1 expression.

4. Small molecule inhibitors of PIM1 kinase

All reported small-molecule PIM1 inhibitors are ATP-competitive binders. Crystal structures of PIM1 reveal the typical two-lobe protein kinase architecture connected by a so-called hinge region, with the catalytic ATP-binding domain positioned in a deep intervening cleft (Figure 3) [33,35,89,92]. The N-terminal lobe is comprised primarily of anti-parallel β-sheets and the C-terminal lobe mainly of α-helices. Unique features of PIM1 are a β-hairpin insert located at N-terminal to helix αC and, most importantly, the presence of a proline residue at position 123 within the hinge region. Almost all protein kinases form two canonical hydrogen bonds between the backbone of the hinge region and the adenine nucleobase of ATP, whereas in PIM kinases one hydrogen bond is prevented by this proline residue, which is probably responsible for the relatively high \( K_m \) value for ATP. While no crystal structure has been reported yet for PIM3, PIM1 and PIM2 adopt constitutively active conformations in their crystal structures, regardless of the phosphorylation state of the activation segment [93].

Several classes of PIM1 inhibitors have been described which cover a significant number of different chemical core structures, ranging from indolocarbazoles, bisindolylmaleimides, naphthylindoles, pyridazines and isoxazoles to thiazolidine-2,4-diones, thienopyrimidinones, pyridones and isoxazoloquinolines (Figure 4) [96-92,94-108]. Staurosporine analogs, inhibitors of casein kinases, PKC or phosphatidylinositol 3 kinase have a rather broad and unspecific profile on PIM1 inhibition [52,109]. Recently, more specific imidazopyridazine derivatives like ETP-45299 [110], K00135 [107] or SGI-1776 [111,112] or the dihydropyrrolocarbazole derivative DHPCC-9 [100,113] have been designed [114]. A monoclonal anti-PIM1 antibody, which binds to cytosolic, nuclear and membraneous PIM1 and induces apoptosis in human and murine cancer cells has been described in preclinical models [115]. So far, only SGI-1776 has been investigated in preclinical toxicity screening [116] and in two early phase clinical trials against refractory leukemia (NCT01239108), lymphoma or prostate cancer (NCT00848601). All mentioned compounds have been shown to inhibit cell proliferation and migration and to influence apoptosis by resensitizing tumor cells to conventional chemotherapeutic agents [110,112,117] or small-molecule kinase inhibitors [118] at low micromolar concentrations.

While all reported compounds have an ATP-competitive mode of action on inhibiting PIM1, one can classify them into two distinct binding modes: close ATP mimics and non-hinge binders. The unspecific broad-spectrum protein kinase inhibitor staurosporine and its maleimide analogs such as bisindoylmaleimides or the class of organometallic
pyridocarbazole complexes represent the first class and closely mimic the binding of ATP to the hinge region. For example, Figure 5 shows the binding of the highly potent organoruthenium complex (S)-DW12 to the ATP binding site of PIM1 (IC$_{50}$ = 0.2 nM at 100 µM ATP) [92]. The pyridocarbazole heterocycle mimics the hydrogen-bonding pattern of the adenine base of ATP by establishing one hydrogen bond between the NH group of the maleimide moiety and the backbone carbonyl group of Glu121, whereas crystal structures of this class of organometallic complexes to other protein kinases typically reveal a second hydrogen bond to one of the carbonyl groups of the maleimide; this is prevented in PIM1 by the proline residue at position 123 [119]. Additional water-mediated hydrogen bonds are formed between the hydroxyl group at the indole of (S)-DW12 and Lys67, Glu89 and Asp186. The organometallic inhibitor is tightly bound in the ATP binding site through a large number of hydrophobic contacts. Maybe most interestingly, although

**Figure 4. Selection of ATP-competitive PIM1 inhibitors.** The reported IC$_{50}$ or $K_i$ values are shown.
this organometallic scaffold is a conventional ATP competitive binder, the unique shape of the complex permits novel interactions with the glycine-rich loop as illustrated in Figure 5. The residues Leu44, Gly45, Phe49 and Val52 together form a small hydrophobic pocket that appears perfectly suited for the CO ligand. In contrast to organic carbonyl functional groups, metal coordinated CO lacks any significant dipole moment and therefore behaves like a very hydrophobic ligand. It is worth noting that in this and related crystal structures the metal is not involved in any direct interaction and merely serves as a structural center. Therefore, it is not surprising that replacing ruthenium in (S)-DW12 against osmium leads to a compound with identical inhibition properties [120]. Metallo-pyridocarbazole complexes currently represent the class of compounds with the highest affinity for PIM1 with IC₅₀ values reaching down to 0.075 nM and demonstrating exquisite PIM1 selectivities [121,122].

The second class of ATP competitive inhibitors is distinguished by the absence of any canonical hydrogen bonds with the hinge region. For example, Figure 6 displays the binding of a benzo[c]isoxazole derivative to the active site of PIM1 [96]. No classical hydrogen bond is observed with the hinge region but a pyrimidine moiety forms a hydrogen bond with Lys67 which is located at the opposite site of the pocket (Figure 6A). However, the authors discuss an unusual weak hydrogen bond between an aromatic CH and the backbone carbonyl group of Glu121. The binding of the imidazo [1,2-b]pyridazine inhibitor K00135, a compound that served as a lead structure for the development of SGI-1776, is shown in Figure 6B and also exploits a hydrogen bond with Lys67 [107]. K00135 was demonstrated to be surprisingly selective for PIM1 over more than 50 diverse serine/threonine kinase catalytic domains and the authors speculated that this is due to the combination out of the unusual PIM1 active site architecture, together with the unconventional binding mode. Interestingly, PIM2 was inhibited significantly weaker by a factor of around 100-fold although the amino acid residues of PIM1 and PIM2 are almost identical so that this might be the result of a dynamic effect. Overall, it appears to be a trend of many PIM inhibitors that selectivity for PIM1 and PIM3 is observed over PIM2. This selectivity might be the result of differences in the dynamic properties of the individual PIM isoforms [52].

5. Expert opinion

Effective and well-tolerable agents are still an unmet medical need in today’s cancer therapy. Despite the introduction of so-called targeted therapies, the overall survival of cancer patients is still disappointing. Commonly, these targeted therapies use tyrosine kinase inhibitors that show a high level

Figure 5. Structure of organoruthenium half-sandwich complex (S)-DW12 bound to the ATP-binding site of PIM1 in an ATP-mimetic fashion (pdb code 2BZI). A. Overall cartoon representation including van der Waals surface. B. Hydrogen bonding interactions of (S)-DW12 with the hinge region and water-mediated interactions to Glu89 and Asp186. C. Interactions with the glycine-rich loop.
of side effects and rapidly lead into resistance as tyrosine kinase pathways are highly redundant in tumor cells. PIM1 represents a constitutively active serine/threonine kinase which is distinct from other classes of kinases by both its molecular structure and its molecular regulation. PIM1 controls oncogenic signaling pathways like hypoxia, cell cycle control or apoptosis resistance and has been shown to be overexpressed in various human cancers and to be associated with metastasis and overall prognosis. Therefore, PIM1 is an interesting novel target for targeted drug therapy.

PIM1 inhibition by small molecules has been accomplished with a variety of chemical scaffolds through the development of ATP competitive binders, either by mimicking closely the hinge-binding of ATP or by exploiting other areas of the active site. Selected compounds have demonstrated efficacy in preclinical models and are currently undergoing early-phase clinical trials. However, the class of organometallic PIM1 inhibitors displays a significantly higher specificity and binding affinity down to picomolar $K_i$ values compared to purely organic inhibitors. This can be traced back to a perfect shape complementarity provided by the structural metal center combined with the ability to undergo unique interactions with the flexible glycine-rich loop of PIM1.

It is, of course, still necessary to better characterize the effect of these organometallic inhibitors on the molecular level in vitro. Here, the numerous interactions and signaling pathways influenced by PIM1 open further possibilities for combination approaches with tyrosine kinase inhibitors or direct pro-apoptotic stimulators to further increase the cytotoxic effects at probably even lower concentrations. Yet, the high number of available compounds demands further pharmacokinetic and pharmacodynamic studies in animal models that are at present still missing for most of these highly potent inhibitors. Based on their chemical properties, excellent in vivo applicability is expected and we therefore believe that clinical trials in human patients can be conducted in the near future.

Acknowledgements

We apologize to all colleagues whose work could not be cited due to space limitations.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

This paper describes how PIM1 is stabilized by the binding of Hsp90, but not by the binding of Hsp70 and that it remains active even after the binding of these proteins.


This paper shows that the cyclin-dependent kinase inhibitor p21cip1/waf1 can be phosphorylated by PIM1 which influences its subcellular localization and promotes cell cycle progression.


This paper demonstrates that PIM1 activity contributes to MYC-dependent transforming capacity.


This paper shows a mechanism how PIM1 is able to regulate chemoresistance.


61. Schenone S, Tintori C, Botta M. Using insights into pim1 structure to design new anticancer drugs. Curr Pharm Des 2010;16:3964-78


PIM1 kinase as a target for cancer therapy


This is the first paper describing the induction of PIM1 expression in epithelial tumors.


First reported structure of an organometallic compound bound to the ATP binding site of a protein kinase.


A. L. Merkel et al.


**Early example of the identification and biological evaluation of a non-hinge binding PIM1 inhibitor.**


**Early example of the identification and biological evaluation of a non-hinge binding PIM1 inhibitor.**


**This paper describes a selection of very selective metal-based protein kinase inhibitors including a picomolar inhibitor for PIM1.**

Affiliation
Anna Lena Merkel1, Eric Meggers2 & Matthias Ocker1†

1Author for correspondence
2Philips University Marburg, Institute for Surgical Research, Baldingerstrasse, Marburg, 35033, Germany
E-mail: Matthias.Ocker@staff.uni-marburg.de

1Department of Chemistry, Philips University Marburg, Institute for Surgical Research, Baldingerstrasse, Marburg, 35033, Germany

112