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Targeting PIM Kinases to Overcome Therapeutic Resistance in Cancer

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ABSTRACT

Cancer progression and the onset of therapeutic resistance are often the results of uncontrolled activation of survival kinases. The proviral integration for the Moloney murine leukemia virus (PIM) kinases are oncogenic serine/threonine kinases that regulate tumorigenesis by phosphorylating a wide range of substrates that control cellular metabolism, proliferation, and survival. Because of their broad impact on cellular processes that facilitate progression and metastasis in many cancer types, it has become clear that the activation of PIM kinases is a significant driver of resistance to various types of anticancer therapies. As a result, efforts to target PIM kinases for anticancer therapy have intensified in recent years. Clinical and preclinical studies indicate that pharmacologic inhibition of PIM has the potential to significantly improve the efficacy of standard and targeted therapies. This review focuses on the signaling pathways through which PIM kinases promote cancer progression and resistance to therapy, as well as highlights biological contexts and promising strategies to exploit PIM as a therapeutic target in cancer.

Introduction

The proviral integration for the Moloney murine leukemia virus (PIM) kinases were first identified as oncogenes in hematopoietic malignancies (1). However, PIM kinases have since been found to be overexpressed in many solid tumors, including prostate cancer (2), breast cancer (3), colon cancer (4), endometrial cancer (5), gastric cancer (1), and pancreatic cancer (6). High expression of PIM1 is a prognosticator of poor survival in multiple cancer types (6–9). The PIM kinases are a family of serine/threonine kinases composed of three different isoforms, PIM1, PIM2, and PIM3. The three isoforms share approximately 60% sequence similarity at the amino acid level (10). PIM3 is translated from a single translation start site, but PIM1 and PIM2 both have multiple start sites, leading to variants of different molecular weights. The shorter form of PIM1 (~33 kDa) localizes primarily to the nucleus, whereas the longer form tends to be more cytoplasmic (11). The additional N-terminal sequence found in the long form of PIM1 (PIM-1L) can anchor it to the cell membrane (12), and a PXXP domain in this N-terminal region also allows PIM-1L to interact with SRC homology 3 domains (13). These observations suggest that differences between the long and short form of PIM1 might impart some substrate specificity. Less is known about the different PIM2 variants, although it has been suggested that the 34-kDa variant is more associated with antiapoptotic activity than the medium and longer forms (14).

PIM activity is thought to be directly correlated with protein levels. Unlike most kinases, the PIMs do not have regulatory domains (15); therefore, it is thought that, once translated, PIM kinases are constitutively active (for an in-depth review, see Warfel and Kraft; ref. 10). As a result, the cellular function and activity of PIM kinases are primary regulated by altering the rates of protein synthesis and degradation. The turnover of PIM isoforms appears to be largely mediated through ubiquitination and proteasomal degradation; however, the relevant E3-ligase complexes that target PIM remain unknown. Growing evidence suggests that PIM levels are controlled by phosphorylation events. Dephosphorylation of PIM kinases by the serine/threonine phosphatase PP2A promotes their ubiquitination and subsequent proteasomal degradation (16, 17). Several groups have observed that small-molecule PIM inhibitors lead to an increase in PIM protein levels (18, 19), suggesting that PIM may control its own degradation through auto- or transphosphorylation. PIM protein stability is also controlled by protein interactions. Binding to the chaperone proteins, HSP90 and HSP70, regulates PIM stability by altering its proteasomal degradation. Specifically, binding of PIM1 to HSP90 shields it from degradation, whereas HSP70 binds to ubiquitinated PIM1 and facilitates its degradation (20, 21). Together, these results suggest that the activity of upstream kinases and/or phosphatases is likely to have an important role in PIM signaling. PIM kinases are transcriptionally regulated downstream of JAK/STAT signaling (22) or, in some cases, T-cell receptor β (23). Being a downstream mediator of JAK/STAT signaling places PIM kinases as key mediators of the cytokine response. However, a broad range of PIM substrates have been described that regulate protein translation, cell motility, angiogenesis, the cell cycle, and apoptosis. As a result, PIM expression contributes to multiple aspects of tumorigenesis.

Protumorigenic Roles of PIM Kinase

Tumor metabolism

One well-known target of the PIM kinases is eukaryotic translation initiation factor 4E binding protein 1 (eiF4EBP1 or 4EBP1), the molecule that binds to eukaryotic translation initiation factor 4E (eIF4E) and blocks the recruitment of the initiation complex and 40S ribosome to mRNAs (24). This is the rate-limiting step of translation. Phosphorylation of 4EBP1 blocks its ability to bind to eIF4E; therefore, regulation of 4EBP1 can control protein production (25). Increased protein production is required for sustained proliferation, a hallmark of cancer. Interestingly, 4EBP1 is also a substrate of mTOR, highlighting a role for PIM kinases as a mechanism of resistance to mTOR inhibition (24). PIM kinases also play a role in regulating cellular energy production. Glycolytic intermediates and glycolytic enzymes are decreased upon the loss of PIM (26–28),

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suggesting that PIM is a driver of glycolysis. PIM2 overexpression in colorectal cancer cells promotes glycolysis over aerobic respiration, a common feature of tumors, and knockdown of PIM2 decreases glucose consumption and pyruvate/lactate production (29). In addition, phosphorylation of pyruvate kinase M2 (PKM2) by PIM2 activates PKM2 to promote glycolysis (30). In gallbladder cancer, PIM inhibition shifts cell metabolism from glycolysis to oxidative phosphorylation (27). Furthermore, c-Myc, a transcriptional activator of glycolysis-associated genes, such as lactate dehydrogenase, has been shown to act synergistically with PIM kinases in many cancer types, and their levels are correlated (31). In addition, PIM1 phosphorylates Myc to enhance its transcriptional activity (28). PIM kinases also impact key regulators of cell-cycle progression. PIM2 can phosphorylate checkpoint kinase 2, which promotes the transition of the cell through mitosis. PIM kinases also phosphorylate p27 (32), which results in its nuclear export and proteasomal degradation and promotes progression through the G_1–S-phase transition. These studies demonstrate the importance of PIM kinases for cell growth and proliferation through multiple pathways that promote tumorigenesis.

Survival

The most well-described role for PIM kinases in the context of cancer is in aiding cancer cells to evade apoptosis. Several PIM targets, including Bcl2 agonist of cell death (Bad) and apoptosis signaling kinase 1 (ASK1), function directly in the intrinsic pathways of apoptosis. Bad is a key Bcl2 family member that can act in either a pro- or antiapoptotic function, depending on its phosphorylation state. All three PIM isoforms phosphorylate Bad at Ser112, which blocks its binding with BCL-X_L and impedes its proapoptotic function (33). ASK1 plays an important role in the MAPK signaling that regulates stress-induced cell death, and phosphorylation by PIM1 inactivates this pathway (34). Newer studies indicate that PIM also prevents cell death by allowing tumor cells to maintain physiologic levels of oxidative stress. PIM kinases reduce reactive oxygen species (ROS) in cancer cells, allowing them to escape ROS-induced cell death (26). They do this, in part, through regulation of nuclear factor-erythroid 2 p45-related factor 2 (Nrf2), a major regulator of the cellular response to ROS (35). Under conditions of oxidative stress, Nrf2 translocates into the nucleus and activates a transcriptional program that upregulates antioxidant molecules and reduces ROS. However, PIM inhibition blocks this nuclear accumulation and does not allow cells to adapt to oxidative stress. PIM1 overexpression is sufficient to increase Nrf2 signaling even in normal mouse prostate cells and in prostate cancer cells without cellular oxidative stress (36). Thus, PIM kinases can block apoptosis caused by excess ROS, a common mechanism of many chemotherapeutics. PIM kinases have also been implicated in maintaining mitochondrial integrity and mitochondrial membrane potential, which is an integral aspect of the apoptotic response and can also contribute to cellular ROS levels. Overexpression of PIM1 or PIM2 increases the mitochondrial membrane potential, and a dominant-negative PIM1 worsens mitochondrial dysfunction (14, 37). PIM kinases also regulate dynamin-related protein 1 (DRP1), the key regulator of mitochondrial fission. PIM inhibition leads to increased levels of DRP1, as well as increased activation of DRP1, which promotes mitochondrial fragmentation (38). Therefore, PIM kinases can protect cancer cells from apoptosis through multiple mechanisms, and the potential to block these mechanisms through PIM inhibition provides a promising approach to improve the efficacy of cancer therapies. This is illustrated by the synergistic effects seen when combining PIM and Bcl2 inhibitors in solid tumors (31, 39). Resistance to Bcl2 inhibitors is thought to arise due to the inability to inhibit myeloid cell leukemia 1 (MCL1). Combination treatment of prostate cancer cells with a Bcl2 inhibitor and a pan-PIM kinase inhibitor showed synergistic cytotoxicity, which coincided with decreased MCL1 levels with PIM inhibition (31). The synergistic effect of this combination therapy was confirmed using a xenograft model of prostate cancer. Similarly, treatment with a BH3-mimetic alone was not sufficient to induce apoptosis in glioblastoma cells, whereas the addition of pan-PIM inhibitors (SGI-1776 and AZD1208) produced a synergistic cytotoxic effect. However, the cell death observed with the combination therapy in glioblastoma cells was not dependent on MCL1, but instead appeared to be because of loss of mitochondrial membrane potential and the unfolded protein response (39). This is consistent with the role of PIM kinases in promoting mitochondrial fusion. In lung cancer cells and lung cancer xenografts, PIM inhibitors promoted mitochondrial fission (38), and that fission sensitized cells to classic chemotherapies, such as docetaxel and cisplatin (38). These findings suggest that PIM might utilize different mechanisms to evade apoptosis in different tumor types, and PIM inhibition should be considered as a mechanism to induce apoptosis in combination with other therapies.

Metastasis

Metastasis is the major cause of cancer-related deaths. Therefore, identifying druggable targets involved in the metastatic cascade is vital to improving patient outcomes. PIM kinases may be promising targets to oppose tumor metastasis. In mouse models, tumors with PIM1 or PIM3 overexpression are more likely to metastasize, and PIM inhibition can decrease metastasis (40). PIM1 overexpression is associated with worse distant metastasis-free survival among patients with triple-negative breast cancer (41). Moreover, PIM kinases have been implicated in multiple processes associated with metastasis, including epithelial-to-mesenchymal transition (EMT) and angiogenesis. PIM2 has been shown to activate STAT3 signaling, which leads to increased levels of *vimentin* and *N-cadherin*, genes associated with a mesenchymal phenotype (42). This phenotype was observed even in cells treated with media conditioned by PIM2 overexpression (43). The triple-negative breast cancer line, MB-MDA-231, was transfected with either PIM2 siRNA or a PIM2 overexpression construct, and conditioned media (CM) from each cell line was placed on naïve MB-MDA-231 cells. Cells cultured in the PIM2-knockdown CM showed decreased STAT3 phosphorylation, whereas cells incubated in the PIM2 overexpression CM displayed increased STAT3 phosphorylation. Cells cultured in the PIM2-knockdown CM also showed decreased migration and invasion in transwell assays, suggesting that PIM kinases may activate extracellular signaling cascades to promote EMT. PIM kinases also regulate Smad2 and Smad3, promoting the expression of key transcription factors associated with EMT, including ZEB, Snail, and Twist (43). Experimental evidence also suggests that PIM facilitates cell migration and invasion. PIM inhibition decreases the migration of multiple cancer cell types when assessed by wound-healing assay and Boyden chamber assay (44, 45). This antimigratory effect of PIM inhibition is modulated through multiple pathways. PIM can phosphorylate and interact with Notch1, promoting the migration of prostate cancer cells (46). PIM also modulates cell adhesion through regulation of forkhead box P3 (FoxP3; ref. 46). N-Myc downstream regulated 1 (NDRG1) is another PIM substrate that suppresses migration and invasion of cancer cells. Phosphorylation of NDRG1 by PIM leads to its degradation, releasing another brake that opposes metastasis (47). Recent studies have identified a new role for PIM kinases in promoting tumor angiogenesis, another hallmark of cancer. Angiogenesis is necessary to supply solid tumors with oxygen and

nutrients, in addition to providing a mechanism for hematogenous tumor spread. Overexpression of PIM1 in xenograft models of prostate and colon cancer significantly increased microvessel density along with tumor volume (48). Increased tumoral PIM may also lead to increased lymphangiogenesis (40). PIM kinases are also expressed in endothelial cells, and they promote new vessel formation via phosphorylation of TNFα and endothelial nitric oxide synthase (49). *PIM1* is upregulated in endothelial cells during angiogenesis by VEGF signaling, and endothelial PIM1 is required for angiogenesis (50). PIM3 also promotes the spreading and migration of endothelial cells (51). Taken together, these studies provide evidence of multiple mechanisms to explain the correlation between PIM kinases and increased metastatic potential. Therefore, further study of the role of PIM kinases as risk factors for metastasis and potential therapeutic targets to block metastasis is warranted.

Immune evasion and inflammation

Immune evasion and inflammation have recently gained interest as critical factors in tumor initiation and progression. PIM1 is upregulated in mouse models of inflammation (52), and PIM inhibition is sufficient to reverse inflammatory diseases (53). The anti-inflammatory effects of PIM inhibitors can likely be attributed to the role of PIM in cytokine signaling. PIM inhibition in Hodgkin lymphoma cells decreases the levels of programmed death ligand (PD-L)1 and PD-L2, immune checkpoint molecules that allow cancer cells to escape the antitumor immune response (54). PIM2 has also been shown to increase PD-L1 expression in cancer cells through the phosphorylation of heat shock transcription factor 1 (55). PIM inhibition in tumor cells increases the activation of T cells in coculture, suggesting that PIM may promote immune escape (56). Furthermore, PIM kinases are increased in tumor-infiltrating immune cells, and they may have a role in regulating the differentiation of inflammatory immune cells (54). Regulating the immune populations within a tumor can enhance the ability of the tumor to evade the immune response. PIM2 knockout increases the proliferative ability of T cells and PIM2-knockout T cells have increased activity, suggesting that PIM kinases block the function of T cells against immune threats (23). In addition, PIM2 knockout in the host, but not in the tumor, leads to a complete antitumor immune response (23). Furthermore, PIM2 blocks the suppressive function of regulatory T cells through its regulation of Foxp3 (57), and PIM-knockout mice show increased levels of natural killer cells (23). Combination therapy of immune checkpoint inhibitors (ICI) and PIM inhibitors may also improve the response to immunotherapy. A recent study (58) examined PIM inhibition in a mouse model of adoptive T-cell therapy (ACT), a treatment method wherein the patient's own T cells are removed, grown *in vitro*, and then reinjected to improve the antitumor immune response. This study found that treatment of the adoptive T cells with PIM inhibitors led to prolonged survival. Although the mice were also treated with PIM inhibitors after T-cell transfer, mice treated only with inhibitor (i.e., no T-cell transfer) did not exhibit increased survival. The authors also examined this combination using xenografts of a melanoma cell line that is resistant to ICIs. Although the combination of ACT and PIM inhibition was sufficient to prolong survival in this model, the addition of an anti-programmed death 1 antibody significantly decreased tumor growth, indicating that PIM inhibition in immune cells may prolong survival and promote sensitivity to ICIs. Although the role of PIM kinases in immune regulation warrants further study, these findings also suggest that PIM kinases might be attractive theragnostic biomarkers for ICI treatment. Despite their clinical promise, many patients do not respond to ICIs, and there have been many studies trying to identify biomarkers that might predict the response to ICIs. The increase in ICI targets, such as PD-L1, with increased PIM expression suggests that patients with increased PIM expression might have a good response to these targeted therapies.

PIM Kinases Promote Therapeutic Resistance

Because of their extensive signaling network, PIM kinases have been shown to promote resistance to a wide range of anticancer therapies (**Table 1**). Increased PIM1 expression is associated with poor response to radiotherapy (59, 60), and PIM overexpression has also been shown to protect cancer cells from various forms of chemotherapy, including platinum- and taxane-based therapies (61). The ability of PIM to evade chemotherapy-induced apoptosis may be, in part, because PIM regulates drug transporters. Treatment with PIM inhibitors sensitizes cells to chemotherapies that would normally be exported by ATP-binding cassette (ABC) drug transporters (62), and this has been shown to be mediated through both a decrease in the activity of ABC transporters, as well as reduced levels (63). The drug transporter ABCG2 is directly phosphorylated by PIM-1L, which promotes drug resistance in prostate cancer cells (64). However, growing evidence supports the idea that PIM kinases represent a survival pathway that cancer cells frequently utilize to manipulate their biology and avoid apoptosis.

PI3k/mTOR inhibitors

The PI3K/Akt pathway is constitutively active in multiple tumor types, and, as such, PI3K or Akt inhibitors are frequently administered as targeted therapy. However, many patients develop resistance to these inhibitors. PIM1 and PIM3 were identified in an *in vitro* screen in breast cancer cells for genes that conferred resistance to PI3K inhibitors, and PIM1 expression predicts whether multiple breast cancer cell lines are responsive to PI3K inhibition (65). In addition to PI3K inhibitors, PIM confers resistance to PI3K/mTOR dual inhibitors, pyruvate dehydrogenase 1 inhibitors, Akt inhibitors, and multiple classes of mTOR inhibitors *in vitro*. This does not require reactivation of Akt; rather, PIM is able to reactivate signaling cascades downstream of Akt to promote translation and suppress apoptosis. This may be of particular interest in cancers in which Akt is constitutively active, such as prostate and breast cancers. In lung cancer cells cultured in the presence of a dual PI3K/mTOR inhibitor, PIM1 was shown to be significantly upregulated, and these cells were more sensitive to combined inhibition of mTOR and PIM than nonresistant control cells (66). Indeed, these three pathways are so intertwined in cancer that single-molecule inhibitors that target more than one of these pathways have been developed. IBL-302, which inhibits PIM, PI3K, and mTOR, has shown efficacy in both neuroblastoma (67) and breast cancer (68). In a screen of more than 700 cell lines, neuroblastoma lines were the most sensitive to IBL-302; however, these cells were not sensitive to PI3K inhibition alone, whereas 14 of 16 lines were sensitive to IBL-302 at low micromolar concentrations, indicating that concurrently inhibiting PIM, PI3K, and mTOR has potential in neuroblastoma (67). The triple inhibitor also had better results than PIM inhibitors or mTOR inhibitors alone. IBL-302 treatment caused both differentiation and cell death in neuroblastoma cell lines and in patient-derived xenograft models. In another study, a panel of breast cancer lines was treated with IBL-302 to determine their sensitivities. Triple-negative cell lines were more sensitive to IBL-302 than HER2-positive or luminal cell lines (68). As there are no targeted therapies for

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Table 1. PIM kinases promote resistance to anticancer therapies.

Drug class	Mechanism of resistance
Chemotherapy	Regulates drug transporters
	Blocks chemotherapy-induced oxidative stress
Radiotherapy	Decreases apoptosis
PI3K/mTOR inhibitors	Activates downstream targets
	Blocks ROS generated by PI3K inhibitors
EGFR inhibitors	Activates downstream targets
	Blocks inhibitory factors
	Activates MET signaling
MET inhibitors	Promotes cell protective, antiapoptotic signaling
	Increases protein translation
FLT3-ITD inhibitors	Supports mitochondrial integrity
Antiangiogenic therapies	Upregulated in hypoxia to promote secretion of proangiogenic factors

Abbreviation: MET: c-MET oncogene.

triple-negative breast cancer, finding therapeutic targets is vital to improving patient outcomes. IBL-302 was even effective in breast cancer cells resistant to traditional chemotherapies. Furthermore, subcutaneous xenografts of breast cancer lines grew significantly more slowly in mice treated with IBL-302, although these mice still experienced tumor growth. An earlier generation inhibitor that only inhibits PI3K and PIM, IBL-202, was developed on the basis of the fact that combining PIM and PI3K inhibitors was more detrimental to cell viability than either agent alone (69). Chronic lymphocytic leukemia (CLL) cells grown in hypoxia, which mimics the low oxygen tension in the lymph nodes, tend to be more resistant to treatment. However, IBL-202 still induced cell death in CLL cell lines and primary cells from patients with CLL grown in hypoxia, whereas a PI3K inhibitor was unable to induce cell death in these conditions (69). IBL-202 was also found to increase mitochondrial ROS, highlighting the import of mitochondrial regulation by PIM kinases in the potential for PIM inhibitors as therapeutic options.

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKI) have become a staple for the personalized treatment of multiple cancer types. Although many patients develop resistance to TKIs through an activating mutation in the targeted receptor tyrosine kinase, other pathways, including PIM, have been shown to be activated after TKI treatment. In lung cancer cells that have become resistant to third-generation EGFR-TKIs, treatment with MET inhibitors increases the expression of PIM1 and PIM3 (70). Interestingly, treatment with the pan-PIM kinase inhibitor, AZD1208, was effective at inducing apoptosis in both EGFR-TKI–sensitive and -resistant cells, suggesting that this may not be dependent on MET amplification (70). In MET-addicted lung and gastric cancer cell lines, treatment with MET inhibitors killed a majority of the cells, but led to colony outgrowth, which was blocked by combination treatment with MET and PIM inhibitors. Furthermore, PIM1 and PIM3 expression was upregulated in MET inhibitor–resistant clones, suggesting that compensatory PIM expression is a mechanism of acquired resistance. Moreover, stable overexpression of PIM1 was sufficient to confer *de novo* resistance to MET inhibitors in these cells (71). Treatment of EGFR-mutant lung cancer cells with AZD1208 increased their sensitivity to osimertinib, a third-generation EGFR-TKI (72), and it has been suggested that this can be attributed to PIM signaling through MET in these tumors, although PIM may activate other downstream signals to overcome TKIs, such as STAT3 and mitogen-inducible gene 6 (MIG6; ref. 73). MIG6 is a negative regulator of EGFR signaling and can block EGFR phosphorylation even in the presence of EGF. Treatment of prostate cancer cells with a pan-PIM kinase inhibitor increased *MIG6* expression, although as it was increased at the mRNA level, this is likely an indirect effect. PIM also confers resistance to anaplastic lymphoma kinase (ALK) inhibition. A CRISPR screen in neuroblastoma cell lines harboring ALK mutations indicated that PIM1 expression could be increased as a mechanism of resistance. PIM1 also acted as a mechanism of resistance to ALK TKIs in anaplastic large cell lymphoma cell lines (74).

As PIM kinases have shown themselves to a mechanism of resistance to TKIs, combination of PIM inhibitors with TKIs has been an

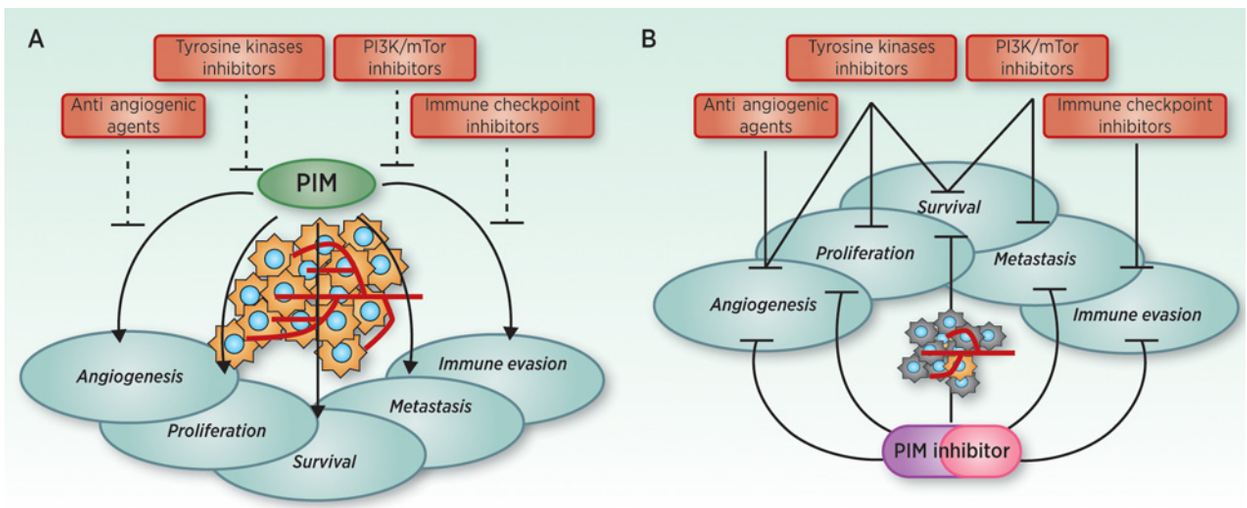

Figure 1.

Targeting PIM kinases to overcome therapeutic resistance. **A,** PIM kinases promote resistance to many classes of anticancer therapy by activating signaling pathways that increase angiogenesis, proliferation, survival, metastasis, and immune evasion. **B,** Small-molecule PIM inhibitors increase the efficacy of chemotherapy and targeted agents by inhibiting various signaling pathways used by cancer cells to escape therapy.

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Table 2. PIM inhibitors in clinical trials.

Drug	Clinical trial number	Phase	Disease	Result
INCB053914	NCT02587598 (85)	I/II	AML, high-risk MDS, MDS/MPN, multiple myeloma, lymphoma, and other lymphoproliferative neoplasms	Preliminary safety (AEs reversible with drug interruption/dose reduction) Estimated completion: 2021
INCB053914 with pomalidomide and dexamethasone	NCT04355039 (86)	II	Refractory/relapsed multiple myeloma	Not yet recruiting
INCB053914 with INCB050465 (PI3K inhibitor)	NCT03688152 (84)	I	Diffuse large B-cell lymphoma	Not yet recruiting
TP-3654	NCT03715504 (87)	I	Advanced solid tumors	Preliminary safety (no DLTs, no grade >3 AEs); prolonged SD
TP-3654	NCT04176198 (88)	I	Myelofibrosis	Ongoing (estimated completion 2022)
SEL24/MEN1703	NCT03008187 (89)	I/II	AML	Acceptable safety profile, five DLTs; one CR, one CRi
AZD1208	NCT01489722 (82)	I	AML	Serious AEs in 23 patients (discontinued in 8) and DLTs in 5; no clinical response
AZD1208	NCT01588548 (82)	I	Advanced solid malignancies	Serious AEs in 8 patients and DLTs in 4; best response SD ≥ 6 weeks (13 patients; ORR, 0%)
SGI-1776	NCT01239108 (90)	I	Relapsed/refractory leukemia	Withdrawn due to cardiac QTc prolongation
SGI-1776	NCT00848601 (91)	I	Prostate cancer/Hodgkin lymphoma	Withdrawn due to cardiac QTc prolongation
PIM447	NCT01456689 (81)	I	Relapsed/refractory multiple myeloma	Hematologic grade 3/4 AEs; disease control rate of 72.2%, ORR of 8.9%
PIM447 with ruxolitinib and LEE011	NCT02370706 (92)	Ib	Myelofibrosis	Ongoing (estimated completion 2020)
CXR1002	Not registered (93)	I	Advanced refractory solid tumors	MTD not reached SD > 12 weeks in 8 patients

Abbreviations: AE, adverse event; CR, complete response; CRi, complete response with incomplete recovery of platelet count; DLT, dose-limiting toxicity; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; ORR, overall response rate; SD, stable disease.

area of great interest. In *in vitro* studies using primary CLL cells, PIM inhibitor treatment cooperated with ibrutinib to induce apoptosis, and combined treatment with PIM inhibitor and ibrutinib reduced the disease burden and increased apoptosis in CLL xenograft studies (75). Acute lymphocytic leukemias (ALL) commonly harbor activating tyrosine kinase mutations, including the Philadelphia chromosome (Ph), and increased signaling through the lymphocyte-specific kinase. In ALL cells, combination therapy with ponatinib, a TKI currently used in the clinic for Ph+ ALL, and a pan-PIM kinase inhibitor decreased cell proliferation and synergistically induced cell death. Dasatinib, another TKI approved for leukemia, showed similar results. Furthermore, in a mouse model, combination of AZD1208 and ponatinib led to decreased tumor burden compared with either agent alone (76). Even when the leukemia was allowed to expand, this combination therapy prolonged survival, suggesting the potential for this therapeutic combination in human patients (76). FMS-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) is a frequent mutation observed in acute myeloid leukemia (AML), and although many patients with AML initially respond to FLT3 TKIs, mutations in the FLT3-ITD often lead to therapeutic resistance. However, this can be overcome by treatment with PIM inhibitors. In FLT3-ITD AML cells that harbored FLT3-ITD mutations and were resistant to FLT3 TKIs, a pan-PIM inhibitor reduced the IC_{50} of FLT3 TKIs (77). This synergism was observed with multiple FLT3 TKIs. This effect may have been because of decreased mitochondrial membrane potential, again highlighting the import of PIM kinases in regulating mitochondrial integrity. Furthermore, in a mouse model of FLT3-ITD AML, AZD1208 and quizartinib (a FLT3 inhibitor) reduced tumor growth significantly, more than quizartinib alone (AZD1208 alone had no tumor reduction effect), and mice treated with the combination therapy had longer survival (78). Combination of AZD1208 and quizartinib also induced apoptosis in blasts taken from patients with FLT3-ITD AML, but not in those taken from patients with FLT-WT AML, suggesting that this combination therapy may be particularly useful in those patients who develop resistance to FLT3 TKIs.

Antiangiogenic therapies

In accordance with their roles in angiogenesis, PIM kinases have also shown the ability to confer resistance to VEGF-A- and VEGFR-targeted agents. In a mouse model of prostate cancer, PIM1 overexpression blocked the ability of antiangiogenic drugs (bevacizumab and sunitinib) to effectively reduce angiogenesis and tumor growth (48). Preclinical and clinical investigations have established that inhibitors of angiogenesis ultimately increase intratumoral hypoxia, which upregulates PIM1 levels. Indeed, treatment with antiangiogenic agents significantly upregulates PIM1 in xenograft tumor models, indicating a feedback loop in which hypoxia-induced PIM1 promotes resistance to angiogenic therapies. Notably, PIM inhibitors kill hypoxic tumor cells at significantly lower doses compared with tumor cells grown in normoxia (35). Thus, PIM inhibitors may be

of particular utility in tumors found in hypoxic microenvironments, such as the bone. In prostate cancer, a tumor known to metastasize to the bone, the hypoxic microenvironment led to decreased response to PI3K inhibitors, and treatment with PIM inhibitors restored this sensitivity (79).

Given the overexpression of PIM kinases in hypoxia and their role in angiogenesis, combination therapy with angiogenesis inhibitors and PIM inhibitors has great potential. Treatment of prostate xenografts with antiangiogenic agents or PIM inhibitors alone had modest antitumor effects and modest reductions in vascular perfusion, but combining antiangiogenic agents and PIM inhibitors significantly blocked tumor growth and vascular perfusion (48). Moreover, combined PIM and VEGF inhibition produced a synergistic antitumor response characterized by marked reductions in angiogenesis and cell proliferation in orthotopic models of colon and prostate cancer (40). This effect was attributed to the ability of PIM inhibitors to reduce levels of hypoxia inducible factor-1α, the major driver of proangiogenic gene transcription. This combination also reduced metastasis in an orthotopic prostate model, likely because decreased neovascularization blocked the hematogenous spread of tumor cells. Recently, novel VEGFR2-PIM kinase inhibitors were developed in an attempt to overcome the resistance to antiangiogenic therapies by PIM kinases (80). These agents were selective for cancer cells *in vitro* and inhibited both VEGFR2 and PIM. However, they have not been tested in animal models.

Targeting PIM in the clinic

The overexpression of PIM kinases in cancer, their roles in multiple aspects of cancer biology, and their ability to promote resistance to multiple types of therapies suggest their utility as therapeutic targets (**Fig. 1**). However, they have shown limited efficacy in clinical trials (**Table 2**). A phase I study of the pan-PIM inhibitor PIM447 in multiple myeloma indicated a clinical benefit rate of 25.3% and a disease control rate of 72.2% (81). The study also showed that patients receiving a higher dose had a longer progression-free survival. However, PIM447 appeared to have a cytostatic, rather than an apoptotic, effect. Similarly, a trial of AZD1208 in AML had a 0% disease control rate, with the best response being stable disease (82). These clinical results suggest that PIM inhibitors produce a cytostatic response and would be best used in combination therapy, which is in agreement with the many preclinical models showing synergistic effects of PIM inhibitors with multiple treatment modalities. Several clinical trials examining pan-PIM inhibitors in combination with other therapies are currently ongoing, including a trial with standard therapies in AML (83) and combination with a PI3K inhibitor in diffuse large B-cell lymphoma (84). In conclusion, PIM kinases are important protumorigenic molecules that play numerous roles in cancer. Emerging evidence indicates that they act as a central mechanism of resistance to many types of anticancer therapy, which should be taken into account when designing treatment for patients with cancer. Although PIM inhibitors have potential as therapeutic targets, their real value seems to lie in their potential to be used in combination therapy with clinically approved drugs. Therefore, future studies, including clinical trials, should focus on combination therapy.

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