Current perspectives on targeting PIM kinases to overcome mechanisms of drug resistance and immune evasion in cancer

Tom Malone, Lea SchÓ“fer, Nathalie Simon, Susan Heavey, Sinead Cuffe, Stephen Finn, Gillian Moore, Kathy Gately

PII: S0163-7258(19)30206-2

DOI: https://doi.org/10.1016/j.pharmthera.2019.107454

Reference: JPT 107454

To appear in: *Pharmacology and Therapeutics*

Please cite this article as: T. Malone, L. SchÓ“fer, N. Simon, et al., Current perspectives on targeting PIM kinases to overcome mechanisms of drug resistance and immune evasion in cancer, *Pharmacology and Therapeutics*(2019), https://doi.org/10.1016/j.pharmthera.2019.107454

P&T #23626

Current perspectives on targeting PIM kinases to overcome mechanisms of drug resistance and immune evasion in cancer

Tom Malone[1], Lea Schäfer[1], Nathalie Simon[1], Susan Heavey[3], Sinead Cuffe[1], Stephen Finn[1], Gillian Moore[2§], Kathy Gately[1*§]

[1]Dept. of Clinical Medicine, Trinity Translational Medicine Institute, St. James's Hospital, Dublin, Ireland.

[2] School of Pharmacy and Biomolecular Sciences, RCSI, Dublin, Ireland

[3] Molecular Diagnostics and Therapeutics Group, University College London, London, UK

[*] **Corresponding author:**

Kathy Gately

Dept. of Clinical Medicine

Trinity Translational Medicine Institute

St. James's Hospital Dublin, Ireland.

Telephone: +353189632[illegible]

E-mail Address: gatelyk@tcd.ie

§ These authors share senior authorship

Abstract

PIM kinases are a class of serine/threonine kinases that play a role in several of the hallmarks of cancer including cell cycle progression, metabolism, inflammation and immune evasion. Their constitutively active nature and unique catalytic structure has led them to be an attractive anticancer target through the use of small molecule inhibitors. This review highlights the enhanced activity of PIM kinases in cancer that can be driven by hypoxia in the tumour microenvironment and the important role that aberrant PIM kinase activity plays in resistance mechanisms to chemotherapy, radiotherapy, anti-angiogenic therapies and targeted therapies. We highlight an interaction of PIM kinases with numerous major oncogenic players, including but not limited to stabilisation of p53, synergism with c-Myc, and notable parallel signalling with PI3K/Akt. We provide a comprehensive overview of PIM kinase's role as an escape mechanism to targeted therapies including PI3K/mTOR inhibitors, MET inhibitors, anti-HER2/EGFR treatments and the immunosuppressant rapamycin, providing a rationale for co-targeting treatment strategies for a more durable patient response. The current status of PIM kinase inhibitors and their use as a combination therapy with other targeted agents, in addition to the development of novel multi-molecularly targeted single therapeutic agents containing a PIM kinase targeting moiety are discussed.

Key words : PIM kinase, PI3K/Akt/mTOR, MET, EGFR, c-Myc, drug resistance mechanisms

Table of Contents:

1. Introduction

Precision medicine has heralded an era of change and challenge in the treatment of patients with cancer. Although targeted therapies and immunotherapies have made significant advances in the personalised treatment of patients, clinicians still face the persistence of disease recurrence and drug resistance. A subset of patients will display innate resistance upon treatment commencing while others will inevitably develop acquired resistance to targeted agents over time (Spaans & Goss, 2014). Cancer cells utilise multiple intercellular signalling cascades mediated by oncogenes such as PIM kinases to maintain cell growth and survival (Lilly & Kraft, 1997; Wang et al., 2001; Santio & Koskinen, 2017). Activation of PIM kinases is tightly regulated in normal cells however their sustained activation promotes apoptotic resistance and uncontrolled proliferation in multiple tumour types (Fox et al., 2003; Deneen et al., 2003). Significant cross-talk between parallel proliferative pathways provides cancer cells with an effective escape mechanism where upon inhibition of a single pathway the cells can adapt by molecularly switching to an alternative and parallel oncogenic signalling pathway. PIM kinase activation has been shown to play a significant role in this bypass signalling mechanism when the PI3K pathway is blocked (Cen et al., 2013; Song et al., 2018; Rebello et al., 2018; Gately et al., 2018). Both pathways are frequently activated in cancer, and they phosphorylate overlapping substrates that regulate the balance between cell survival and apoptosis. Both kinases among others, represent promising targets for cancer therapy (Tursynbay et al., 2016; Zhang et al., 2018) and are the focus of significant drug development efforts (Jeyapal et al., 2018; Asati et al., 2019). A better understanding of PIM kinase synergism with other signalling pathways is important to further strengthen the rationale for co-targeted treatment strategies and combination therapy to improve the efficacy of PIM kinase inhibitors in the clinic.

2. The PIM kinase family

2.1 Expression and function of PIM kinases

The proviral insertion site in Moloney murine leukaemia virus (PIM) proteins are a group of evolutionarily conserved serine/threonine kinases in eukaryotes consisting of PIM1, PIM2 and PIM3. PIM1 is located on chromosome 17, PIM2 on the X chromosome and PIM3 on chromosome 15. The amino acid sequence of these genes is very similar with PIM1 and PIM2 sharing a 61% homology and PIM1 and PIM3 being 71% homologous (Narlik-Grassow et al., 2014). This suggests some redundancy in function of the proteins (Eichmann et al., 2000). The three protein family members are differentiated by their respective genes and composed of six exons that are transcribed into the mRNA transcripts. While PIM3 gene is thought to encode a single transcript, PIM1 and PIM2 consist of 2 and 3 isoforms respectively arising from alternative translation initiation sites along with additional codons at the 5' end, leading to proteins with different molecular masses (Saris et al., 1991). PIM1 is highly expressed in haematopoietic and lymphoid cells as well as prostate cells (Bachmann et al., 2004). PIM2 is expressed in lymphoid and brain tissues while high expression of PIM3 can be observed in breast, kidney and brain tissue (Mikkers et al., 2004).

In contrast to most other kinases, PIM kinase activity is not dependant on post-translational modification. They are constitutively active due to an acidic residue (Asp) in the activation loop that is capable of forming a salt bridge with basic residues of the catalytic loop. This mimics the serine or threonine residue to be phosphorylated and hence lacks a regulatory domain (Bullock et al., 2009). This would suggest that the kinases are regulated at the transcriptional and translational level and via proteasomal degradation (Mondello et al., 2014). This lack of a regulatory domain was confirmed by X-ray crystallography studies of

PIM1 (Qian et al., 2005). The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is one of the key upstream transcriptional regulators of PIM kinases. JAK/STAT signalling is an important pathway for cell processes such as cell differentiation, division, migration, immunity and apoptosis. STAT3 and STAT5 have been found to bind to the promotor region of PIM1 and upregulate its expression. Negative feedback can be seen also, as PIM1 binds to and activates (via phosphorylation) the suppressor of cytokine signalling (SOCS) proteins which act as a negative feedback regulator to prevent activation of the JAK/STAT pathway (Peltola et al., 2004). PIM kinases are also downstream effectors of ABL, Flt-3 and nuclear factor kappa-B (NF-kB) signalling (Mendello et al., 2014). Constitutively activated FLT3 signalling leads to an upregulation in PIM1 expression and enhances FLT3 anti-apoptotic and pro-proliferative activity in leukemic cells (Kim et al., 2005). PIM1 expression is upregulated by BCR-ABL via STAT5 activation in leukemic cells and is thought to play a role in BCR-ABL mediated cell transformation (Nieborowska-Skorska et al., 2002).

The mRNA transcripts of PIM genes are quite short-lived owing to multiple destabilizing sequences of AUUU(A) in the 3'UTR region (Warfel & Kraft, 2015). PIM genes also require cap-dependent translation due to G-C rich sequences near the 5'UTR which makes PIM a 'weak' transcript (Wang et al., 2005). Overexpression of eIF4E allows for complex assembly which binds to the 5'-m^7G cap, enhancing translation of the PIM protein (Hoover et al., 1997). Interestingly, PIM2 can phosphorylate and thus inhibit eIF4E-binding repressor 4EBP1, promoting cap-dependant translation (Fox et al., 2003).

PIM protein stability is regulated through ubiquitination and subsequent proteasomal degradation. It is believed that the chaperone protein Hsp90 binds to PIM1 and helps

stabilise it while Hsp70 when bound, leads to ubiquitination of PIM1 targeting it for proteasomal degradation (Shay et al., 2005). PIM activity can be regulated by ETK tyrosine kinase which phosphorylates PIM1 within the activation loop enhancing its activity (Kim et al., 2004). However, dephosphorylation of the kinases by serine/threonine phosphatase PP2A promotes ubiquitination and leads to degradation (Losman et al., 2003). So, while PIM kinases are constitutively active and do not require post-translational modifications for their activity, phosphorylation and dephosphorylation can still alter PIM's ability to function.

A recent study by Miyakawa et al. identified a novel function of PIM kinases in which they facilitate promotion of viral infectivity by counteracting the host antiviral system. The auxiliary protein Vpx enables lentiviruses to counteract the intrinsic host restriction factor SAMHD1. PIM1 and PIM3, phosphorylate HIV-2 Vpx at Ser13 and stabilize the interaction of Vpx with SAMHD1 thereby promoting ubiquitin-mediated proteolysis of SAMHD1. Inhibition of PIM kinases promotes the antiviral activity of SAMHD1, ultimately reducing viral replication and highlights a novel therapeutic strategy to restore the hosts antiviral response (Miyakawa et al., 2019).

2.2 PIM kinases in cancer

Activated PIM kinases have been identified in many types of cancer including haematological malignancies such as myeloid and lymphoblastic leukaemias and solid epithelial tumours such as prostate and pancreatic cancer (Amson et al., 1989; Dhanasekaran et al., 2001; Arrouchi et al., 2019a). The PIM1 gene was initially identified via insertion of the proviral integration sites in Moloney murine leukaemia virus (MMLV)-induced lymphomas which resulted in over 50% of T-cell lymphomas displaying integration near to the PIM locus (Cuypers et al., 1984). Further experiments revealed an increase in

PIM1 mRNA when the PIM1 gene in transgenic mice was supplied with an upstream immunoglobulin enhancer and a downstream MMLV. 5-10% of the transgenic mice developed T-cell lymphomas by 7 months as opposed to zero percent of the control mice, providing evidence that PIM1 is an oncogene (van Lohuizen et al., 1989). MMLV proviral integration in mice that lacked the PIM1 gene resulted in the identification of PIM2 which was expressed in the late stages of MMLV-induced lymphomas and appeared to act as a compensatory protein in the absence of PIM1 (Allen et al., 1997). Similarly, mice lacking PIM1 and PIM2 genes led to the discovery of PIM3 by proviral tagging of the c-Myc transgenic mice (Mikkers et al., 2002). The frequency of MMLV-induced lymphomas in PIM1 transgenic mice is quite low while the tumours latency is relatively high suggesting that the PIM genes are 'weak' oncogenes despite being present in many types of cancers. PIM proteins do not appear to drive cancer single-handedly, however, working in concert with other robust oncogenes, such as c-Myc they can considerably boost the pro-tumorigenic effects of these oncogenes compared to their effects alone (van Lohuizen et al., 1989).

This synergism was first suggested when it was shown that 95% of MMLV-induced tumours in Eμ-PIM1 transgenic mice, engineered to overexpress PIM1 in lymphocytes resulted in an overexpression of c-Myc or N-Myc due to insertion of the provirus (van Lohuizen et al., 1989). Under normal conditions, c-Myc has a half-life of approximately 20 minutes, however, experiments both *in vitro* and *in vivo* show that phosphorylation on Ser329 along with other possible phosphorylation sites by PIM1 and PIM2 stabilises c-Myc increasing its oncogenic transforming potential (Amati et al., 1998; Yeh et al., 2004; Zhang et al., 2008).

PIM1 is overexpressed in over half of prostate cancers (Dhanasekaran et al., 2001) and in murine models of Myc-driven prostate cancer (Ellwood-Yen et al., 2003). Stable

overexpression of PIM1 did not result in the transformation of benign RWPE-1 cells into malignant ones but did enhance tumorigenesis in human prostate cancer cell lines LNCaP and DU145**.** PIM1 phosphorylates androgen receptor at Ser213 in hormone refractory prostate cancer impacting gene transcription and correlates to aggressive disease (Ha et al., 2013). Studies have indicated that PIM1 is expressed in androgen-dependent prostate cancer (ADPC) and castration-resistant prostate cancer (CRPC) (Holder et al., 2014). Androgen deprivation therapy (ADT) did not affect PIM1 expression and it is hypothesized that PIM1 may be important in the proliferation and differentiation of prostate cancer during ADT (Wang et al., 2017) and hence a potential target for treatment particularly in CRPC. PIM kinase inhibitors halted the growth of human triple-negative breast cancer tumours with elevated Myc expression in patient-derived tumor xenograft (PDX) and Myc-driven transgenic mouse models of breast cancer by inhibiting the oncogenic transcriptional activity of Myc and restoring the function of the endogenous cell cycle inhibitor, p27. Therefore, PIM kinase inhibition presents a novel targeted therapy against triple-negative breast tumors with elevated Myc expression (Horiuchi et al., 2016).

PIM kinases may also have a role in the stromal cells of the tumour microenvironment such as cancer associated fibroblasts (CAFs). CAFs secrete growth factors and extracellular matrix (ECM) proteins involved in supporting cancer cell growth and regulating cancer cell migration and tumour associated angiogenesis (Tao et al., 2017). Evaluation of prostate cancer biopsies demonstrated PIM1 overexpression in stromal fibroblasts. *In vitro* PIM1 overexpressing fibroblasts demonstrated an enhanced ability to differentiate into myofibroblasts in co-cultures with primary prostate cells, BPH1s, and an enhanced expression of CAF-associated markers, including COL1A1, CCL5 and PDGFR. Inhibition of PIM1 with AZD1208, decreased the number of αSMA-positive myofibroblasts and reduced

the secretion of collagens including COL1A1 and the expression of PDGFR. While AZD1208 had no effect on the growth of primary prostate cells alone, when AZD1208 was added to co-cultures of BPH1 cells and PIM1 overexpressing fibroblasts, the ability of the fibroblasts to enhance BPH1 proliferation was reduced. These data suggest that the fibroblast to CAF transition and the ability of CAFs to stimulate prostate epithelial cell growth is enhanced by PIM1 activity. More research in this area is essential to further unravel the regulatory role of PIM kinase in CAFs in driving tumorigenesis in prostate and other cancer types (Zemskova et al., 2015).

2.3 PIM kinase substrates

The preferred substrate specificity of PIM1 is the amino-acid sequence K/R-X-X-X-S/T-X, where X is any residue that is not basic or large and hydrophobic (Friedmann et al., 1992). Further studies utilising stepwise replacement of this sequence yielded a target site of R-X-R-H-X-S where X is any amino acid, providing a more precise recognition sequence for both PIM1 and PIM2 (Peng et al., 2007).

PIM kinases exert their oncogenic effects in many ways including regulation of tumour cell proliferation, metabolism and motility via phosphorylation of downstream substrates. PIM1 is capable of directly phosphorylating the cell cycle suppressor and CDK2 inhibitor p21 at Thr145 and indirectly at Ser146, stabilising and localising it within the cytoplasm thereby enhancing tumour cell proliferation as cytoplasmic p21 has been linked to oncogenicity (Zhang et al., 2007). PIM kinases also regulate p27 both indirectly at the transcriptional level and directly via phosphorylation of the protein. PIM kinases are capable of phosphorylating and thereby inactivating the transcription factors FoxO1a and FoxO3a, two members of the Forkhead box O (FoxO) family. These transcription factors are responsible for the expression

of p27 and their phosphorylation by PIM leads to a suppression of the gene. All three PIM kinases are also capable of directly phosphorylating p27 at Thr157 and Thr198, leading to p27 binding to 14-3-3 protein, causing its export from the nucleus and subsequent proteasome-dependant degradation (Morishita et al., 2008). Due to its role as a regulator of the cell cycle, its degradation by PIM kinases result in proliferation of tumour cells.

PIM kinases are known to regulate other tumour suppressor proteins such as p53, an important transcription factor responsible for cell cycle arrest and apoptosis in DNA damaged cells. PIM1 was found to phosphorylate Mdm2, the E3 ubiquitin ligase responsible for p53 degradation at Ser166 and Ser186, thereby increasing p53 expression which may cause the cellular senescence observed in primary murine embryo fibroblasts (Hogan et al., 2008). Cell cycle progression is also mediated through PIM kinase regulated phosphorylation on the dual-specificity phosphatases, Cdc25A and Cdc25C. Cdc25A is also a substrate for c-Myc and may provide a link for crosstalk between the two in terms of their synergistic capabilities (Mochizuki et al., 1999). PIM1 is capable of phosphorylating Cdc25A directly, enhancing its phosphatase activity (Bachmann et al., 2006). PIM1 was also found to phosphorylate C-TAK1 kinase, a negative regulator of Cdc25C. This results in reducing its ability to phosphorylate and inactive Cdc25C, thus, promoting cell cycle progression at the G_2/M phase (Bachmann et al., 2004; Bachmann et al., 2006).

PIM kinases can enhance cell survival through phosphorylation of the protein BAD at Ser112, leading to its dissociation from Bcl-2 promoting anti-apoptotic activity (Yan et al., 2003). All three PIM kinases are capable of phosphorylating NOTCH1 at Ser2152, leading to nuclear localisation and transcriptional activity of the NOTCH1 intracellular domain (N1ICD). PIM kinase phosphorylation of NOTCH1 was found to promote migration of prostate cancer

cells while it regulates glucose metabolism and mitochondrial function in breast cancer cells (Santio et al., 2016). Glucose metabolism is highly important in tumorigenesis as proliferating cells need energy to fuel their growth. In studies concerning hepatocellular carcinoma (HCC), high PIM1 expression was observed and associated with metastatic potential. Knockdown of PIM1 reduced glucose uptake by HCC cells and decreased levels of glycolytic pathway intermediates was observed, indicating the importance of PIM kinase activity in metabolism and its effects on tumour progression. In this study, Akt was found to be a substrate for PIM1, promoting Akt-mediated glycolysis allowing survival of the hypoxic tumour microenvironment (Leung et al., 2015).

2.4 PIM kinases and immune modulation

The role of PIM kinases in immune modulation is complex and not fully elucidated. However it has been shown that they modulate the survival and function of immune cells by employing glycolysis and IRES translation targets to redirect the immune cells against antitumor immunity to defend cancer cells (Jinesh et al., 2016). Tumor necrosis factor and Toll-like receptor ligands can induce the expression of PIM1. PIM2 is regulated by FoxP3 in Treg cells causing Treg cell expansion and stimulating immune tolerance and hence protecting cancer cells against immune attack (Basu et al., 2008; Deng et al., 2015). Daenthanasanmak et al. demonstrated that PIM2 negatively regulated T cell responses to alloantigen in contrast to PIM1 and PIM3 which acted as positive regulators (Daenthanasanmak et al., 2018). Silencing PIM2 in polyclonal or antigen specific CD8+ T cells enhanced their antitumour response in adoptive T cell immunotherapy. PIM kinases were recently shown to orchestrate tumour immune escape and support Reed-Sternberg cell survival and are a promising target in Hodgkin lymphoma (Szydłowski et al., 2017). Given the role of PIM kinases in tumour immunity they have the potential to interact directly or

indirectly with immunotherapies and may benefit a combination approach with checkpoint inhibitors.

2.5 Parallel pathways of PIM kinase

The phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) make up crucial interconnected signalling pathways involved in many cell processes such as growth, survival and metabolism. As such, they play a key role in promoting tumorigenesis as their aberrant activation leads to dysregulated cell growth, survival, angiogenesis and metastasis (Porta et al., 2014). These signaling pathways interplay with PIM kinases extensively, often with parallel and overlapping mechanisms of action (Figure 1). PI3K is a lipid kinase that can phosphorylate PIP2 to form PIP3, allowing for recruitment of Akt and PDK1 to the plasma membrane for further signalling. PIK3CA, the gene coding for the p110α catalytic subunit of PI3K is often mutated in different cancer types resulting in activation without an upstream initiating signal from receptor tyrosine kinases (Gyori et al., 2017). Akt is phosphorylated and activated by PDK1 and mTORC2, allowing it to activate mTORC1 (Mabuchi et al., 2015). Dual inhibition of PIM and PI3K's catalytic subunit P110α, produced antineoplastic effects in glioblastoma cells suggesting a relationship between the two kinases that is not well understood (Aziz et al., 2018). An indirect relationship has been elucidated whereby IRS-1, an upstream activator of PI3K, is phosphorylated by PIM kinases at Ser1101 (Song et al., 2016). Further research is necessary to determine whether PIM kinases and PI3K have other link points.

PI3K/Akt and PIM have notable overlapping pathways which can primarily be explained by their similar substrate target sequence (Figure 1) (Obata et al., 2000). Akt is capable of phosphorylating p21 and p27, Mdm2 and BAD along with others similar to PIM kinase (del

Peso et al., 1997; Fujita et al., 2002; Zhou et al., 2001a; Zhou et al., 2001b). Along with having the same substrates, both PIM and Akt show similar activity against cisplatin in ovarian cancer cells and are important in controlling the chemosensitivity of the cells. PIM2 was found to be induced upon treatment with cisplatin resulting in increased BAD phosphorylation and cell resistance to DNA damaging agents. Furthermore, sensitivity of ovarian cancer cells to cisplatin could be enhanced through targeted inhibition of PIM2 (Musiani et al., 2014). Akt was also found to induce resistance to cisplatin with an increase in cells in S and G2/M phases via its activation of checkpoint kinase 1 (Chk1) (Zhang et al., 2016). Thus, with so much in common ranging from substrates to chemoresistance, PIM and Akt dual inhibition is an exciting emerging therapeutic strategy.

An effector protein of Akt is mTOR kinase and it has an important role in controlling the translational machinery including ribosomal protein S6 kinases, and factors involved in the initiation and elongation steps of the protein translation process and thus, it is heavily involved in cell growth and metabolism (Viel et al., 2017). It is the catalytic component of mTORC1 and mTORC2 complexes and its signalling is regulated by both PIM and Akt via its effectors (Yao et al., 2017). PIM and Akt both phosphorylate TSC2, a negative regulator of mTORC1, along with controlling the AMP:ATP ratio where an increase leads to activation of TSC2 (Beharry et al., 2011; Lu et al., 2013). They both also phosphorylate proline rich-Akt substrate 40 (PRAS40) increasing mTOR kinase activity (Aziz et al., 2018). As a result, there is increased 4EBP1 and p70S6 kinase phosphorylation leading to translational activity. PIM kinases were shown in CLL patients to promote cell migration in a CXCR2-mTOR dependant manner (Bialopiotrowicz et al., 2018). Thus, a rationale exists for combinatorial therapy for PIM and mTOR along with Akt and even PI3K due to their overlapping and often parallel pathways.

3. PIM kinase mediated resistance

Due to their integration in many oncogenic signalling pathways it can be expected that PIM kinases contribute a substantial role in mediating resistance to many targeted treatments as well as chemotherapy and radiotherapy (Table 1). Overcoming acquired resistance to treatment is one of the biggest challenges for clinicians today. The mechanisms underlying this resistance are broad and complicated ranging from specific mutational mechanisms to non-mutational and even epigenetic factors (Lackner et al., 2012). PIM kinases mediate their resistance through a number of different mechanisms via its specific substrates.

3.1 Regulation of resistance to chemotherapy

An important effector of resistance to chemotherapy is the ATP-binding cassette (ABC) transporters, a protein family that function by the efflux of ions, lipids, amino acids, peptides, proteins and drugs (Mahadevan & Ghirahatti, 2005). PIM1 phosphorylates the ABC transporter BCRP/ABCG2 at Thr362 promoting BCRP dimerization and ultimately its plasma membrane localization. Both proteins are upregulated in mitoxantrone- and docetaxel-resistant prostate cancer cells. PIM1 knockdown re-sensitised these cells to chemotherapeutic agents. Similarly, the drug resistant activity of BCPR was impaired by a T362A mutation, showing PIMs importance in mediating resistance (Xie et al., 2008). PIM1 has also been shown to phosphorylate the ABC transporter P-glycoprotein (Pgp), a protein that when exported from the endoplasmic reticulum (ER) can either be glycosylated to form an active transporter protein, cleaved by ER proteases or be ubiquitinated and subsequently degraded by the proteasome. PIM1 knockdown was found to decrease cell surface Pgp with an increase in ubiquitinated Pgp suggesting PIM-specific phosphorylation of Pgp promotes its stabilisation, thus preventing its degradation (Xie et al., 2010). PIM1 inhibition sensitised

Pgp-overexpressing cells to doxorubicin. Thus, PIM inhibition may be a novel approach to combatting standard chemotherapy resistance in cancer patients.

3.2 Resistance to radiation therapies

PIM kinase has also been shown to induce resistance to radiation therapies. In particular, prostate cancer patients have a risk of up to 40% failure of primary therapies such as surgery or radiation (Siegel et al., 2014). Prostate tumours often have hypoxic regions that can render them more resistant to radiation therapy (Turaka et al., 2012). This in part, can be attributed to PIM1 activation in response to processes such as hypoxia and radiation which can then enhance tumorigenicity in tandem with c-Myc (Kim et al., 2011). This highlighted the potential to target PIM1 in prostate cancer in combination with radiation therapy using AZD1208. Combination treatment with radiation and AZD1208 resulted in greater and more sustained tumour growth inhibition than either treatment alone in castrate-resistant prostate cancer (CRPC) mice (Kirschner et al., 2015). The success of this treatment plan may be due to the role PIM1 overexpression has in stabilising p53, as inhibition with AZD1208 alone would result in decreased levels of the tumour suppressor (Hogan et al., 2008). Since radiation therapy can strongly induce p53 expression, this may account for the added efficacy of the combination as well as PIM1's role in resensitising tumours to radiation.

In non-small cell lung cancer (NSCLC), PIM1 can also exert radiation desensitising effects in patients leading to treatment resistance. Kim et al. showed that radiation led to PIM1 overexpression, reduction in protein phosphatases PP2A and PP5, resulting in PIM1 translocation to the nucleus. Nuclear PIM1 increases PRAS40 phosphorylation, which can then form a trimer with 14-3-3 and phosphorylated FOXO3a to move into the cytoplasm.

Cytoplasmic FOXO3a has been linked to decreased pro-apoptotic protein expression and radiation therapy resistance (Kim et al., 2013). PIM1 expression is also associated with EGFR expression which is known to promote resistance to radiotherapy (Peltola et al., 2009). Kim et al. used three PIM1 inhibitors to determine their radiosensitising capabilities, SGI-1776, ETP-45299 and tryptanthrin. They were found to disrupt the processes described previously that lead to radiation resistance, resulting in promotion of FOX3A transcriptional activity for expression of proapoptotic proteins in radioresistant cells.

3.3 Regulation of resistance via PI3K/Akt/mTOR pathway

The PI3K/Akt and mTOR pathways, discussed previously, are integral to cell growth and survival and therefore present themselves as key targets for anticancer therapies. Their inhibition has been documented and has proven efficacious in clinical trials. For example, mTOR inhibition with the first-in-class drug CCI-779 paved the way for further clinical programs due to its enhancement of survival rates in patients with advanced renal cell carcinoma (Faivre et al., 2006). However, this treatment proved inefficacious as the trials progressed due to reciprocal feedback loops that counteracted antitumor activity and promoted resistance. Similarly, Akt inhibition resulted in up-regulation of receptor tyrosine kinases (RTKs) which was found to be the result of PIM1 activity. PIM1 increased expression of RTKs in prostate cancer patients in a cap-independent manner via control of internal ribosome entry (Cen et al., 2013; Chandarlapaty et al., 2011). PIM1 mediated resistance to Akt inhibition was confirmed when a combination of Akt and pan-PIM inhibitors, GSK690693 and SMI-4a respectively, led to a synergistic inhibition of prostate tumour growth both *in vitro* and *in vivo* (Cen et al., 2013). In more recent studies, PIM kinases have been implicated in their overlapping role with the PI3K/Akt/mTOR pathway in ovarian cancer.

Work is being considered on how to bring the overlapping components to a clinical treatment for ovarian cancer (Aziz et al., 2018).

3.4 Resistance to angiogenesis inhibitors under hypoxic conditions

Another key mechanism by which PIM kinases exert their resistance to anticancer therapies is their increased expression under hypoxia. Hypoxia in cancer is characterised by a low level of oxygen within a tumour environment (Muz et al., 2015). It is usually due to poor vasculature and is correlated with cell survival, proliferation, resistance and is often a poor diagnostic marker in patients with solid tumours (Warfel & El-Deiry, 2014). It has been found that PIM kinases are expressed due to hypoxia in a HIF-1 independent manner and are implicated in providing protection to the tumour microenvironment by promoting angiogenesis and cell survival (Chauhan & Warfel, 2018). This provided a rationale for a role for PIM kinases in resistance to anti-angiogenic drugs. It was found that PIM was upregulated in response to treatment with anti-VEGF therapies with PIM1 reducing the drug's efficacy in disrupting vasculature and blocking tumour growth (Casillas et al., 2018). HIF-1α is known to bind to the PIM2 promoter and promote its expression, while PIM2 in turn is capable of binding to the transactivation domain of HIF-1α allowing for its transcription in response to hypoxia, creating a positive feedback loop that is key to driving tumour progression (Yu et al., 2014). As well as being upregulated in a HIF-1 dependent manner, PIM1 is regulated in a HIF-1 independent manner in response to hypoxia. It was seen that hypoxia protects the degradation of PIM1 from the proteasome allowing for its continuation in its role in cell survival (Chen et al., 2009).

3.5 Resistance to rapamycin and immune evasion

The immunosuppressive drug rapamycin, targets and disrupts cytokine signalling responsible for lymphocyte growth and differentiation (Dumont & Su, 1996). It also has displayed anti-tumour properties through its inhibition of mTOR, a central component of signalling pathways containing tumour suppressor genes and oncogenes (Law, 2005). Fox et al. showed that PIM1 and PIM2 are regulators of sensitivity in T cells as PIM deficient mice did not respond to pro-survival cytokines (Fox et al., 2005). This was converse to wild-type mice exposed to rapamycin, which failed to fully inhibit T cell proliferation, growth and survival. This immune evasion mediated predominately by PIM2 promoted cell survival through phosphorylation of BAD, retaining the protein within the cytoplasm and attenuating its pro-apoptotic properties (Petros et al., 2004). Therefore, rapamycin serves only as a semi-effective immunosuppressant. PIM kinase inhibition could enhance rapamycin's immunosuppressive properties in a clinical setting for a manner of different treatments such as preventing transplant rejection and as an anticancer therapy.

3.6 Resistance to HER2/ EGFR tyrosine kinase inhibitors

The mechanism of action of HER2 targeted therapies trastuzumab and lapatinib in HER2 positive breast cancer cells is in part through BAD de-phosphorylation and activation and its subsequent inhibition of survival signalling. Akt and RSK are known to phosphorylate BAD therefore it is possible that BAD inactivation represents a common downstream node through which resistance induced by PI3K or MAPK pathway activation is partly mediated and through which HER2 activation itself promotes survival in the absence of anti-HER2 treatment. A study by Moody et al. discovered that expression of the survival kinases PRKACA and PIM1 rescued cells from anti-HER2 therapy (Moody et al., 2015). The resistance effect conferred by overexpression of both PRKACA and PIM1 is at least in part mediated

through the restoration of anti-apoptotic signalling. This study highlights a role for combined anti-HER2 and PIM kinase therapy in HER2-positive breast cancers.

A common cause of many types of cancer lies in gain-of-function mutations of the epidermal growth factor receptors (EGFRs) which propagate growth signals to downstream targets such as PI3K, MAPK and JAK2/STAT3 without mitogen activation (Sigismund et al., 2018). EGFR amplification, transcriptional upregulation and excess ligand production from autocrine and paracrine factors also contribute to their uncontrolled signalling. These receptor tyrosine kinases (RTKs) are responsible for pathways such as cell growth, proliferation, cell cycle progression and survival (Lazzara et al., 2010). The development of EGFR TKIs has led to the replacement of standard chemotherapy in NSCLC patients with sensitising mutations in the EGFR TK domain (Karachaliou et al., 2018). However, like all targeted therapies the emergence of acquired resistance is inevitable (Figure 2). The activation of signalling nodes such as STAT3 and YAP1 as well as the upregulation of different RTKs such as CDCP1 and AXL upon EGFR inhibition also result in EGFR drug resistance (Chaib et al., 2017; Karachaliou et al., 2018). A recent study showed that combining PIM and EGFR inhibitors in EGFR-mutation positive NSCLC cell lines was moderately synergistic but decreased STAT3 phosphorylation, an important signalling node in therapy resistance (Bracht et al., 2019).

Activation of the MET gene is involved in ~ 22% of EGFR-TKI acquired resistant specimens (Engelman et al. 2007) and PIM1 regulates MET expression and signalling through phosphorylation of eIF4B thereby controlling translation of MET (Cen et al., 2014). Therefore, we hypothesise that PIM inhibition may be an effective treatment in MET amplified EGFR TKI resistant cells.

3.7 Resistance to MET inhibition

MET is a cell surface RTK for hepatocyte growth factor and is involved in unrestrained cell growth, angiogenesis and metastasis making it an attractive target for anticancer therapeutics. This has led to the development of many MET inhibitors, and clinical trial data demonstrates that patients with MET amplified tumours benefitting hugely from drugs such as tivantinib (Gentile et al., 2008; Santoro et al., 2013). Other trials demonstrated that NSCLC patients responded rapidly from treatment with crizotinib however, this efficacy was not observed in phase II clinical studies, leading to the possibility of acquired resistance to the drug (Cecchi et al., 2012). PIM1 regulates MET expression and experiments have shown that PIM1 and PIM3 are upregulated in a feedback induction loop upon MET inhibition (Cen et al., 2014). The levels of PIM kinases increase as the cancerous cells adapt to increasing doses of the MET inhibitor. Furthermore, inhibition of PIM whether by siRNA or small molecule inhibitors, was found to re-sensitise the cell to MET inhibitors. Acquired savolitinib (a potent and selective MET inhibitor) resistance in NSCLC arises via multiple mechanisms that converge on MET independent mTOR, Myc activation and a requirement for PIM kinase signalling with PIM inhibition restoring savolitinib sensitivity both *in vitro* and *in vivo* (Henry et al., 2016). This provides a rationale for dual treatment of PIM and MET inhibition for MET-dependant tumours such as, prostate and gastric cancers that have shown decreased efficacy in clinical trials due to acquired resistance (An et al., 2015).

.

Drug Name	Target	Study
Radiation	NSCLC	Kim W et al. 2011

Radiation	NSCLC	Kim A et al. 2013
Docetaxel	ABC transporter BCRP/ABCG2	Xie et al. 2008
CCI-779	mTOR	Kurmasheva et al. 2006
GSK690693	Akt	Cen et al. 2013
AZD5363	Akt	Meja et al. 2014
Anti-VEGF	VEGF	Casillas et al. 2017
Rapamycin	mTOR	Kurmasheva et al. 2006
Trastuzumab	HER2	Moody et al. 2014
Osimertinib	EGFR	Bracht et al. 2019
AZD6094	MET	Ningfei et al. 2015
Doxorubicin	ABC transporter Pgp	Xie et al. 2010

Table 1 Anti-cancer treatments, their targets and the relevant study which highlighted PIM kinase's role in resistance to treatment.

4.0 Targeting PIM kinase

4.1 Pan-PIM kinase inhibitors

Due to their overexpression in different types of cancers and their role in cancer progression pathways such as cell survival, cell cycle progression and metastasis, PIM kinases present themselves as potential therapeutic targets in cancer patients. Due to the redundancy of the three PIM kinase proteins, small molecule inhibitors have greater efficacy in treatment by inhibiting all three PIM kinases. This stems from knockout experiments on mice where those lacking all three PIM kinases displayed reduced size and body weight as well as being less responsive to haematopoietic growth factors. Despite these effects, the mice remained

viable and fertile suggesting that targeting all three PIM isoforms could be efficacious (Mikkers et al., 2004). Another rationale for PIM kinases being desirable targets of small molecule inhibitors is their unique and novel ATP binding pocket that was observed by crystallographic studies and peptide library screens (Bullock et al., 2005). X-ray structure analysis of PIM1 in conjunction with kinase inhibitors, staurosporine and adenosine revealed these novel kinase mechanisms (Jacobs et al., 2005). The enzyme consists of two domains linked by a hinge region. The active site is surrounded by the hinge region (residues 122-127), the activation loop in the C-terminal domain (residues 186-210) and a glycine-rich loop in the N-terminal domain (residues 44-55). The uniqueness in PIM1 can be seen in its employment of Pro123 into a position that in most protein kinases proline is absent. In all other kinases this position is utilised for hydrogen bonding between the main chain amide group and ATP, Pro123 in PIM1 sterically blocks this interaction. Thus, only 1 hydrogen bond exists between the kinase and ATP or ATP mimetic inhibitors while other Van der Waal or hydrophobic forces are capable of aligning correct substrate positioning. The hinge region is also unique in that it possesses a 2-residue insertion compared to CDK-2 and JNK-3 and a single residue insertion relative to PKA which share high sequence homologies (De Bondt et al., 1993; Xie et al., 1998; Jacobs et al., 2005). These insertions result in the hinge region bulging away from the ATP-binding site by approximately 4A. This unique hinge region that is structurally distinct from other kinases along with possible polar interactions with Pro123's carbonyl oxygen could offer scope to designing PIM specific targeted inhibitors.

As PIM kinases are constitutively active, all PIM inhibitors are type I kinase inhibitors, i.e. those that bind to the enzyme in its catalytically active state (Roskoski, 2016). PIM kinase inhibitors can be divided into ATP-mimetics that interact in place of ATP at Glu121 in the hinge region and non-ATP-mimetic inhibitors that disrupt the salt bridge that confers

catalytic activity via interaction with Lys67 (Bogusz et al., 2017). While most reported PIM inhibitors are ATP-mimetics, non-ATP-mimetic inhibitors would prove to be more specific due to their binding at residues opposite the binding pocket.

CX-6258 is a potent non-ATP-mimetic inhibitor of all three isoforms pertaining to a class of oxindole-derivatives. It was found that all PIM kinase proteins along with another kinase Flt-3 were inhibited by over 80% by 0.5µM CX-6258 in a panel of 107 kinases, thus displaying high selectivity. It unsurprisingly showed antiproliferative activity against cell lines derived from both solid tumours and haematological malignancies especially acute leukaemias. In human AML cells, CX-6258 inhibited phosphorylation of the pro-survival proteins BAD and 4EBP1 at PIM kinase specific serine and threonine sites (Haddach et al., 2012).

A class of imidazo[1,2-b]pyridazines were identified as PIM kinase inhibitors with SGI-1776 being identified as a lead compound. These inhibitors are also non-ATP-mimetic compounds that do not interact with the hinge region and have been shown to have antileukemic properties (Pogacic et al., 2007). In CLL patients, the PIM gene was found to be overexpressed, while nanomolar concentrations of SGI-1776 were shown to inhibit all three isoforms (Cohen et al., 2004). It was proposed that the mechanistic action of this inhibitor lies in PIM's synergism with c-Myc and that disruption of c-Myc activation by PIM inhibition could lead to a decrease in oncogenic transformation in primary lymphocytes of CLL patients (Chen et al., 2009). Treatment with SGI-1776 promoted apoptosis as well as inhibiting RNA synthesis. SGI-1776 reached phase I clinical trials for refractory prostate cancer and relapsed/ refractory non-Hodgkin lymphoma but was terminated due to toxicity owing to cardiac QTc prolongation in patients (NCT00848601). Other promising imidazopyradazines include ETP-45299, which displayed PIM specific inhibition with a Ki for PIM of 30nM,

resulting in significantly decreased phosphorylation of BAD and 4EBP1 along with inhibition of proliferation in leukaemia cell lines (Blanco-Aparicio et al., 2011). Further studies, however, were unable to proceed due to ETP-45299's instability *in vivo*.

A class of benzothienopyrimidinones were found to be potent inhibitors of all three PIM isoforms. Tao et al. discovered that compound 14j inhibited the myelogenous leukaemia cell line K562, with high selectivity against a range of functionally distinct kinases. It was shown to have an EC50 value of 1.7μM and Ki values for all three PIM isoforms of 2, 3 and 0.5 nM respectively. Similar to other inhibitors already discussed, this class inhibits phosphorylation of BAD. It was shown to have a bioavailability of 76% after oral dosage and ADME profiling has shown that the compound has a long half-life in human and murine liver microsomes with good permeability and reasonable protein binding (Tao et al., 2009).

Novel benzylidenethiazolinediones were synthesised and found to potently block PIM phosphorylation of BAD in prostate cancer and leukaemia cell lines inducing G1/S cell cycle arrest as well as blocking anti-apoptotic effects of PIM kinase. This cell cycle arrest was suggested to be due to inhibition of CDK2 along with nuclear translocation of p27 (Beharry et al., 2009). Specifically, 5-(3-Trifluoromethylbenzylidene) thiazolidine-2,4-dione (SMI-4a) was screened and found to be a potent PIM1 inhibitor with high selectivity over 50 other protein kinases tested. A model for lead optimisation was found by computational docking of 4a with PIM1 and a number of derivatives were synthesised with new compounds displaying IC50 values of 13nM and 2.3μM for PIM1 and PIM2 respectively. Additional screenings led to higher selectivity of PIM1 by 2,500-fold and PIM to by 400-fold (Xia et al., 2009). Further work on SMI-4a has shown its ability to induce G1 cell-cycle arrest in pre-T-LBL cells along with DU145 prostate cancer cells and MV4-11 myeloid leukaemia cells (Lin et

al., 2010). It is currently under preclinical trials for AML treatment in other haematological cancers including CML and B-cell ALL (Fan et al., 2017).

A potent pan-PIM inhibitor, LGB321 has shown significant antiproliferative activity in haematological malignancies such as ALL, AML, multiple myeloma (MM) and Non-Hodgkin Lymphoma (NHL) (Garcia et al., 2014). In more recent studies, LGB321 has been shown to be efficacious in inducing apoptosis in primary human CLL cells. It is considered a viable treatment option due to blocking PIM1/CXCR4-induced interaction of CLL cells with the protective tumour microenvironment as well as disrupting PIM2 and PIM3 induced CLL-cell survival (Decker et al., 2016). Based on these results, clinical trials are warranted.

A pan-PIM inhibitor, LGH447 displayed activity in relapsed refractory multiple myeloma cell lines by inhibiting proliferation, mTORC1 signalling along with phosphorylation of BAD. It was also shown to inhibit tumour growth in mouse subcutaneous xenograft models of MM and AML suggesting its possible efficacy in AML patients as well (Langowski et al., 2013). In 2012 a phase I trial on oral LGH447 in patients with relapsed/ refractory MM commenced and the outcome of this trial is expected later this year (NCT01456689). However, initial published results have indicated good tolerance overall in patients who have had no effective treatment options (Lin et al., 2010).

INCB053914, an ATP-competitive pan-PIM inhibitor, has been shown preclinically to decrease proliferation and activation/phosphorylation of downstream substrates of PIM kinases (Koblish et al., 2018). Initial results from an ongoing Phase I monotherapy dose escalation study in patients with advanced malignancies has indicated preliminary safety and efficacy (NCT02587598). *Ex vivo* treatment of AML patient derived primary bone marrow blasts with INCB053914 decreased phosphorylation of p70S6K and 4EBP1 and

increased PIM2 expression (Byrne et al., 2017). A recommended dose was identified from this Phase I study for use in the current follow on phase II investigating the combination of NCB053914 with other targeted therapies for the treatment of B-cell lymphoma (NCT03688152).

AZD1208 is another ATP-competitive, pan-PIM inhibitor that was shown to reduce proliferation and increase apoptosis in AML and prostate cancer (Keeton et al., 2013; Kirschner et al., 2014). Recently published results from two parallel phase I studies of AZD1208 monotherapy indicated it had a manageable safety profile with a tolerated dose of up to 700 mg in AML and prostate cancer, however no clinical efficacy was observed.

In conclusion, aside from SGI-1776, there are a number of pan-PIM inhibitors such as LGH447, NCB053914 and AZD1208 that appear to be well tolerated by cancer patients. However, the lack of clinical responses in both haematological and solid tumours suggests that targeting PIM kinases alone may be insufficient to achieve the desired therapeutic effect. Instead dual-targeting agents or combination approaches may be more efficacious and effective in generating durable clinical outcomes. Drug repositioning efforts using molecular docking analysis to investigate whether known drugs have binding ability against the PIM kinases have shown nilotinib, vemurafenib and idelalisib all have binding ability to PIM-1 kinase (Arrouchi et al., 2019b).

4.2 Targeting PIM kinase via dual-targeted inhibitors

Testing of a first-in-class dual PIM and FLT3-ITD inhibitor, SEL24-B489 is underway for AML patients (Czardybon et al., 2018). Fms-like tyrosine kinase 3 internal tandem duplication (FLT3-ITD) is a common genetic lesion in AML patients with 70% of newly diagnosed patients exhibiting its expression with a further 30% showing activating mutations of the receptor

(Thiede et al., 2002). Mutated FLT3 phosphorylates STAT5, MAPK and BAD allowing for cell proliferation and survival (Mizuki et al., 2003). These overlapping substrates with PIM kinases provide a rationale for the development of a dual PIM/FLT3 inhibitor, alongside increased expression of PIM kinases in AML patients treated with a single FLT3 inhibitor (Kim et al., 2005). FLT3 inhibitor-mediated expression of PIM2 induced resistance to inhibition as well, providing further reasoning for the importance of a dual inhibitor. SEL24-B489 showed on-target activity in pre-clinical models of AML at low-micromolar concentrations and was efficacious in blocking FLT3-ITD-triggered pathways along with phosphorylation of PIM kinase substrates, resulting in decreased survival of AML cell lines and primary AML blasts (Green et al., 2015). Dual inhibition also restored sensitivity to FLT3 inhibition by blocking direct PIM phosphorylation of the FLT3-ITD receptor. This phosphorylation by PIM has been shown to reduce receptor-inhibitor affinity (Natarajan et al., 2013). Other results from this study have shown that SEL24-B489 suppresses signalling in many different pathways resulting in decreased proliferation, protein translation and metabolism. The inhibitor has recently been cleared for phase I/II trial in AML patients with further work still continuing (NCT03008187).

The combination of a pan-PIM kinase inhibitor and a PI3K inhibitor indicated synergistic anti-cancer effects *in vitro* and *in vivo* in haematological malignancies including Non-Hodgkinson's lymphoma and CLL , supporting the rationale to design a single dual targeting PIM and PI3K inhibitor (Koblish et al., 2018). In preclinical studies IBL-202, a dual targeting PIM and PI3K inhibitor, has demonstrated superior cytotoxic activity in CLL cells *in vitro* compared to PI3K inhibitor Idelalisib alone, in addition to significantly reducing the proliferative, migratory capacity of leukaemia cells (Crassini, 2018).

A pan-PIM kinase and PI3K/mTOR inhibitor IBL-302 was effective and tolerable in pre-clinical testing on over 700 cell lines with a PI3K mutation and high PIM kinase expression Screening identified neuroblastoma as a strong candidate for PIM/PI3K/mTOR inhibition. IBL-302 treatment alone reduced tumor growth *in vivo*, combination therapy with low-dose cisplatin inhibited neuroblastoma PDX growth (Mohlin et al., 2019). IBL-302 may also benefit one third of patients with breast cancer who have a mutated PI3K pathway and those with resistant triple negative breast cancer who have high PIM kinase expression (Horiuchi et al., 2016).

The design of single agent, multi-molecularly targeted inhibitors is a relatively new and emerging field of research in cancer therapeutics. We speculate that simultaneous inhibition of PIM kinases in addition to other parallel pro-tumorigenic kinases may not only provide a more robust and clinically efficacious approach to cancer treatment but also a less complex and more cost-effective patient treatment regime.

5. Co-targeting PIM kinase via combination therapies

Due to their limited efficacy as a single targeted therapy as well as the role PIM kinases play in mediating resistance to other anticancer therapies, PIM kinase inhibitors may be most efficacious when used in co-targeting treatments strategies. The dual inhibition of PIM kinase along with another pathway inhibitor could prove more advantageous in treatment of patients than either inhibitor acting alone.

5.1 Inhibition of PIM kinase and the translation machinery

The oxindolo-furanyl based pan-PIM inhibitor CX-6258 previously described, was used in combination with the RNA polymerase I inhibitor CX-5461, which blocks transcription of

rRNA. The advantage to inhibiting this particular biological process is that in Myc-driven tumours such as prostate cancer, Myc has been shown to upregulate ribosomal biogenesis and overexpress its synthesis (Grandori et al., 2005). PIM kinases mediate resistance to conventional therapies by their phosphorylation of 4EBP1 at Ser65 to stimulate eIF4E-dependent mRNA translation and increased protein synthesis (Qin et al., 2016). This role of PIM kinases along with their documented synergy with c-Myc, provides a logical reasoning to test the dual inhibition of PIM kinases and RNA polymerase I. While use of CX-6258 alone showed limited antiproliferative activity, its combination with CX-5461 dramatically enhanced the suppression of large invasive lesions and proliferation in Hi-Myc mouse xenografts models of prostate cancer. Furthermore, in PTEN null (low Myc) mouse xenograft models where CX-5461 had minimal effects alone, the combination of CX-5461 and CX-6258 was effective at reducing lesion size. These results provided evidence for the preclinical efficacy of this combination therapy (Rebello et al., 2016).

5.2 Inhibition of PIM kinase and the PI3K/Akt/mTOR pathway

Recently, a combination of the pan-PIM inhibitor AZD1208 and mTOR inhibitor AZD2014 was shown to reduce proliferation and induce apoptosis in AML cell lines (Harada et al., 2015). AZD2014 is a highly specific mTOR kinase inhibitor for mTORC1 and mTORC2, serving to block the mTOR pathway (Eyre et al., 2014). The inhibitor duo activated AMPKα which functions as a negative regulator of mTORC1, which contributed to greater antitumoral effects in comparison to either drug acting alone. Furthermore, protein expression analysis showed there to be a decreased expression of c-Myc from AZD2014 inhibition, Myb from AZD1208 and HSF1 from both inhibitors. HSF1 has been shown to drive translation by regulating ribosome biogenesis (Mamane et al., 2006; Santagata et al., 2013). Suppression

of Myc, Myb and HSF1 culminates in a decrease in protein synthesis, thereby counteracting cell proliferation. Finally, the combination exerted a novel proapoptotic effect due to CH10 (HSPE1), that was not seen in either drug when tested alone (Samali et al., 1999). This combinational therapy proved successful within this study and this data should help support the approval of clinical trials in the future.

PIM1 mediated resistance to Akt inhibition was confirmed when a combination of Akt and pan-PIM inhibitors, GSK690693 and SMI-4a respectively, led to a synergistic inhibition of prostate tumour growth both *in vitro* and *in vivo* (Cen et al., 2013). The pan-PIM selective inhibitor, AZD1897 has been tested in combination with Akt inhibitor AZD5363 in preclinical models of AML and multiple myeloma (MM). In AML cell lines and primary cell cultures, the combined therapy demonstrated a synergistic and enhanced reduction of pro-apoptotic Mcl-1 and other overlapping substrates of PIM1 and Akt compared to either monotherapy alone (Meja et al., 2014). AZ1897 or AZD5363 had modest effects on proliferation in MM cell lines, but their combination increased anti-proliferative activity, a synergistic increase in apoptosis and reduced activation of downstream effectors of mTORC1 signalling (e.g. 4EBP1 and S6) (Meja et al., 2014).

Another study has been carried out, designed to combat intrinsic resistance to PIM inhibition in AML patients. It's been reported that targeted PIM inhibition increases the levels of reactive oxygen species (ROS), activating MAPK p38α along with upregulating downstream signalling of Akt/mTOR. Intrinsic resistance to associated AZD1208 was found to occur through feedback activation of mTOR signalling as a result of ROS, p38 and Akt activation (Brunen et al., 2016; Song et al., 2015). Utilising shRNA-based genetic screening, kinases were identified whose suppression would be synergistic with PIM inhibition. The

experiment concluded that inhibition of p38α with SB202190 or SCIO-469, sensitises haematological cell lines to AZD1208 both *in vitro* and *in vivo*. MAPK p38 suppression was found to counteract PIM inhibitor-mediated resistance through its suppression of mTOR signalling. This combination has a potential for clinical trials as p38 inhibitors which are in clinical trials for a multitude of diseases are less toxic than PI3K, Akt and mTOR inhibitors. This implicates their potential use in combinatorial therapies.

As previously mentioned the pan-PIM inhibitor INCB053914 is undergoing preclinical trials in combination with other targeted therapies. Combination with INCB050465, a selective Phase II PI3K inhibitor indicated synergistic anti-proliferative effects in *in vitro* and *in vivo* models of non-Hodgkinson's lymphoma (Koblish et al., 2018). INCB050465 induced an upregulation of PIM kinase expression, suggesting that targeting both simultaneously may help circumvent resistance mechanisms to PI3K inhibitors. Based on these results, a phase 1/2 dose-escalation trial is currently underway to assess the safety profile and efficacy of INCB053914 and INCB050465 combined (NCT03688152).

5.3 Inhibition of PIM kinase and anti-apoptotic proteins

Combinatorial therapy consisting of PIM kinase inhibitor SMI-4a and the Bcl-2 antagonist ABT-737 was shown to cause a powerful apoptotic effect in prostate cancer cells *in vitro* and *in vivo*. Alone ABT-737 is unable to inhibit Mcl-1, an anti-apoptotic protein of the Bcl-2 family. Thus mcl-2 protein expression dilutes ABT-737's pro- apoptotic activity. The efficacy of combined SMI-4a and ABT-737 is due to the ability of SMI-4a to decrease the levels of Mcl-1 via blocking 5'-cap dependant translation and decreasing protein half-life (Song and Kraft, 2012). More recent work investigated the efficacy of ABT-737 in combination with different PIM inhibitors in CLL lymphocytes, due to the overexpression of Bcl-2 family

proteins as well as PIM mRNA transcripts (Cervantes-Gomez et al., 2016; Kitada et al., 1998; Nawijn et al., 2011). Results showed that the combination resulted in a synergistic cytotoxicity with SGI-1776 surprisingly showing the highest cytotoxic levels of all PIM inhibitors tested, contrary to the respective IC50 values of the PIM inhibitors. It would have been expected that AZD1208 being the most potent inhibitor would have the highest cytotoxicity in CLL cells, showing the complexity of combination therapeutics.

5.4 PIM Kinase inhibition with chemotherapy

Along with combinatorial inhibition, PIM inhibitors can also be used in conjunction with standard-of-care cancer treatments. The novel pan-PIM inhibitor PIM447 was used in multiple myeloma cells in combination with different standard treatments such as bortezomib and dexamethasone, lenalidomide and dexamethasone, and pomalidomide and dexamethasone (Paino et al., 2017). PIM447 exerts its cytotoxicity on myeloma cells by disruption of the cell cycle along with a decrease in BAD phosphorylation, m-Myc levels and inhibition of the mTORC1 pathway resulting in apoptosis. It was also found to significantly reduce the tumour burden and prevent tumour-associated bone loss in disseminated murine models of human myeloma. The antitumoral and bone-protective effects of PIM447 provides a rationale for clinical applications of this therapy. Indeed, PIM447 in combination with Ruxolitinib and LEE011 is currently undergoing phase I clinical trials in patients with myelofibrosis (NCT02370706). The previously discussed pan-PIM inhibitor, INCB053914, is also being investigated in combination with a number of chemotherapy drugs including Ruxolitinib, Azacitidine and intermediate dose cytarabine for the treatment of advanced malignancies including refractory AML and myelofibrosis (NCT02587598).

5.5 PIM Kinase inhibition with immunotherapy

Immunotherapies are a rapidly evolving anticancer treatment option that harnesses the patient's own immune system to attack and destroy cancer cells and tumours. Adoptive T cell therapy utilises *ex vivo*-engineered tumour-reactive epitopes. While they have shown to be successful in multiple cases, the T cell anti-tumour response declines with loss of effector function and survival, providing a need to improve the lifespan and durability of these T cells. One of the key aspects of adoptive cell transfer (ACT) success lies in the cell's metabolic phenotype (Sugiura & Rathmell, 2018). T cells that rely on glycolysis for their effector function energy requirements result in diminished durability while cells that utilise oxidative phosphorylation lead to a central memory (Tcm) phenotype which allows for a longer more persistent anti-tumour function (van der Windt & Pearce, 2012). Strategies to inhibit the glycolytic pathway are therefore beneficial in maintaining the Tcm phenotype and increasing the T cell effector functions. Due to PIM kinases role in regulating protein synthesis and cell growth via mTOR activation, Chatterjee et al. proposed that PIM inhibition in combination with ACT may help increase the durability of T cells by blocking their glycolytic metabolism (Chatterjee et al., 2019). AZD1208 was used in combination with ACT and anti-PD1 to assess whether this triple combination treatment could help improve tumour control as PD1 has a key role in downregulating the immune response and suppressing T cell activity (Jin et al., 2010). T cells treated with this combination therapy were shown to have reduced uptake of the fluorescent glucose analogue 2-NBDG, as well as showing a reduced extracellular acidification rate meaning a lower rate of lactate production when compared to wild-type T cells. Cells treated with AZD1208 also showed increased surface expression of CCR7, a signature chemokine receptor of Tcm cells (Sheng et al., 2017). Chatterjee et al. also observed reduced phosphorylation of the transcription factor Foxo1 in AZD1208 treated cells, leading to its retention in the nucleus with

subsequent increased transcriptional activity and expression of CD62L. As increased transcriptional activity of Foxo1 is associated with anergy prevention and maintaining the CD8+ memory state (Delpoux et al., 2018), PIM kinase inhibition thereby led to an increase in the Tcm phenotype, allowing for improved tumour control in conjunction with ACT. This improved anti-tumour response was enhanced when PIM kinase inhibition was combined with an anti-PD1 antibody further potentiating the Tcm phenotype by checkpoint blockade, thus combatting resistance mechanisms that arose upon ACT single treatment.

6. Conclusion

This review highlights the growing number of studies demonstrating that PIM kinase plays an important role within cancer and mediates a resistance mechanism through its substrates that allows cancer cells to evade cell death and develop a more aggressive phenotype. These findings along with beneficial reports of dual targeting strategies inhibiting PIM kinase and other pathway components such as PI3K/Akt/mTOR or MET simultaneously as well as synergistic approaches with checkpoint inhibitors highlight potentially more effective treatment protocols for patients with cancer and emphasise the need for further combinatorial studies.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest. The work has been approved by all authors. The manuscript has not been published and is not under consideration for publication elsewhere.

References

Allen, J. D., Verhoeven, E., Domen, J., van der Valk, M., & Berns, A. (1997). Pim-2 transgene induces lymphoid tumors, exhibiting potent synergy with c-myc. *Oncogene 15*, 1133–1141.

Amati, B., Alevizopoulos, K., & Vlach, J. (1998). Myc and the cell cycle. *Frontiers in Bioscience 3*, d250-68.

Amson, R., Sigaux, F., Przedborski, S., Flandrin, G., Givol, D., & Telerman, A. (1989). The human protooncogene product p33pim is expressed during fetal hematopoiesis and in diverse leukemias. *Proceedings of the National Academy of Sciences of the United States of America 86*, 8857–8861.

An, N., Xiong, Y., LaRue, A. C., Kraft, A. S., & Cen, B. (2015). Activation of Pim Kinases Is Sufficient to Promote Resistance to MET Small-Molecule Inhibitors. *Cancer Research 75*, 5318–5328.

Arrouchi, H., Lakhlili, W., & Ibrahimi A. (2019a). A review on PIM kinases in tumors. *Bioinformation 15*, 40-45.

Arrouchi, H., Lakhlili, W., & Ibrahimi A.(2019b). Re-positioning of known drugs for Pim-1 kinase target using molecular docking analysis. *Bioinformation 15*, 116-120.

Asati, V., Mahapatra, D.K., & Bharti, S.K. (2019). PIM kinase inhibitors:Structural and pharmacological perspectives. *Eur J Med Chem 172*, 95-108.

Aziz, A. U. R., Farid, S., Qin, K., Wang, H., & Liu, B. (2018). PIM Kinases and Their Relevance to the PI3K/AKT/mTOR Pathway in the Regulation of Ovarian Cancer. *Biomolecules*, *8*, 7.

Bachmann, M., Hennemann, H., Xing, P. X., Hoffmann, I., & Moroy, T. (2004). The oncogenic serine/threonine kinase Pim-1 phosphorylates and inhibits the activity of Cdc25C-associated kinase 1 (C-TAK1): a novel role for Pim-1 at the G2/M cell cycle checkpoint. *The Journal of Biological Chemistry 279*, 48319–48328.

Bachmann, M., Kosan, C., Xing, P. X., Montenarh, M., Hoffmann, I., & Moroy, T. (2006). The oncogenic serine/threonine kinase Pim-1 directly phosphorylates and activates the G2/M specific phosphatase Cdc25C. *The International Journal of Biochemistry & Cell Biology 38*, 430–443.

Basu, S., Golovina, T., Mikheeva, T., June, C.H., & Riley, J.L. (2008). Cutting edge:Foxp3-mediated inductio. of pim 2 allows human T regulatory cells to preferentially expand in rapamycin. *J Immunol 180*, 5794-8.

Beharry, Z., Zemskova, M., Mahajan, S., Zhang, F., Ma, J., Xia, Z., … Kraft, A. S. (2009). Novel benzylidene-thiazolidine-2,4-diones inhibit Pim protein kinase activity and induce cell cycle arrest in leukemia and prostate cancer cells. *Molecular Cancer Therapeutics 8*, 1473–1483.

Beharry, Z., Mahajan, S., Zemskova, M., Lin, Y.W., Tholanikunnel, B. G., Xia, Z., … Kraft, A. S. (2011). The Pim protein kinases regulate energy metabolism and cell growth. *Proceedings of the National Academy of Sciences of the United States of America 108*, 528–533.

Bialopiotrowicz, E., Gorniak, P., Noyszewska-Kania, M., Pula, B., Makuch-Lasica, H., Nowak, G., … Juszczynski, P. (2018). Microenvironment-induced PIM kinases promote CXCR4-triggered mTOR pathway required for chronic lymphocytic leukaemia cell migration. *Journal of Cellular and Molecular Medicine 22*, 3548–3562.

Blanco-Aparicio, C., Collazo, A. M. G., Oyarzabal, J., Leal, J. F., Albaran, M. I., Lima, F. R., … Bischoff, J. R. (2011). Pim 1 kinase inhibitor ETP-45299 suppresses cellular proliferation and synergizes with PI3K inhibition. *Cancer Letters 300*, 145–153.

Bogusz, J., Zrubek, K., Rembacz, K. P., Grudnik, P., Golik, P., Romanowska, M., … Dubin, G. (2017). Structural analysis of PIM1 kinase complexes with ATP-competitive inhibitors. *Scientific Reports 7*, 13399.

Bracht, J., Karachaliou, N., Berenguer, J., Fernandez-Bruno, M., Filipska, M., Pedraz Valdunciel, C., … Rosell, R. (2019). PIM-1 inhibition with AZD1208 to prevent osimertinib-induced resistance in EGFR-mutation positive non-small cell lung cancer. *Journal of Cancer Metastasis and Treatment 5*, 22.

Brunen, D., Garcia-Barchino, M. J., Malani, D., Jagalur Basheer, N., Lieftink, C., Beijersbergen, R. L., … Bernards, R. (2016). Intrinsic resistance to PIM kinase inhibition in AML through p38alpha-mediated feedback activation of mTOR signaling. *Oncotarget 7*, 37407–37419.

Bullock, A. N., Debreczeni, J., Amos, A. L., Knapp, S., & Turk, B. E. (2005). Structure and substrate specificity of the Pim-1 kinase. *The Journal of Biological Chemistry 280*, 41675–41682.

Bullock, A. N., Russo, S., Amos, A., Pagano, N., Bregman, H., Debreczeni, J. E., … Knapp, S. (2009). Crystal structure of the PIM2 kinase in complex with an organoruthenium inhibitor. *PloS One 4*, e7112.

Byrne, M., Donnellan, W., M. Zeidan, A., Cherry, M., R. Baer, M., Fathi, A., … Savona, M. (2017). Preliminary Results from an Ongoing Phase 1/2 Study of INCB053914, a Pan-Proviral Integration Sites for Moloney Virus (PIM) Kinase Inhibitor, in Patients with Advanced Hematologic Malignancies. *ASH Annual Meeting*.

Casillas, A. L., Toth, R. K., Sainz, A. G., Singh, N., Desai, A. A., Kraft, A. S., & Warfel, N. A. (2018). Hypoxia-Inducible PIM Kinase Expression Promotes Resistance to Antiangiogenic Agents. *Clinical Cancer Research 24*, 169–180.

Cecchi, F., Rabe, D. C., & Bottaro, D. P. (2012). Targeting the HGF/Met signaling pathway in cancer therapy. *Expert Opinion on Therapeutic Targets 16*, 553–572.

Cen, B., Mahajan, S., Wang, W., & Kraft, A. S. (2013). Elevation of receptor tyrosine kinases by small molecule AKT inhibitors in prostate cancer is mediated by Pim-1. *Cancer Research 73*, 3402–3411.

Cen, B., Xiong, Y., Song, J. H., Mahajan, S., DuPont, R., McEachern, K., … Kraft, A. S. (2014). The Pim-1 protein kinase is an important regulator of MET receptor tyrosine kinase levels and signaling. *Molecular and Cellular Biology 34*, 2517–2532.

Cervantes-Gomez, F., Lavergne, B., Keating, M. J., Wierda, W. G., & Gandhi, V. (2016).

Combination of Pim kinase inhibitors and Bcl-2 antagonists in chronic lymphocytic leukemia cells. *Leukemia & Lymphoma 57*, 436–444.

Chaib, I., Karachaliou, N., Pilotto, S., Codony Servat, J., Cai, X., Li, X., … Rosell, R. (2017). Co-activation of STAT3 and YES-Associated Protein 1 (YAP1) Pathway in EGFR-Mutant NSCLC. *Journal of the National Cancer Institute 109*, djx014.

Chandarlapaty, S., Sawai, A., Scaltriti, M., Rodrik-Outmezguine, V., Grbovic-Huezo, O., Serra, V., … Rosen, N. (2011). AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. *Cancer Cell 19*, 58–71.

Chatterjee, S., Chakraborty, P., Daenthanasanmak, A., Iamsawat, S., Andrejeva, G., Luevano, L. A., … Mehrotra, S. (2019). Targeting PIM Kinase with PD1 Inhibition Improves Immunotherapeutic Antitumor T-cell Response. *Clinical Cancer Research 25*, 1036–1049.

Chauhan, S. S., & Warfel, N. A. (2018). Targeting PIM kinases to oppose hypoxia-mediated therapeutic resistance. *Oncoscience 5*, 254–255.

Chen, J., Kobayashi, M., Darmanin, S., Qiao, Y., Gully, C., Zhao, R., … Lee, M.-H. (2009). Hypoxia-mediated up-regulation of Pim-1 contributes to solid tumor formation. *The American Journal of Pathology 175*, 400–411.

Chen, L. S., Redkar, S., Bearss, D., Wierda, W. G., & Gandhi, V. (2009). Pim kinase inhibitor, SGI-1776, induces apoptosis in chronic lymphocytic leukemia cells. *Blood 114*, 4150–4157.

Cohen, A. M., Grinblat, B., Bessler, H., Kristt, D., Kremer, A., Schwartz, A., … Don, J. (2004). Increased expression of the hPim-2 gene in human chronic lymphocytic leukemia and

non-Hodgkin lymphoma. *Leukemia & Lymphoma 45*, 951–955.

Crassini, K. (2018). The dual inhibitor of the phosphoinositol-3 and PIM kinases, IBL-202, is effective against chronic lymphocytic leukaemia cells under conditions. *British Journal of Haematology 182,* 654-669.

Cuypers, H. T., Selten, G., Quint, W., Zijlstra, M., Maandag, E. R., Boelens, W., … Berns, A. (1984). Murine leukemia virus-induced T-cell lymphomagenesis: integration of proviruses in a distinct chromosomal region. *Cell 37*, 141–150.

Czardybon, W., Windak, R., Golas, A., Galezowski, M., Sabiniarz, A., Dolata, I., … Brzozka, K. (2018). A novel, dual pan-PIM/FLT3 inhibitor SEL 24 exhibits broad therapeutic potential in acute myeloid leukemia. *Oncotarget 9*, 16917–16931.

De Bondt, H. L., Rosenblatt, J., Jancarik, J., Jones, H. D., Morgan, D. O., & Kim, S. H. (1993). Crystal structure of cyclin-dependent kinase 2. *Nature 363*, 595–602.

Daenthanasanmak, A., Wu, Y., Iamsawat, S., Nguyen, H.D., Bastian, D. Zhang, M.,…Yu, X.Z. (2018) PIM-2 protein kinase negatively regulates T cell responses in transplantation and immunity. J Clin Invest 128, 2787-2801.

Decker, S., Kissel, S., Aumann, K., Zenz, T., Zirlik, K., Claus, R., … Dierks, C. (2016). The Pan-PIM Kinase Inhibitor LGB321 Affects Apoptotic Pathways and Microenvironmental Interactions in CLL. *Blood 128*, 4370.

del Peso, L., Gonzalez-Garcia, M., Page, C., Herrera, R., & Nunez, G. (1997). Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. *Science 278*, 687–689.

Delpoux, A., Michelini, R. H., Verma, S., Lai, C.-Y., Omilusik, K. D., Utzschneider, D. T., … Hedrick, S. M. (2018). Continuous activity of Foxo1 is required to prevent anergy and

maintain the memory state of CD8(+) T cells. *The Journal of Experimental Medicine 215*, 575–594.

Deneen, B., Welford, S.M., Ho, T., Hernandez, F., Kurland, I., & Denny, C.T. (2003). PIM3 proto-oncogene kinase is a common transcriptional target of divergent EWS/ETS oncoproteins. *Mol Cell Biol 23*, 3897–908.

Deng, G., Nagai, Y., Xiao, Y., Li, Z., Dai, S., Ohtani, T.,… Greene, M.I (2015). Pim-2 kinase influences regulatory T cell function and stability by mediating Foxp3 protein N-terminal phosphorylation. *J Biol Chem 290*, 20211–20220.

Dhanasekaran, S. M., Barrette, T. R., Ghosh, D., Shah, R., Varambally, S., Kurachi, K., … Chinnaiyan, A. M. (2001). Delineation of prognostic biomarkers in prostate cancer. *Nature 412*, 822–826.

Dumont, F. J., & Su, Q. (1996). Mechanism of action of the immunosuppressant rapamycin. *Life Sciences 58*, 373–395.

Eichmann, A., Yuan, L., Bréant, C., Alitalo, K., & Koskinen, P. J. (2000). Developmental expression of Pim kinases suggests functions also outside of the hematopoietic system. *Oncogene 19*, 1215–1224.

Ellwood-Yen, K., Graeber, T. G., Wongvipat, J., Iruela-Arispe, M. L., Zhang, J., Matusik, R., … Sawyers, C. L. (2003). Myc-driven murine prostate cancer shares molecular features with human prostate tumors. *Cancer Cell 4*, 223–238.

Engelman, J.A., Zejnullahu, K., Mitsudomi, T., Song, Y., Hyland, C., Oh Park, J., … A Jänne, P. (2007). MET Amplification Leads to Gefitinib Resistance in Lung Cancer by Activating ERBB3 Signaling. *Science 316*, 1039–1043.

Eyre, T. A., Collins, G. P., Goldstone, A. H., & Cwynarski, K. (2014). Time now to TORC the TORC? New developments in mTOR pathway inhibition in lymphoid malignancies. *British Journal of Haematology 166*, 336–351.

Faivre, S., Kroemer, G., & Raymond, E. (2006). Current development of mTOR inhibitors as anticancer agents. *Nature Reviews. Drug Discovery* 5, 671–688.

Fan, R.-F., Lu, Y., Fang, Z.-G., Guo, X.-Y., Chen, Y.-X., Xu, Y.-C., … Liu, X.-F. (2017). PIM-1 kinase inhibitor SMI-4a exerts antitumor effects in chronic myeloid leukemia cells by enhancing the activity of glycogen synthase kinase 3beta. *Molecular Medicine Reports 16*, 4603–4612.

Fox, C. J., Hammerman, P. S., Cinalli, R. M., Master, S. R., Chodosh, L. A., & Thompson, C. B. (2003). The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptotic inhibitor. *Genes & Development 17*, 1841–1854.

Fox, C. J., Hammerman, P. S., & Thompson, C. B. (2005). The Pim kinases control rapamycin-resistant T cell survival and activation. *The Journal of Experimental Medicine 201*, 259–266.

Friedmann, M., Nissen, M. S., Hoover, D. S., Reeves, R., & Magnuson, N. S. (1992). Characterization of the proto-oncogene pim-1: kinase activity and substrate recognition sequence. *Archives of Biochemistry and Biophysics 298*, 594–601.

Fujita, N., Sato, S., Katayama, K., & Tsuruo, T. (2002). Akt-dependent phosphorylation of p27Kip1 promotes binding to 14-3-3 and cytoplasmic localization. *The Journal of Biological Chemistry 277*, 28706–28713.

Garcia, P. D., Langowski, J. L., Wang, Y., Chen, M., Castillo, J., Fanton, C., … Burger, M. T.

(2014). Pan-PIM kinase inhibition provides a novel therapy for treating hematologic cancers. *Clinical Cancer Research 20*, 1834–1845.

Gately, K., Heavey, S., Cuffe, S., Finn, S., Byrne, K., O'Neill, M., & Moore, G. (2018). PO-505 Targeting PIM kinase to overcome resistance to PI3K-mTOR inhibition in NSCLC. *ESMO Open* 3.

Gentile, A., Trusolino, L., & Comoglio, P. M. (2008). The Met tyrosine kinase receptor in development and cancer. *Cancer Metastasis Reviews 27*, 85–94.

Grandori, C., Gomez-Roman, N., Felton-Edkins, Z. A., Ngouenet, C., Galloway, D. A., Eisenman, R. N., & White, R. J. (2005). c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nature Cell Biology 7*, 311–318.

Green, A. S., Maciel, T. T., Hospital, M. A., Yin, C., Mazed, F., Townsend, E. C., … Tamburini, J. (2015). Pim kinases modulate resistance to FLT3 tyrosine kinase inhibitors in FLT3-ITD acute myeloid leukemia. *Science Advances 1*, e1500221.

Gyori, D., Chessa, T., Hawkins, P. T., & Stephens, L. R. (2017). Class (I) Phosphoinositide 3-Kinases in the Tumor Microenvironment. *Cancers 9*, 24.

Ha, S., Iqbal, N.J., Mita, P., Ruoff, R., Gerald, W.L., Lepor, H., … Logan, S.K. (2013) Phosphorylation of the androgen receptor by PIM1 in hormone refractory prostate cancer. *Oncogene 32*, 3992-4000.

Haddach, M., Michaux, J., Schwaebe, M. K., Pierre, F., O'Brien, S. E., Borsan, C., … Ryckman, D. M. (2012). Discovery of CX-6258. A Potent, Selective, and Orally Efficacious pan-Pim Kinases Inhibitor. *ACS Medicinal Chemistry Letters 3*, 135–139.

Harada, M., Benito, J., Yamamoto, S., Kaur, S., Arslan, D., Ramirez, S., … Konopleva, M. (2015). The novel combination of dual mTOR inhibitor AZD2014 and pan-PIM inhibitor AZD1208 inhibits growth in acute myeloid leukemia via HSF pathway suppression. *Oncotarget 6*, 37930–37947.

Henry, R. E., Barry, E. R., Castriotta, L., Ladd, B., Markovets, A., Beran, G., … Schuller, A. G. (2016). Acquired savolitinib resistance in non-small cell lung cancer arises via multiple mechanisms that converge on MET-independent mTOR and MYC activation. *Oncotarget 7*, 57651–57670.

Hogan, C., Hutchison, C., Marcar, L., Milne, D., Saville, M., Goodlad, J., … Meek, D. (2008). Elevated levels of oncogenic protein kinase Pim-1 induce the p53 pathway in cultured cells and correlate with increased Mdm2 in mantle cell lymphoma. *The Journal of Biological Chemistry 283*, 18012–18023.

Holder, S.L., & Abdulkadir, S.A. (2014) PIM1 kinase as a target in prostate cancer: Roles in tumorigenesis, castration resistance, and docetaxel resistance. *Current Cancer Drug Targets 14*, 105–114.

Hoover, D. S., Wingett, D. G., Zhang, J., Reeves, R., & Magnuson, N. S. (1997). Pim-1 protein expression is regulated by its 5'-untranslated region and translation initiation factor elF-4E. *Cell Growth & Differentiation : The Molecular Biology Journal of the American Association for Cancer Research 8*, 1371–1380.

Horiuchi, D., Camarda, R., Zhou, A. Y., Yau, C., Momcilovic, O., Balakrishnan, S., … Goga, A. (2016). PIM1 kinase inhibition as a targeted therapy against triple-negative breast tumors with elevated MYC expression. *Nature Medicine 22*, 1321–1329.

Jacobs, M. D., Black, J., Futer, O., Swenson, L., Hare, B., Fleming, M., & Saxena, K. (2005). Pim-1 ligand-bound structures reveal the mechanism of serine/threonine kinase inhibition by LY294002. *The Journal of Biological Chemistry 280*, 13728–13734.

Jeyapal, G.P., Chandrasekar, M.J.N., Krishnasamy, R., Selvaraj, J., Mohammad, M., & Nanjan, M.J. (2018). Potential Pharmacological Inhibitors of Pim Kinase Under Clinical Trials. *Anticancer Agents Med Chem 18,* 1100-1114.

Jin, H. T., Ahmed, R., & Okazaki, T. (2010). Role of PD-1 in regulating T-Cell immunity. *Current Topics in Microbiology and Immunology 350*, 17–37.

Jinesh, G.G., Mokkapati, S., Zhu, K., & Morales, E.E. (2016). Pim kinase isoforms: devils defending cancer cells from therapeutic and immune attacks. *Apoptosis 21*, 1203–1213.

Karachaliou, N., Chaib, I., Cardona, A. F., Berenguer, J., Bracht, J. W. P., Yang, J., … Rosell, R. (2018). Common Co-activation of AXL and CDCP1 in EGFR-mutation-positive Non-small cell Lung Cancer Associated With Poor Prognosis. *EBioMedicine 29*, 112–127.

Karachaliou, N., Fernandez-Bruno, M., Bracht, J. W.P, & Rosell, R. (2018). EGFR first- and second-generation TKIs- there is still place for them in EGFR -mutant NSCLC patients. *Translational Cancer Research 8*, S23-S47.

Keeton, E., McEachern, K., S Dillman, K., Palakurthi, S., Cao, Y., R Grondine, M., … Huszar, D. (2013). AZD1208, a potent and selective pan-PIM kinase inhibitor, demonstrates efficacy in preclinical models of acute myeloid leukemia. *Blood 123,* 905-913.

Kim, K.-T., Baird, K., Ahn, J.-Y., Meltzer, P., Lilly, M., Levis, M., & Small, D. (2005). Pim-1 is up-regulated by constitutively activated FLT3 and plays a role in FLT3-mediated cell survival. *Blood 105*, 1759–1767.

Kim, O., Jiang, T., Xie, Y., Guo, Z., Chen, H., & Qiu, Y. (2004). Synergism of cytoplasmic kinases in IL6-induced ligand-independent activation of androgen receptor in prostate cancer cells. *Oncogene 23*, 1838–1844.

Kim, W., Youn, H., Seong, K. M., Yang, H. J., Yun, Y. J., Kwon, T., … Youn, B. (2011). PIM1-activated PRAS40 regulates radioresistance in non-small cell lung cancer cells through interplay with FOXO3a, 14-3-3 and protein phosphatases. *Radiation Research 176*, 539–552.

Kim, W., Youn, H., Kwon, T., Kang, J., Kim, E., Son, B., … Youn, B. (2013). PIM1 kinase inhibitors induce radiosensitization in non-small cell lung cancer cells. *Pharmacological Research 70*, 90–101.

Kirschner, A. N., Wang, J., van der Meer, R., Anderson, P. D., Franco-Coronel, O. E., Kushner, M. H., … Abdulkadir, S. A. (2015). PIM kinase inhibitor AZD1208 for treatment of MYC-driven prostate cancer. *Journal of the National Cancer Institute 107,* dju407.

Kitada, S., Andersen, J., Akar, S., Zapata, J. M., Takayama, S., Krajewski, S., … Reed, J. C. (1998). Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with In vitro and In vivo chemoresponses. *Blood 91*, 3379–3389.

Koblish, H., Li, Y., Shin, N., Hall, L., Wang, Q., Wang, K., … Scherle, P. (2018). Preclinical characterization of INCB053914, a novel pan-PIM kinase inhibitor, alone and in combination with anticancer agents, in models of hematologic malignancies. *PLOS ONE 13*, e0199108.

Lackner, M. R., Wilson, T. R., & Settleman, J. (2012). Mechanisms of acquired resistance to targeted cancer therapies. *Future Oncology 8*, 999–1014.

Langowski, J. L., Holash, J., Burger, M., Zang, R., Zavorotinskaya, T., Fanton, C., … Vanasse, K. G. (2013). The Pan-PIM Kinase Inhibitor LGH447 Shows Activity In PIM2-Dependent Multiple Myeloma and In AML Models. *Blood 122*, 1666.

Law, B. K. (2005). Rapamycin: an anti-cancer immunosuppressant? *Critical Reviews in Oncology/Hematology 56*, 47–60.

Lazzara, M. J., Lane, K., Chan, R., Jasper, P. J., Yaffe, M. B., Sorger, P. K., … Lauffenburger, D. A. (2010). Impaired SHP2-mediated extracellular signal-regulated kinase activation contributes to gefitinib sensitivity of lung cancer cells with epidermal growth factor receptor-activating mutations. *Cancer Research 70*, 3843–3850.

Leung, C. O., Wong, C. C., Fan, D. N., Kai, A. K., Tung, E. K., Xu, I. M., … Lo, R. C. (2015). PIM1 regulates glycolysis and promotes tumor progression in hepatocellular carcinoma. *Oncotarget 6*, 10880–10892.

Lilly, M.,& Kraft, A. (1997). Enforced expression of the Mr 33,000 Pim-1 kinase enhances factor-independent survival and inhibits apoptosis in murine myeloid cells. *Cancer Research 57*, 5348-55.

Lin, Y.-W., Beharry, Z. M., Hill, E. G., Song, J. H., Wang, W., Xia, Z., … Kraft, A. S. (2010). A small molecule inhibitor of Pim protein kinases blocks the growth of precursor T-cell lymphoblastic leukemia/lymphoma. *Blood 115*, 824–833.

Losman, J. A., Chen, X. P., Vuong, B. Q., Fay, S., & Rothman, P. B. (2003). Protein phosphatase 2A regulates the stability of Pim protein kinases. *The Journal of Biological Chemistry 278*, 4800–4805.

Lu, J., Zavorotinskaya, T., Dai, Y., Niu, X.-H., Castillo, J., Sim, J., … Garcia, P. D. (2013). Pim2 is

required for maintaining multiple myeloma cell growth through modulating TSC2 phosphorylation. *Blood 122*, 1610–1620.

Mabuchi, S., Kuroda, H., Takahashi, R., & Sasano, T. (2015). The PI3K/AKT/mTOR pathway as a therapeutic target in ovarian cancer. *Gynecologic Oncology 137*, 173–179.

Mahadevan, D., & Shirahatti, N. (2005). Strategies for targeting the multidrug resistance-1 (MDR1)/P-gp transporter in human malignancies. *Current Cancer Drug Targets 5*, 445–455.

Mamane, Y., Petroulakis, E., LeBacquer, O., & Sonenberg, N. (2006). mTOR, translation initiation and cancer. *Oncogene 25*, 6416–6422.

Meja, K., Smith, D., Percy, L., Huszar, D., Davies, P. R., Yong, K. L., & Khwaja, A. (2014). Synergistic Induction of Cell Death By Combined Inhibition of PIM and AKT Kinases in Cytogenetically Defined Standard and High-Risk Multiple Myeloma. *Blood 124*, 4723.

Mikkers, H., Allen, J., Knipscheer, P., Romeijn, L., Hart, A., Vink, E., & Berns, A. (2002). High-throughput retroviral tagging to identify components of specific signaling pathways in cancer. *Nature Genetics 32*, 153–159.

Mikkers, H., Nawijn, M., Allen, J., Brouwers, C., Verhoeven, E., Jonkers, J., & Berns, A. (2004). Mice deficient for all PIM kinases display reduced body size and impaired responses to hematopoietic growth factors. *Molecular and Cellular Biology 24*, 6104–6115.

Mizuki, M., Schwable, J., Steur, C., Choudhary, C., Agrawal, S., Sargin, B., … Serve, H. (2003). Suppression of myeloid transcription factors and induction of STAT response genes by AML-specific Flt3 mutations. *Blood 101*, 3164–3173.

Mochizuki, T., Kitanaka, C., Noguchi, K., Muramatsu, T., Asai, A., & Kuchino, Y. (1999).

Physical and functional interactions between Pim-1 kinase and Cdc25A phosphatase. Implications for the Pim-1-mediated activation of the c-Myc signaling pathway. *The Journal of Biological Chemistry 274*, 18659–18666.

Mohlin S., Hansson K., Radke K., Martinez S., Blanco-Apiricio C., Garcia-Ruiz C., … Bexell D. (2019).

Anti-tumor effects of PIM/PI3K/mTOR triple kinase inhibitor IBL-302 in neuroblastoma. *EMBO Molecular Medicine* 11, e10058.

Mondello, P., Cuzzocrea, S., & Mian, M. (2014). Pim kinases in hematological malignancies: where are we now and where are we going? *Journal of Hematology & Oncology*, *7*, 95.

Moody, S. E., Schinzel, A. C., Singh, S., Izzo, F., Strickland, M. R., Luo, L., … Hahn, W. C. (2015). PRKACA mediates resistance to HER2-targeted therapy in breast cancer cells and restores anti-apoptotic signaling. *Oncogene 34*, 2061–2071.

Morishita, D., Katayama, R., Sekimizu, K., Tsuruo, T., & Fujita, N. (2008). Pim kinases promote cell cycle progression by phosphorylating and down-regulating p27Kip1 at the transcriptional and posttranscriptional levels. *Cancer Research 68*, 5076–5085.

Musiani, D., Hammond, D. E., Cirillo, L., Erriquez, J., Olivero, M., Clague, M. J., & Di Renzo, M. F. (2014). PIM2 kinase is induced by cisplatin in ovarian cancer cells and limits drug efficacy. *Journal of Proteome Research 13*, 4970–4982.

Muz, B., de la Puente, P., Azab, F., & Azab, A. K. (2015). The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia 3*, 83–92.

Miyakawa K, Matsunaga S, Yokoyama M, Nomaguchi M, Kimura Y, Nishi M, Kimura H,

Sato H, Hirano H, Tamura T, Akari H, Miura T, Adachi A, Sawasaki T, Yamamoto N, Ryo A. PIM kinases facilitate lentiviral evasion from SAMHD1 restriction via Vpx phosphorylation. Nat Commun. 2019 *10*, 1844.

Narlik-Grassow, M., Blanco-Aparicio, C., & Carnero, A. (2014). The PIM family of serine/threonine kinases in cancer. *Medicinal Research Reviews 34*, 136–159.

Natarajan, K., Xie, Y., Burcu, M., Linn, D. E., Qiu, Y., & Baer, M. R. (2013). Pim-1 kinase phosphorylates and stabilizes 130 kDa FLT3 and promotes aberrant STAT5 signaling in acute myeloid leukemia with FLT3 internal tandem duplication. *PloS One 8*, e74653.

Nawijn, M. C., Alendar, A., & Berns, A. (2011). For better or for worse: the role of Pim oncogenes in tumorigenesis. *Nature Reviews. Cancer 11*, 23–34.

Nieborowska-Skorska, M., Hoser, G., Kossev, P., Wasik, M. A., & Skorski, T. (2002). Complementary functions of the antiapoptotic protein A1 and serine/threonine kinase pim-1 in the BCR/ABL-mediated leukemogenesis. *Blood 99*, 4531–4539.

Obata, T., Yaffe, M. B., Leparc, G. G., Piro, E. T., Maegawa, H., Kashiwagi, A., … Cantley, L. C. (2000). Peptide and protein library screening defines optimal substrate motifs for AKT/PKB. *The Journal of Biological Chemistry 275*, 36108–36115.

Okada, K., Nogami, A., Ishida, S., Akiyama, H., Chen, C., Umezawa, Y., & Miura, O. (2018). FLT3-ITD induces expression of Pim kinases through STAT5 to confer resistance to the PI3K/Akt pathway inhibitors on leukemic cells by enhancing the mTORC1/Mcl-1 pathway. *Oncotarget 9*, 8870–8886.

Paino, T., Garcia-Gomez, A., Gonzalez-Mendez, L., San-Segundo, L., Hernandez-Garcia, S., Lopez-Iglesias, A.-A., … Ocio, E. M. (2017). The Novel Pan-PIM Kinase Inhibitor, PIM447,

Displays Dual Antimyeloma and Bone-Protective Effects, and Potently Synergizes with Current Standards of Care. *Clinical Cancer Research 23*, 225–238.

Peltola, K. J., Paukku, K., Aho, T. L. T., Ruuska, M., Silvennoinen, O., & Koskinen, P. J. (2004). Pim-1 kinase inhibits STAT5-dependent transcription via its interactions with SOCS1 and SOCS3. *Blood 103*, 3744–3750.

Peltola, K., Hollmen, M., Maula, S.-M., Rainio, E., Ristamaki, R., Luukkaa, M., … Jalkanen, S. (2009). Pim-1 kinase expression predicts radiation response in squamocellular carcinoma of head and neck and is under the control of epidermal growth factor receptor. *Neoplasia 11*, 629–636.

Peng, C., Knebel, A., Morrice, N. A., Li, X., Barringer, K., Li, J., … Wang, L. (2007). Pim kinase substrate identification and specificity. *Journal of Biochemistry 141*, 353–362.

Petros, A. M., Olejniczak, E. T., & Fesik, S. W. (2004). Structural biology of the Bcl-2 family of proteins. *Biochimica et Biophysica Acta 1644*, 83–94.

Pogacic, V., Bullock, A. N., Fedorov, O., Filippakopoulos, P., Gasser, C., Biondi, A., … Schwaller, J. (2007). Structural analysis identifies imidazo[1,2-b]pyridazines as PIM kinase inhibitors with in vitro antileukemic activity. *Cancer Research 67*, 6916–6924.

Porta, C., Paglino, C., & Mosca, A. (2014). Targeting PI3K/Akt/mTOR Signaling in Cancer. *Frontiers in Oncology 4*, 64.

Qian, K. C., Studts, J., Wang, L., Barringer, K., Kronkaitis, A., Peng, C., … Farmer, B. (2005). Expression, purification, crystallization and preliminary crystallographic analysis of human Pim-1 kinase. *Acta Crystallographica 61*, 96–99.

Qin, X., Jiang, B., & Zhang, Y. (2016). 4E-BP1, a multifactor regulated multifunctional protein.

Cell Cycle 15, 781–786.

Rebello, R. J., Kusnadi, E., Cameron, D. P., Pearson, H. B., Lesmana, A., Devlin, J. R., … Furic, L. (2016). The Dual Inhibition of RNA Pol I Transcription and PIM Kinase as a New Therapeutic Approach to Treat Advanced Prostate Cancer. *Clinical Cancer Research 22*, 5539–5552.

Rebello, R.J., Huglo, A.V., & Furic, L. (2018). PIM activity in tumours: A key node of therapy resistance. *Adv Biol Regul 67*, 163-169.

Roskoski, R. J. (2016). Classification of small molecule protein kinase inhibitors based upon the structures of their drug-enzyme complexes. *Pharmacological Research 103*, 26–48.

Samali, A., Cai, J., Zhivotovsky, B., Jones, D. P., & Orrenius, S. (1999). Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. *The EMBO Journal 18*, 2040–2048.

Santagata, S., Mendillo, M. L., Tang, Y., Subramanian, A., Perley, C. C., Roche, S. P., … Lindquist, S. (2013). Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state. *Science 341*6143, 1238303.

Santio, N. M., Landor, S. K.-J., Vahtera, L., Yla-Pelto, J., Paloniemi, E., Imanishi, S. Y., … Koskinen, P. J. (2016). Phosphorylation of Notch1 by Pim kinases promotes oncogenic signaling in breast and prostate cancer cells. *Oncotarget 7*, 43220–43238.

Santio, N.M., Koskinen, P.J. (2017) PIM kinases: From survival factors to regulators of cell motility. *Int J Biochem Cell Biol 93*, 74-85.

Santoro, A., Rimassa, L., Borbath, I., Daniele, B., Salvagni, S., Van Laethem, J. L., … Porta, C. (2013). Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a

randomised, placebo-controlled phase 2 study. *The Lancet. Oncology 14*, 55–63.

Saris, C. J., Domen, J., & Berns, A. (1991). The pim-1 oncogene encodes two related protein-serine/threonine kinases by alternative initiation at AUG and CUG. *The EMBO Journal 10*, 655–664.

Shay, K. P., Wang, Z., Xing, P.-X., McKenzie, I. F. C., & Magnuson, N. S. (2005). Pim-1 kinase stability is regulated by heat shock proteins and the ubiquitin-proteasome pathway. *Molecular Cancer Research 3*, 170–181.

Sheng, S. Y., Gu, Y., Lu, C. G., Zou, J. Y., Hong, H., & Wang, R. (2017). The distribution and function of human memory T cell subsets in lung cancer. *Immunologic Research 65*, 639–650.

Siegel, R., Ma, J., Zou, Z., & Jemal, A. (2014). Cancer statistics, 2014. *CA: A Cancer Journal for Clinicians 64*, 9–29.

Sigismund, S., Avanzato, D., & Lanzetti, L. (2018). Emerging functions of the EGFR in cancer. *Molecular Oncology 12*, 3–20.

Song, J.H., & Kraft, A. S. (2012). Pim kinase inhibitors sensitize prostate cancer cells to apoptosis triggered by Bcl-2 family inhibitor ABT-737. *Cancer Research 72*, 294–303.

Song, J.H., An, N., Chatterjee, S., Kistner-Griffin, E., Mahajan, S., Mehrotra, S., & Kraft, A. S. (2015). Deletion of Pim kinases elevates the cellular levels of reactive oxygen species and sensitizes to K-Ras-induced cell killing. *Oncogene 34*, 3728–3736.

Song, J.H., Padi, S. K. R., Luevano, L. A., Minden, M. D., DeAngelo, D. J., Hardiman, G., … Kraft, A. S. (2016). Insulin receptor substrate 1 is a substrate of the Pim protein kinases. *Oncotarget 7*, 20152–20165.

Song, J.H., Singh, N., Luevano, L.A., Padi, SKR., Okumura, K., Olive, V.,…Kraft AS. (2018) Mechanisms Behind Resistance to PI3K Inhibitor Treatment Induced by the PIM Kinase. *Mol Cancer Ther* 17, 2710-2721.

Spaans, J.N., & Goss, G.D. (2014). Drug Resistance to Molecular Targeted Therapy and Its Consequences for Treatment Decisions in Non-Small-Cell Lung Cancer. In *Frontiers in oncology 4,* 190.

Sugiura, A., & Rathmell, J. C. (2018). Metabolic Barriers to T Cell Function in Tumors. *Journal of Immunology 200*, 400–407.

Szydłowski, M., Prochorec-Sobieszek, M., Szumera-Ciećkiewicz, A., Derezińska, E., Hoser, G., Wasilewska, D.,… Juszczyński, P. (2017) Expression of PIM kinases in Reed-Sternberg cells fosters immune privilege and tumor cell survival in Hodgkin lymphoma. *Blood 130*, 1418-1429.

Tao, L., Huang, G., Song, H., Chen, Y., & Chen, L. (2017). Cancer associated fibroblasts: An essential role in the tumor microenvironment. *Oncology Letters 14*, 2611–2620.

Tao, Z.-F., Hasvold, L. A., Leverson, J. D., Han, E. K., Guan, R., Johnson, E. F., … Penning, T. D. (2009). Discovery of 2H-benzo[4,5]thieno[3,2-d]pyrimidin-4-ones as potent, highly selective, and orally bioavailable inhibitors of the human protooncogene proviral insertion site in moloney murine leukemia virus (PIM) kinases. *Journal of Medicinal Chemistry 52*, 6621–6636.

Thiede, C., Steudel, C., Mohr, B., Schaich, M., Schakel, U., Platzbecker, U., … Illmer, T. (2002). Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood 99*, 4326–4335.

Turaka, A., Buyyounouski, M. K., Hanlon, A. L., Horwitz, E. M., Greenberg, R. E., & Movsas, B. (2012). Hypoxic prostate/muscle PO2 ratio predicts for outcome in patients with localized prostate cancer: long-term results. *International Journal of Radiation Oncology, Biology, Physics 82*, e433-9.

Tursynbay, Y., Zhang, J., Li, Z., Tokay, T., Zhumadilov, Z., Wu, D., & Xie, Y. (2016). Pim-1 kinase as cancer drug target: An update. *Biomed Rep 4*, 140-146.

van der Windt, G. J. W., & Pearce, E. L. (2012). Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunological Reviews 249*, 27–42.

van Lohuizen, M., Verbeek, S., Krimpenfort, P., Domen, J., Saris, C., Radaszkiewicz, T., & Berns, A. (1989). Predisposition to lymphomagenesis in pim-1 transgenic mice: cooperation with c-myc and N-myc in murine leukemia virus-induced tumors. *Cell56*, 673–682.

Viel, S., Besson, L., Marotel, M., Walzer, T., & Marcais, A. (2017). Regulation of mTOR, Metabolic Fitness, and Effector Functions by Cytokines in Natural Killer Cells. *Cancers 9,* 132.

Wang, Z., Bhattacharya, N., Weaver, M., Petersen, K., Meyer, M., Gapter, L., Magnuson, N.S.(2001). Pim-1: a serine/threonine kinase with a role in cell survival, proliferation, differentiation and tumorigenesis. *J Vet Sci 3*, 167-79.

Wang, Z., Weaver, M., & Magnuson, N. S. (2005). Cryptic promoter activity in the DNA sequence corresponding to the pim-1 5'-UTR. *Nucleic Acids Research 33*, 2248–2258.

Wang, J., Li, G., Li, B., Song. H., Shang, Z., Jiang, N., Niu, Y. (2017) Androgen deprivation therapy has no effect on Pim-1 expression in a mouse model of prostate cancer. *Oncology Letters 13*, 4364-4370.

Warfel, N A, & El-Deiry, W. S. (2014). HIF-1 signaling in drug resistance to chemotherapy. *Current Medicinal Chemistry 21*, 3021–3028.

Warfel, N. A., & Kraft, A. S. (2015). PIM kinase (and Akt) biology and signaling in tumors. *Pharmacology & Therapeutics 151*, 41–49.

Xia, Z., Knaak, C., Ma, J., Beharry, Z. M., McInnes, C., Wang, W., … Smith, C. D. (2009). Synthesis and evaluation of novel inhibitors of Pim-1 and Pim-2 protein kinases. *Journal of Medicinal Chemistry 52*, 74–86.

Xie, X., Gu, Y., Fox, T., Coll, J. T., Fleming, M. A., Markland, W., … Su, M. S. (1998). Crystal structure of JNK3: a kinase implicated in neuronal apoptosis. *Structure 6*, 983–991.

Xie, Yingqiu, Xu, K., Linn, D. E., Yang, X., Guo, Z., Shimelis, H., … Qiu, Y. (2008). The 44-kDa Pim-1 kinase phosphorylates BCRP/ABCG2 and thereby promotes its multimerization and drug-resistant activity in human prostate cancer cells. *The Journal of Biological Chemistry 283*, 3340–3356.

Xie, Yingqiu, Burcu, M., Linn, D. E., Qiu, Y., & Baer, M. R. (2010). Pim-1 kinase protects P-glycoprotein from degradation and enables its glycosylation and cell surface expression. *Molecular Pharmacology 78*, 310–318.

Yan, B., Zemskova, M., Holder, S., Chin, V., Kraft, A., Koskinen, P. J., & Lilly, M. (2003). The PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. *The Journal of Biological Chemistry 278*, 45358–45367.

Yao, Y., Jones, E., & Inoki, K. (2017). Lysosomal Regulation of mTORC1 by Amino Acids in Mammalian Cells. *Biomolecules 7*, 51.

Yeh, E., Cunningham, M., Arnold, H., Chasse, D., Monteith, T., Ivaldi, G., … Sears, R. (2004). A signalling pathway controlling c-Myc degradation that impacts oncogenic transformation of human cells. *Nature Cell Biology 6*, 308–318.

Yu, Z., Zhao, X., Ge, Y., Zhang, T., Huang, L., Zhou, X., … Huang, G. (2014). A regulatory feedback loop between HIF-1alpha and PIM2 in HepG2 cells. *PloS One 9*, e88301.

Zemskova, M. Y., Song, J. H., Cen, B., Cerda-Infante, J., Montecinos, V. P., & Kraft, A. S. (2015). Regulation of prostate stromal fibroblasts by the PIM1 protein kinase. *Cellular Signalling 27*, 135–146.

Zhang, D., Piao, H.-L., Li, Y.-H., Qiu, Q., Li, D.-L., Du, M.-R., & Tsang, B. K. (2016). Inhibition of AKT sensitizes chemoresistant ovarian cancer cells to cisplatin by abrogating S and G2/M arrest. *Experimental and Molecular Pathology 100*, 506–513.

Zhang, X., Song, M., Kundu, J.K., Lee, M.H.,& Liu ZZ. (2018). PIM Kinase as an Executional Target in Cancer. *J Cancer Prev 23,* 109-116.

Zhang, Y., Wang, Z., & Magnuson, N. S. (2007). Pim-1 kinase-dependent phosphorylation of p21Cip1/WAF1 regulates its stability and cellular localization in H1299 cells. *Molecular Cancer Research 5*, 909–922.

Zhang, Y., Wang, Z., Li, X., & Magnuson, N. S. (2008). Pim kinase-dependent inhibition of c-Myc degradation. *Oncogene 27*, 4809–4819.

Zhou, B. P., Liao, Y., Xia, W., Spohn, B., Lee, M. H., & Hung, M. C. (2001a). Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-

overexpressing cells. *Nature Cell Biology 3*, 245–252.

Zhou, B. P., Liao, Y., Xia, W., Zou, Y., Spohn, B., & Hung, M. C. (2001b). HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nature Cell Biology 3*, 973–982.

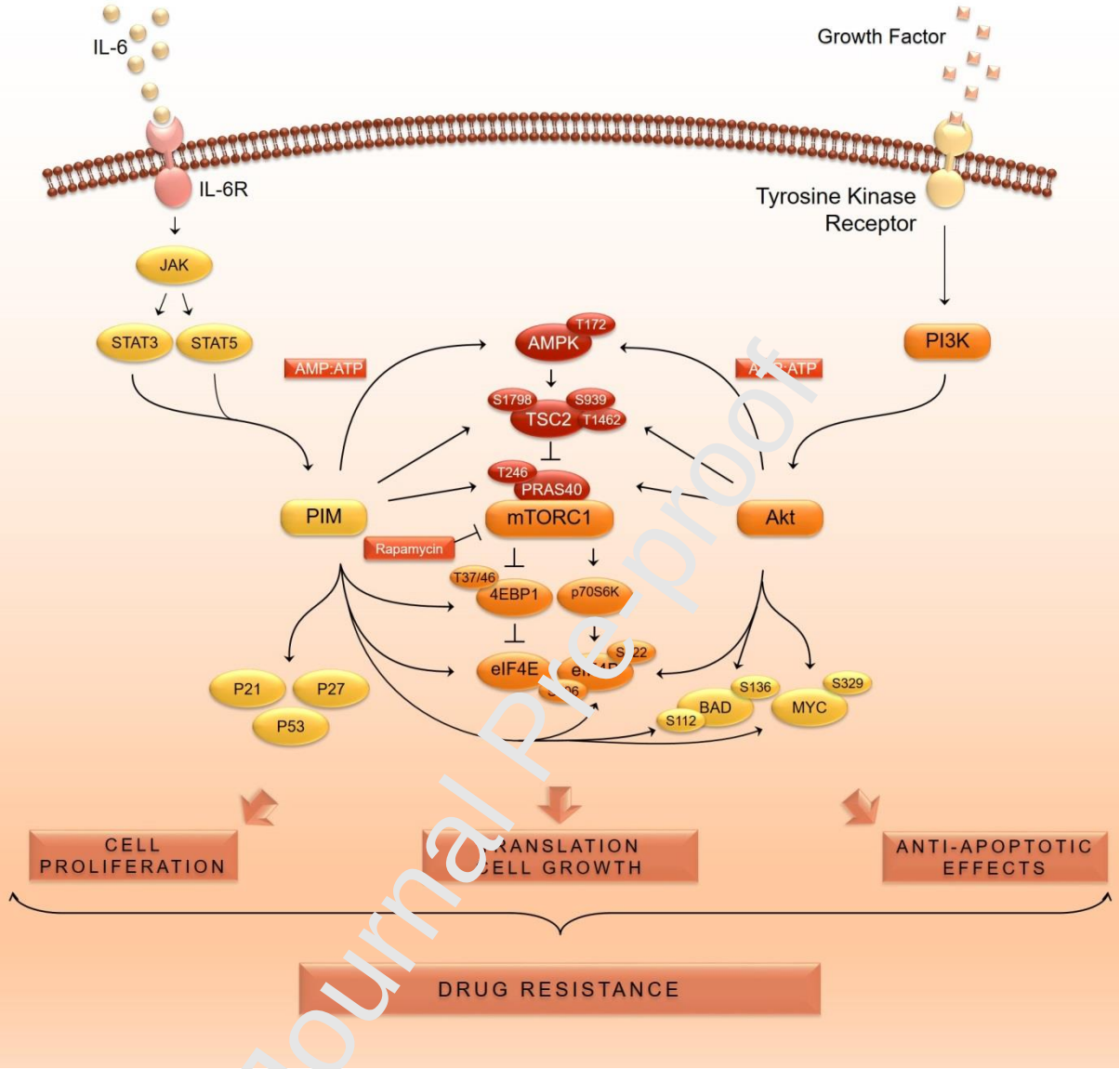

Figure 1 PIM kinase and PI3K/Akt signalling pathways with overlapping substrates.

PIM kinase and PI3K/Akt share substrates that are both upstream and downstream effectors of mTORC1 resulting in control of its signalling axis and regulation of translation and cell growth.

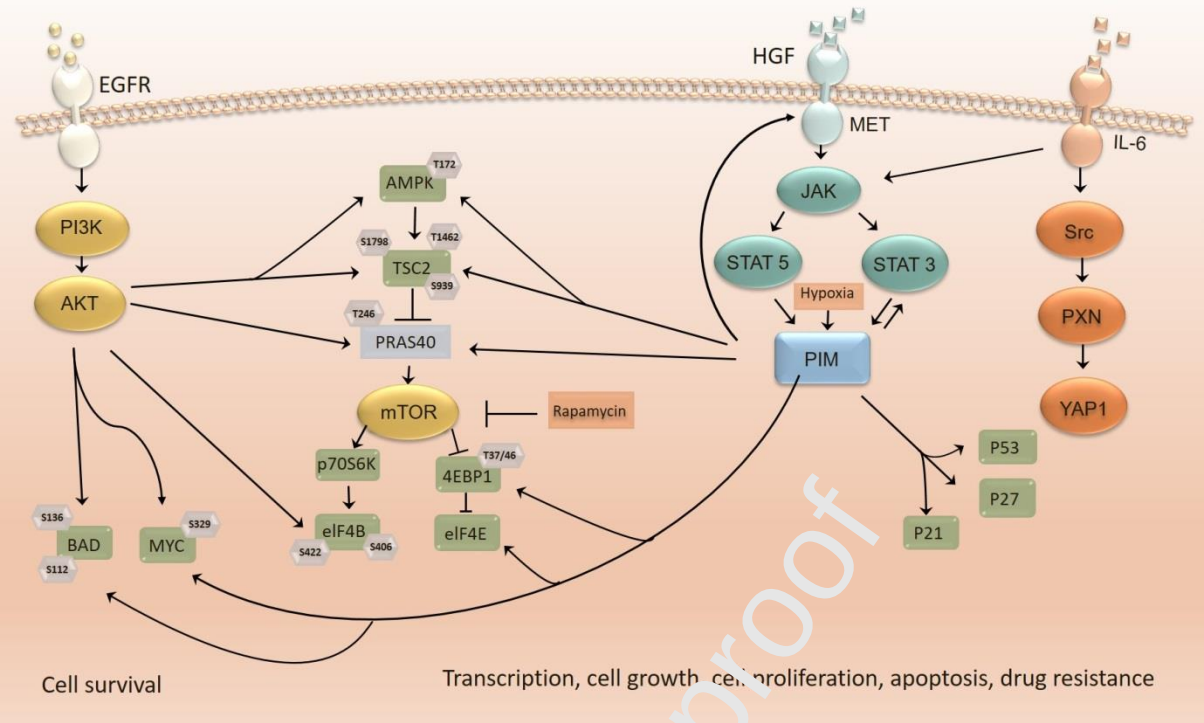

Figure 2 Pathway crosstalk driving resistance mechanisms to EGFR TKIs

The cross-talk between EGFR, HGF and IL-6 signalling pathways results in the emergence of acquired drug resistance to EGFR TKIs. PIM kinases share a common node within these signalling pathways and their activation has emerged as a significant mechanism of resistance to several pathway inhibitors