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PIM-1 KINASE: A NOVEL TARGET FOR CANCER CHEMOTHERAPY- A REVIEW

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ABSTRACT: Cancer is the major cause of mortality in most of the developing countries. Recent times have seen an exceptional advancement in cancer biology Research. Researchers undertook the development of enormous chemotherapeutic agents. Pim family of serine/threonine kinases regulated by calcium/calmodulin have been identified as unique molecular targets in oncogenesis. It constitutes three members: Pim-1, Pim-2, and Pim-3 discovered by cloning the proviral integration site in Moloney murine leukemia virus (M-MuLV). Pim-1 was found to bear two isoforms Pim-1S and Pim-1L. It plays a dominant role in various processes of cell regulation like replicative senescence, drug resistance, apoptosis, epidynamics regulator, diagnostic tool, prostate cancer biomarker, and an immunotherapy agent. An insight into the structure of Pim kinases by crystal studies laid down a molecular basis for the development of selective and synergistic anti-cancer therapies. These proto-oncogenes are overexpressed in B lymphoid, myeloid cell lines, hematopoietic malignancies, and prostate cancer. This review emphasizes the importance and role of Pim-1 kinase in various molecular signaling pathways involved in tumorigenesis and the potential Pim-1 inhibitors reported so far.

INTRODUCTION: Proviral Integration site of Moloney murine leukemia virus abbreviated as PIM kinases are a family of serine/threonine kinases calcium/calmodulin-dependent [1]. The Pim family primarily consists of three genes; pim-1,pim-2 and pim3 which show high homology amongst the constituents. Pim family of genes was indigenously identified as proto-oncogenes in transgenic mouse models. Their discovery dates back to the 1980's by cloning of retroviral integration site in Moloney murine leukemia virus (M-MuLV) generated lymphomas [2].

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These proto-oncogenes are highly expressed in B-lymphoid, Myeloid cell lines, hematopoietic malignancies, and prostate cancer [3]. These proto-oncogenes play a crucial role in multiple cellular functions such as cell cycle, cell survival epigenetic dynamics regulation, cellular, replicative senescence, apoptosis and as an immunotherapy to name a few [4].

Transgenic mice with pim 1 proto-oncogene showed more susceptibility to T-cell lymphoma when compared to non-transgenic mice. These pim-1 transgenic mice developed T-cell lymphoma much faster when infected with M-MuLV. The oncogenic activity of deregulated pim-1 in lymphomagenesis was strongly supported through the cooperation among pim-1, c-myc and *n-myc* which showed high expression in T-cell lymphoma. *Myc* family of proteins is a group of basic helix-loop helix -leucine zipper transcription factors that

are predominately expressed in cancer up-regulation and overexpression of *myc* is observed in a wide range of human malignancies. In various lymphomagenesis either *c-myc* or *n-myc* activation bolstered strong cooperation between pim-1 and *myc* in the tumorigenesis [5, 6].

1. PIM Kinases: Types: Pim kinases are unique and divergent from other kinases with greater than 60% parity amongst each member. For a better understanding of the role of each member of Pim family in tumorigenesis, the equality between the three constituents, in terms of amino acid sequence, peptide length and domain identification have been outlined [7].

Percentage of amino acid homology between various members of the pim family: Pim-1 and Pim-3: 66%, Pim-2 and Pim-3: 68%, Pim-1, and Pim-2: 55% as shown in **Fig. 1**.

Amino acid composition of each of the Pim members: Pim-1 406 AA, Pim-2 326 AA, Pim-3 370 AA.

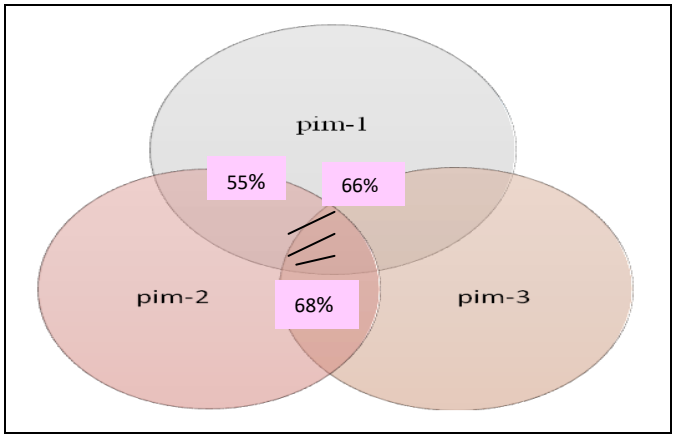

FIG. 1: PERCENTAGE OF AMINO ACID PARITY

Each Pim gene encompasses 6 axons.

The regions of Pim mRNA: 5 untranslated regions (UTR) comprised of 7-methyl guanine cap followed by a GC (Guanyl cyclase) rich region conferring them a weak transcription property which relies on cap-dependent translation [8]. The 3 UTR houses an AUUUA motif which according to the triple knockout studies in *myc* shortens the Pim mRNA half-life [9, 10].

2. PIM-1 Kinase: It was discovered that the Pim-1 kinase gene encodes for two isoforms: Pim-1L (44kDa) and Pim-1S (33kDa). The major discriminations between the two iso-forms were in terms of their length: Pim-1L being longer in comparison to Pim-1S. In terms of their location, the 44kDa isoform is primarily located on the plasma membrane and the 33kDa isoform resides both in the cytosol and the nucleus. This indicates an additional difference in the regulation of the respective substrates in the prostate carcinoma cells. In recent times, Pim-1 came forth as a potential diagnostic tool in the carcinoma of the prostate. An increased upregulation of Pim-1 was seen in human prostate cancer as well as in the malignant tissues of various mouse models. It can be inferred from an experiment undertaken in 2004, the regulation of Pim-1 kinase by IL-6 in prostate cancer cells and played a crucial role in the activation of the androgen receptor by IL-6 independent of ligand activation. This experiment also demonstrated the synergistic association of the 33kDa Pim-1 and the tec family tyrosine kinase Etk to induce the transcriptional activity of the androgenic receptor in the prostate cancer cells. This was further corroborated when the treatment with chemotherapeutic drugs caused the downregulation and inactivation of the Etk tyrosine kinase activity by the binding between the proline-rich domain of p53 (tumor suppressor gene) and the SH_3 domain of the Etk. The increased length of the Pim-1L was due to the addition of a proline-rich motif: PXXP motif. There is competitive inhibition of the interaction between the tumor suppressor p53 gene and the SH_3 domain of Etk by the interaction between the proline-rich motif and the Etk gene. The results also suggested an increased prominence of Pim-1L in the antiapoptotic property of anti-cancer agents [11].

2.1 Crystal Structure of Pim-1 Kinase: The crystal structure of Pim-1 has been experimentally determined by various researchers [12, 13]. As per the data**,** Pim kinases exhibit an aberrant behavior by showing less than 30% parity with the other kinases. According to the crystal structure experiments, the Pim-1 kinase assumes a conventional kinase fold arrangement with two lobes and a cleft intervening between the C-terminus and N-terminus. The N-terminus lobe is comprised of two beta sheets (βH_1 and βH_2) anti-parallel to each other. The C-terminus lobe constitutes alpha sheets parallel to each other. These two domains are joined together by a hinge

region (contained in the residues 121-126). The further detailed analysis supports the constitutive activation of the kinase without the requirement of A loop phosphorylation. Pim-1 kinase has a novel proline residue at the 123 position which confers the ATP binding pocket a distinctive form. In kinases this position is traditionally localized by a residue that donates a hydrogen atom to the adenine of the ATP, making the glutamine residue in position 124 a donor of a hydrogen bond to the adenine moiety of ATP. Moreover, there are two hypotheses as to how AMP-PNP (Adenylyl Imidophospate, a non-hydrolyzable analog of ATP) binds to the Pim-1. An absence of the following two features was observed. There is a common hydrogen bond association with the hinge portion and formation of a salt bridge between the lysine moiety at 169-position and the phosphate group AMP-PNP. The evidence that suggests the constitutive activity of Pim kinases is the resemblance in the conformation of the catalytic region of non-phosphorylated Pim-1 with that of the phosphorylated Pim-1 [14, 15].

2.2 Pim-1 - Cell Cycle Regulator: Pim-1phosphorylates many cellular substrates and thus regulates many cellular processes like cell cycle progression, cellular division, differentiation, and apoptosis. Pim-1 kinase accelerates the process of the cell cycle by modifying and interfering with numerous processes of cell cycle regulation. Amongst these regulators, interaction with p21 a G1 specific inhibitor, C-TAK1, Cdc25A, and Cdc25B play an important role. Phosphorylation of Cdc25A and Cdc25B enables them to translate into S and M phases from G1 and G2 phases respectively. Here Pim-1 positively regulates the cell cycle. Cdc25A being a direct transcriptional substrate for c-Myc plays a prominent role in apoptosis and cell cycle progression [16, 17]. Nuclear mitotic apparatus (NumA) that resides in the spindle fibers has two peptide sites that preferentially get phosphorylated by Pim-1 kinase. A recent study showed an interaction between Pim-1 and NumA peptide sequences in HcLA cells induced with nocodazole. Mechanistically, this interaction in cells with abnormal mitotic poles enables them to bypass the checkpoints thus conferring the daughter cells with polyploidy and multinucleation [18]. Due to the absence of regulatory domains in the amino acid sequences of the Pim oncoproteins, their kinase property is constitutively active. Thus, the conventional regulation mechanisms of other kinases like phosphorylation or binding to the plasma membrane are absent and their activity is regulated primarily by the following processes: transcription, translation and proteo-somal degradation. The genetic expression of Pim 1 is rapidly upregulated by exposure to various cytokines, mitogens, and hormones such as G-CSF, GN-CSF, erythropoietin, interleukins (IL-2, IL-3, and IL-6), Con A, PMA, Interferons and prolactin [19]. Pim-1 kinases are primarily regulated by the JKAT/STAT pathway [20].

2.3 Pim-1 Kinase - In Metastasis: Pim-1 is overexpressed in a variety of cancers and plays a prominent role in various cellular functions. The role of Pim-1 in secondary tumor progression and its metastasis into organs is still being experimented and studied. CXCR4 also termed as "fusin" is traditionally found to aid the entry of HIV into genital dendritic cells. In recent findings, it plays a prominent role in the metastasis of various malignancies like leukemia, multiple myeloma, breast cancer, prostate cancer, ovarian cancer, lung cancer, gastrointestinal cancer, retinal cell carcinoma, melanoma, brain tumors, and soft tissue sarcomas. The only known specific ligands for CXCR4 are CXCL12 (Chemokine stromal cell-derived factor 1) and chemokine receptor CXC Receptor-7 [21, 22]. Hematopoietic cell types, stem cells in blood and bone marrow, embryonic stem cells are a few cell lines on which CXCR4 is expressed. Prostate carcinoma is fatal in advanced stages by forming metastases into draining lymph nodes and lungs.

These stages of orthotopic prostate cancer show overexpression of Pim-1 kinase. In a recent study, Xenografts of mice when regularly treated with DHPCC-9 (An inhibitor selective and specific to Pim-1) showed a drastic reduction in the size and invasive potential of Pim-1. Mechanistically, Pim-1 in these xenografts promotes secondary tumor-progression by promoting angiogenesis and migration to those distant areas localizing secondary tumors. Additionally, phosphorylation of CXCR4 at serine residue in 339 positions enabled the invasion of tumor cells towards nearby draining lymph nodes and lungs where the ligand-specific to CXCR4: CXCL-12 is overexpressed [23]. Pim-1 also

regulates GSK-3B, which is a potential target for therapeutic intervention in cancer [24]. It plays a significant role in the invasion of malignancy to secondary tumor locations. Epithelial cells and fibroblasts of the prostate showed overexpression of Pim-1 oncogene. It regulated the cross signaling between epithelial and stromal cells. Prostate fibroblasts subjected to increased expression of Pim-1 kinase are prone to the secretion of collagen 1A1, an extracellular matrix molecule, pro-inflammatory chemokine CCL5, and platelet-derived growth factor receptors (PDGFr). When various assays utilizing the co-cultivation technique were conducted, there was an enhanced functional ability of Pim-1 overexpressing fibroblasts to transform into myofibroblasts. These differentiated myofibroblasts also expressed biochemical markers of cancer known as Cancer-associated fibroblasts (CAP's). Thus this study revealed Pim-1 kinase as a potential regulator of prostate fibroblast differentiation and maturation [25]. This provides cancer researchers worldwide an opportunity to synthesize novel Anticancer agents specific to this molecular signaling pathway.

2.4 Pim-1 Kinase - Immunotherapy Agent: Men of the Native American origin are at a higher risk of prostate cancer. Pim-1 kinase is emerging to be a unique target for various chemotherapeutic agents. The transcription of Pim-1 being regulated by various interleukins, mainly IL-6 gives physicians and healthcare professionals an opportunity to use it as a target in various immunotherapy-based medications. A neutralized IL-6 antibody, when directed against Pim-1 kinases in prostate cancer cells leads to a decrease in their expression. There are various preclinical studies being undertaken against Pim-1 kinases using monoclonal antibodies specific to them. A study was conducted on mice having severe combined immunodeficiency disorder (SCID) [26]. These animals, when fed subcutaneously with human prostate cancer cell lines (DU145) and subsequent treatment with mAbpa (an antibody specific to Pim-1) led to suppression in neoplasm proliferation [27]. Thus Pim-1 kinase is evolving to be a potential immuno-therapy target in numerous malignancies [28].

2.5 Pim-1 kinase – In Drug Resistance: Cancer has emerged as a deadly lethal disease worldwide. One of the reasons that make cancer a fatal condition is various hurdles leading to the failure of chemotherapeutic agents. This brings a serious problem in the treatment and management protocol of cancer patients. A patient can acquire resistance at the initiation of the treatment termed as intrinsic resistance or as the treatment progresses termed as acquired resistance [29]. Mechanistically this acquired resistance originates as a result of any of the three following phenomena: mutations or chemical modifications in proteins against which the drug is targeted, activation of survival pathways and enhanced effluence of the drug.

The proteins that increase the discharge of drugs from the body belong to the Adenosine triphosphate Binding cassette (ABC) superfamily localized in the trans-membranes. MDR1 also known as ABCB1 or P-glycoprotein is a protein belonging to this superfamily exerting the above-mentioned effects. Although these proteins show high specificity to respective receptors, they are known to discharge a large number of lipophilic compounds. It was initially established that a mechanism by which Pim-1 kinase mediates drug resistance is the protection of malignant cells from chemotherapeutic agents induced programmed cell death [30].

Further exploration by a screening of yeast 2-hybrid, revealed BCRP/ABCG 2 belonging to the class of ABC transporters as an important mediator co-working with Pim-1 in drug resistance. It activated the breast cancer resistance protein (BCRP) by phosphorylating threonine at position 362, which was essential for its actions. This was further bolstered by a revelation from the study that mutation of this particular threonine residue at 362 positions made BCRP protein devoid of this drug resistance activity. Pim-1 kinase also interacts with the ABC transported p. glycoprotein (Pgp/ABCB1) to show its drug resistance actions. Translocation of 150kDa Pgp from the Endoplasmic reticulum to 170kDa Pgp in the cell membrane by glycosylation promotes the affluence of various structurally divergent antineoplastic drugs.

Studies like immunoblotting, flow cytometry, and stability testing show the inconspicuous interaction of Pgp with Pim-1 and subsequent phosphorylation. Thus, Pim-1 kinase aids the Pgp mediated drug resistance by conferring it a protection against

proteasomal and proteolytic breakdown [31]. Acute myeloid leukemia (AML) is associated with treatment failure due to internal tandem duplication (ITD) mutations of FLT3, a receptor Fms- like tyrosine kinase.

A 10 fold decrease in Pim-1 expression as a result of FLT3 mutation was ascertained by a qualitative polymerase chain reaction (QPCR). Autophospho-rylation of FLT3 at a serine residue at 93-position with Pim-1 kinase leads to an enhanced threshold to apoptosis and cytotoxicity associated with FLT3 inhibition. This contributes to the kinase inhibitor drug resistance activated by FLT3 pathway [32].

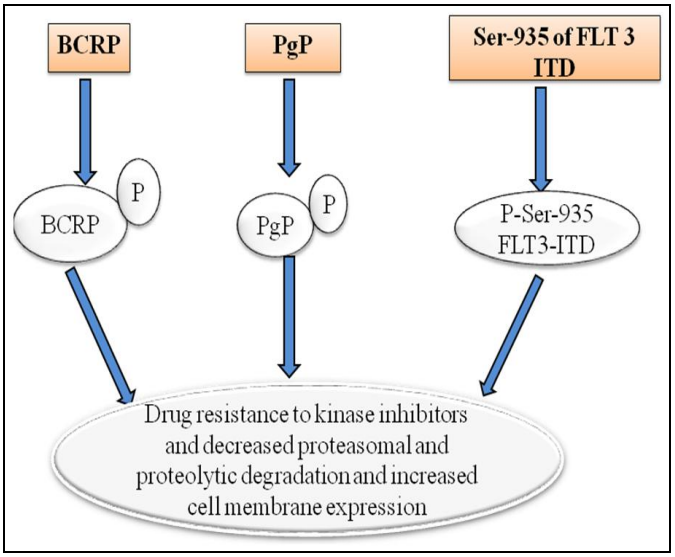

FIG. 2: REPRESENTATION OF VARIOUS MOLECULAR SIGNALLING PATHWAYS TO REGULATE DRUG RESISTANCE

2.6 Pim-1 kinase - In Senescence: Cellular senescence is delineated as a condition in which there is a permanent cessation of the dividing capability of the cell. It was first observed in 1960's, by Leonard Hayflick and Paul Moorhead in human fetal fibroblast cells. These cells showed senescence after reaching a maximum of 50 replications. This phenomenon went onto be termed as Hayflick's limit or "Replicative senescence" [33, 34, 35]. There are many cellular mechanisms proposed to explain the Replicative senescence. Reduction in the length of telomeres leading to DNA damage is a potential trigger of senescence. Overexpression of reactive oxygen species (ROS) and oncogene activation are a few other triggers [36]. It arrests tumor progression and acts as a threshold that the proto-oncogene must bypass to become an oncogene.

This phenomenon was first observed in a mutated model of transcription factor E235 [37, 38]. Pim-1 kinase exhibits conflictive actions in the regulation of senescence [39, 40, 41]. Human cardiac progenitor cells (hCPC's) have the ability to differentiate and dedifferentiate into mature cardiomyocytes and improve the pumping abilities of a heart with a compromised pumping action. These cells find an increased and appreciated application in regenerative therapy for patients with pathologically disabled stem cells.

The results of the experiment conducted show an increased rejuvenation and regeneration of these stem cells following modification with Pim-1 kinase at genetic levels. There was an increase in the rate of proliferation, reduction in the shortening of telomere length and a deficiency in the expression of senescence biomarkers [42]. Thus, Pim-1 kinase alleviated the senescence exhibited by hCPC cells. Conversely, there is an increased expression of Pim-1 in senescent cells. This overexpressed Pim-1 enhances the phosphorylation of Heterochromatin protein 1-gamma on serine residue at 93-position.

This enhanced the binding ability of HP1-gamma To H3KP me3 lead to an over expression of various genes like CCNA2 and PCNA. These genes showed an abnormal expression in rapidly dividing cells which were segmented because of this phosphorylation. Mechanistically, this phenomenon was aided by IL-6, which proves to be an important component of the senescence regulation. Hence Pim-1 kinase is upregulated by IL-6/STAT3 pathway and aids the antioncogenic properties of senescence mediated by cytokines.

The major pathway which regulates cellular senescence is controlled by p53, a tumor suppressor gene, and pRb. Activation and function of p53 gene is mediated by binding of $p12^{ARF}$ and MDM2 thus restricting the degradation of p53. The transcriptional property of p53 is also regulated by direct binding to MDM2. This activation of p53 brings about the induction and activation of genes containing the protein-coding regions like the p21 gene as shown in **Fig. 3** [43]. Thus, various aspects of cellular machinery and triggers that confer Pim-1 kinase with this contradictive property is still an area of ongoing research.

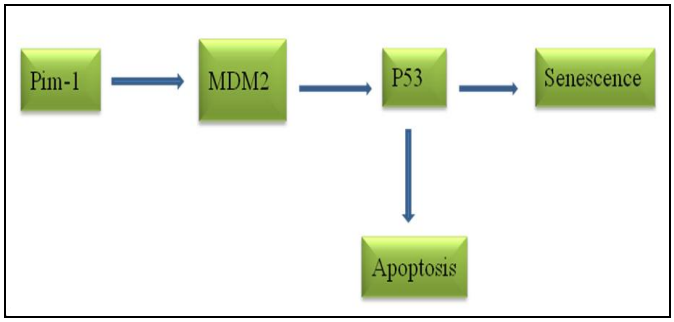

FIG. 3: THE MAJOR PATHWAY WHICH REGULATES CELLULAR SENESCENCE

3. Inhibitors of PIM-1: Since Pim-1 is over expressed in various types of malignancies, it has proven to be a potential target for cancer therapy. This is further enhanced by intricate and delineated study of its crystal structure conducted by various researchers [44, 45, 46]. The hinge design of Pim-1 kinase is very novel owing to the presence of an additional proline residue at 123 position, which is absent in conventional kinases. This proline residue fails to donate protons for ATP binding. This lead to the synthesis and formulation of highly specific, structurally diverse small molecular inhibitors directed against the Pim group of threonine/serine kinases [47]. In conventional kinases, the ATP binding occurs by the formation of dual hydrogen bonds with the hinge portion. This is absent in Pim-1 kinases due to the non-availability of a canonical hydrogen bond donor in the hinge architecture. This arises due to the placement of proline residue at 123-position at the equivalent site [48, 49].

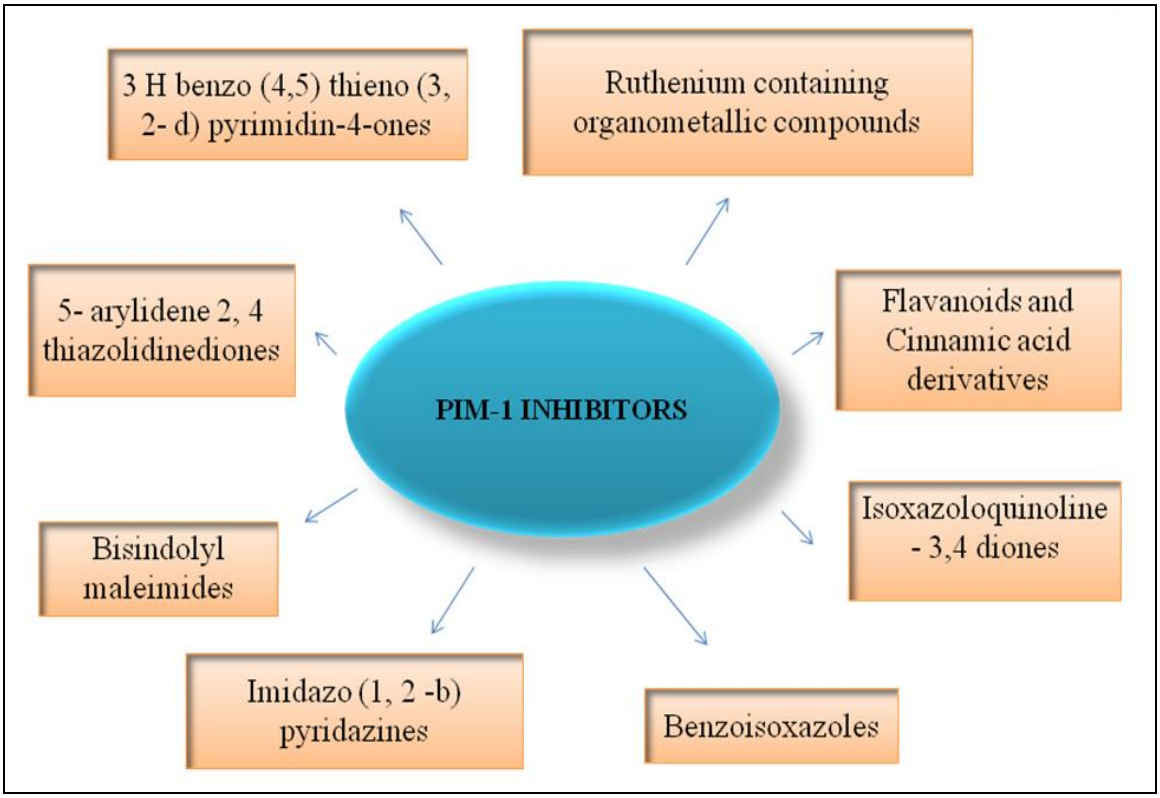

FIG. 4: INHIBITORS OF PIM-1

3.1. Binding Mechanisms of Pim-1 Inhibitors: The most protein kinases contain the binding site for ATP in a deep depression formed by the bi-local arrangement and hinge region. This differs in Pim kinases [50]. The diverse (greater than 50) inhibitors of Pim that are reported basically fall into two classifications based on their mechanism of binding. The first group of inhibitors being ATP-mimetic compounds mimicking the adenine ring of ATP and an intermolecular hydrogen bond with the oxygen moiety of glutamine residue at 121 position in the Pim backbone [51, 52]. The second group of inhibitors is ATP competitive inhibitors. There is no interaction with the hinge portion by formation of hydrogen bonds. Polar interactions are seen with the active site housing lysine and a water molecule present in most co-crystal structures. These interactions aid the binding of the inhibitor to the dorsal portion of the ATP pocket [53]. Additionally, the third class of inhibitors were discovered which mainly act by the interaction between the halogen atoms and the hinge region of Pim kinase [54, 55].

3.2. Individual Compounds:

3.2.1. SGI-1776: Lisa S Chen *et al*., reported the apoptotic activity of SGI-1776 in chronic lymphocytic leukemia cells. Chemically, this compound is Imidazopyridazine **Fig. 5**. The development of this Pim-1 inhibitor has been very actively going on in recent times. This interesting compound found its application by promoting apoptosis in various malignancies like chronic lymphocytic leukemia, acute myeloid leukemia (AML) and cell lines with myeloproliferative neoplasms [56]. It interfered with G1 phase, leading to a decrease in phosphorylation of BAD and subsequent apoptosis of cell lines with prostate neoplasm. It ameliorated multidrug resistance cancer by reviving sensitivity and responsiveness to taxane treatments in drug-resistant cells [57]. A recent study showed the activity of this compound against FLT3 Kinase. It showed enhanced inhibition of FLT3 Kinase with internal tandem duplications (ITD) in mice cell lines [58]. Its inhibitive action is shown against Pim-1, Pim-2, and Pim-3. IC_{50} values being 7nm for Pim-1, 363nm for Pim-2 and 69nm for Pim-3. It also inhibits Haspin and FLT3 at an IC_{50} of 34 nm and 44nm, respectively [59]. However, this interesting compound faced rejection in phase 1 clinical trials due to cardiac toxicity.

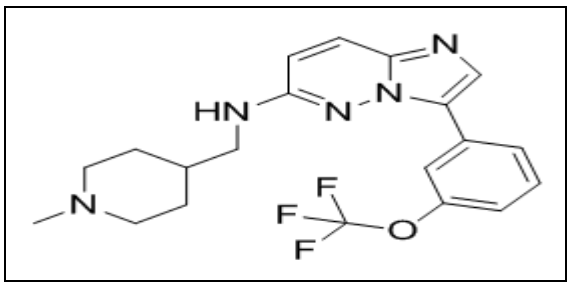

FIG. 5: IMIDAZOPYRIDAZINE

3.2.2. AZD 1208: Daken *et al*., reported this novel compound as a selective and novel inhibitor of all the three kinases of Pim family. Various studies have identified substituted benzylidiene-1, 3-thiazolidne-2, 4 diones (TZD's) as strong and specific inhibitors of Pim kinase [60] **Fig. 6**. Their synthesis, structure-activity relationship, X-ray crystallographic studies have been conducted. Various modifications have been considered after confirming that the thiazolidiene ring was essential for ATP binding. Further investigations, tested the incorporation of a basic amine at a position ortho to the scaffold. This lead to the design of molecules potent against all three isoforms of Pim kinases. Being a specific and targeted PAM-PIM kinase inhibitor, it was found to be highly efficacious in cell lines with AML, Xenografts and FLT3 kinase with internal tandem duplications (ITD). It exhibited a Cell cycle stoppage and programmed cell death in MOLM-16 cells. Phosphorylation of Bcl-2 protein, an inhibitor of cell death was restricted. Additionally, phosphorylation of proteins like p70S6k, 4EBP1, and S6 was inhibited in a dose-dependent manner. With this, the potential of these interesting compounds against AML with Pim kinase overexpression was established. Synthesis of AZ1208 was supported and performed by Astra Zeneac R&D. The results of the study were established after carefully performing immuno-blotting techniques, Catabolite activator protein (Cap) dependent translation assay, profiling of polysome in 5-6 weeks old female mice infected with CB17 SCID and bone marrow samples from AML suffering patients with a recent diagnosis. IC_{50} values of PIM-1, PIM-2, and PIM-3 were found to be 0.4nm, 5.0 nm and 1.9 nm respectively. A synergism was seen between AZD 1208 and cytarabine in tumor xenografts. This provides a beneficial ground for incorporating AZD 1208 with standardized anticancer drugs [61].

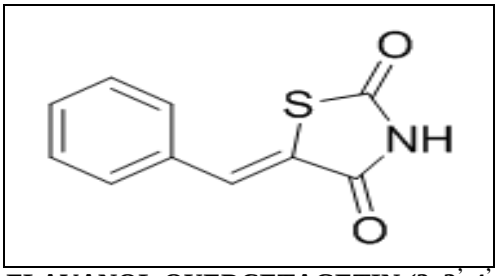

FIG. 6: FLAVANOL QUERCETAGETIN (3, 3', 4', 5, 6, 7-HYDROXYFLAVONE)

3.2.4. CX-6258: Haddach MNJ *et al*., discovered and reported CX-6258 as a novel pan-pim inhibitor. Chemically, this is a 3-(5-((2-oxoindolin-3-ylidene) methyl) furan-2-yl) amide derivative **Fig. 7** which is an orally active and selective inhibitor of Pam-Pim kinases. This study shows the inhibitory activity of these oxindole derivatives against all the three forms of Pim kinases in xenografts. A prototype molecule bearing an oxindole group was identified by High-throughput screening (HTS) which showed Pim-1 kinase inhibition at an IC_{50} of 368nm. SAR of this compound outlined the importance of hydrogen bond donor present in the lactam NH. Replacement of this group with a CH_3

group showed a loss in activity. Thus this hydrogen bond donor present in the lactam NH interacts with lysine in 67 positions and Aspertamine in 186 of Pim-1 to show inhibition. IC_{50} values were found to be 15nm for Pim-1, 25nm for Pim-2 and 16nm for Pim-3. A concentration of 134nm produced inhibition of FLT3. Mechanistically, it inhibited the phosphorylation of BAD and 4E-BPI inducing apoptosis of hematological, acute leukemia and human solid tumor cell lines.

Autophosphorylation of FLT-3 was not affected. It also showed synergistic actions with doxorubicin and placitaxel in 10:1 and 100:1 molar ratios respectively. It is currently undergoing preclinical studies [65].

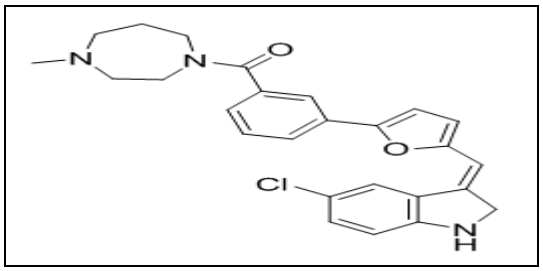

FIG. 7: 7-(H-1, 2, 4- TRIAZOL-3-YL) BENZOL [2, 6] NAPTHYRIDINE

3.2.5. CX1002: Barnett Ab *et al*., reported CX1002 as a highly selective inhibitor of Pim kinases. It is an ionized form of an ammonium salt of perfluroacetic acid derivative **Fig. 8**. Tumor xenografts and in vitro cell lines were used to demonstrate its Pim kinase inhibitory activity. It induced Endoplasmic reticulum stress. An assay technique which used ATP uptake and depletion was used to test cytotoxicity *in-vitro*. It was used in combination with eight other anticancer drugs in a series of malignant cell lines. Western blotting and microarray analyses were performed to identify its mechanism of action of cytotoxic activity in pancreatic, ovarian carcinoma and sarcoma cell lines. Moreover, hematological cell lines showed the highest specificity to CXR1002. An effective synergistic combination of CXR1002 and gemcitabine was established by testing a panel of eight control anti-cancer drugs in eleven malignant cell cultures [66].

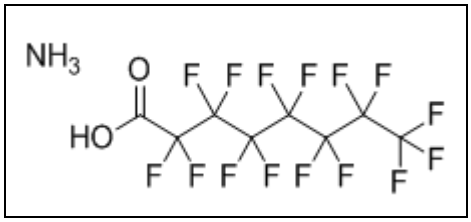

FIG. 8: PERFLUROACETIC ACID DERIVATIVE

4. Clinical Trials of PIM-1 Kinases: Clinical trials and difficulties faced by researchers worldwide in the development of potential inhibitors of Pim-1 kinase have been outlined in **Table 1**.

TABLE 1: INTERPRETATIONS OF THE PERFORMED CLINICAL TRIALS

Drug	Objectives	Type of malignancy	Dose Range	ADR's	Conclusion
AZD1208 [67.] Phase :1	*Evaluate safety, tolerability and maximum tolerable dose (MTD) by dose-responsive studies. *Analyse and elucidate pharmacodynamics, pharmacokinetics and preliminary efficacy of the drug	Subjects with acute myeloid leukemia (AML) that is recrudescent and solid tumors	AML: 120-900mg. Solid tumor: 800mg	Dose-independent: gastrointestinal. Dose-dependent: rash fatigue, vomiting	Ineffectiveness of monotherapy in the treatment of Pim regulated malignancies
SGI-1776 [68,69.] Phase : 1	*Evaluate maximum tolerable dose (MTD) by dose-responsive studies and dose-limiting toxicities. *Analyse and elucidate pharmacodynamics pharmacokinetics, PSA response and renal elimination	Subjects with non-Hodgkin's lymphoma that is refractory or relapsed and prostate cancer refractory to hormones and docetaxel therapy	Undertaken by SuperGen Inc. 100 mg initial dose given as 50mg every 12 hours for 14 days of a 21-day cycle	QT wave prolongation and cardiac toxicity	Discontinued the clinical studies based on the dose-limiting toxicity. However, SuperGen moves ahead with its objective of developing Pim inhibitors
CXR1002 [70]. Phase : 1	*Evaluate safety, tolerability and pharmacokinetics. * Confirming the dose for Phase 2 clinical trials to be given per orally once a week	Subjects with advanced cancer	One cohort-single dose after which one dose/ week for 6 weeks. Other cohorts one dose/ Week	Abnormally high and prolonged $t_{1/2}$ was exhibited. Minimal toxicity up to a dose of 750mg once weekly	Toxicity with higher doses is still ongoing

CONCLUSION: Pim (Pro-viral integration site in Moloney murine leukemia virus) kinases are a family of serine/threonine protein kinases that are highly conserved through evolution in multicellular organisms. They have a significant role in cell cycle regulation, cell survival, apoptosis, cellular senescence and drug resistance. It is emerging as a potential biomarker in a number of human malignancies. More than 100 pharmacological inhibitors of this interesting kinase have been developed and are undergoing Phase 1 and 2 clinical trials. Although, these compounds have exhibited good potency and minimal toxicities in Xenograft studies and *in-vitro* cell lines, their clear toxicity analysis is still being undertaken by researchers worldwide. Studies reported a common cross-inhibition between Pim kinases and Haspin and FLT3 kinases. This interesting feature if tested *in vivo*, will aid in the development of agents for the treatment of hematological neoplasms. The inhibitors also showed a synergistic action when combined with traditional chemotherapeutic agents. Their efficacies and combined toxicities are yet to be evaluated. Thus, Pim-1 kinases are promising targets for novel anti-tumor therapies.

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