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Jak2 Inhibitors are the solution for Myelo Proliferative Disorders

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Abstract:

The recent discovery of JAK2 –activating mutations as a casual event in the majority of patients with Philadelphia chromosome negative (ph-)myeloproliferative disorders (MPDs) prompted many pharmaceutical companies to develop JAK2- selective inhibitors for the treatment of MPD'S . JAK2 inhibitors effectively reduce JAK2 driver phosphorylation of signal-transducer and activator of transcription 5,and cell proliferation and cell survival in JAK 2 activated cells in vitro and invivo. The results of ongoing clinical trials will allow further evaluation of clinical benefits and safety of these compounds. In this review the authors summarize the status of JAK2 inhibitors in development and discuss their benefits and challenges.

Keywords: Essential thrombocythemia (ET), JAK2V617F, Myeloproliferativeneoplasms (MPN), Polycythemiavera (PV), Thrombopoietin receptor(TPO).

INTRODUCTION:

JAK2V617F was the first mutation to be described in patients with ph- negative MPN'S and the fact that it can be detected in patients with all 3 subtypes of MPN's gives to their interrelationship, Which was recognized in the 1950's by Dameshek in a classic blood editorial[1].After discovery of JAK2V617F;other investigators discovered mutation in related genes of hemopoietic growth factor signaling pathways, including exon 12 mutations in JAK2,Mutation of MPL (Thrombopoietin receptor (TPO)) and more recently. Mutations in the adapter protein LNK.[2]It appears that mutations of signaling pathways are responsible for response to Hematopoietic growth factors are a common feature of these disorders. Besides providing the pathophysiology of pH-negative MPN the JAK2V617F mutation also represents a potential therapeutic target. In fact, since mid-2007, several clinical trials were started that explored the therapeutic potential of JAK2 inhibitors in patients with PV, ET and MF. Identification of other mutations and potential therapeutic targets in MPN is under investigation.

THE JAK FAMILY OF TYROSINE KINASE:

There are four members of the JAK family; JAK1, JAK2, JAK3 and TYK. JAKs are cytoplasmic kinases

that associate with the intracellular portion of cytokine receptors that do not possess intrinsic kinase activity, such as receptors for hematopoietic growth factors (erythropoietin receptor (EPOR), G-CSF receptor (G-CSFR),and thrombopoietin receptor (C-MPL)[3]

Binding of the putative ligand leads to receptor dimerization and subsequent approximation of two JAK kinases, which trans phosphorylates and activate each other, initiating intracellular signalling pathways (FIG.1)

One of the most important intracellular signalling pathways activated by JAKs is the JAK-STAT (signal transducer and activator of transcription) pathway.[4]STAT is latent,cytoplasmic transcription factors. JAKS phosphorylate STATs tyrosine residues; leading to STAT dimerization, translocation to the nucleus and activation of transcription. Aberrant activation of STAT3 and STAT5 has been linked to neoplastic transformation. Other signalling pathways which can be activated by JAKs include the Ras/RAF/MAPK pathway and the PBK/Akt pathway. Activation of these pathways leads to increased cellular proliferation and resistance to apoptosis, and deregulation could cause the development hematological malignancies.

Structurally,JAKs consists of seven different domains(fig.2)[5]. The tyrosine kinase domain (JAK homology domain 1 (JH1)) and the pseudokinase domain (JH2) are located in the c-terminal portion of the molecule. The kinase domain has all the features of an active TK domain, while the pseudokinase domain has no kinase activity. It is believed that JH2 domain interacts and inhibits the activity of the kinase domain, as deletion of the JH2 domain leads to increased kinase activity [6.] Due to the mutation of V617F, which is located in the JH2 domain leads to increased kinase activity instead of inhibitory activity of JH2 domain.JH3,JH4 domains have structural similarity. The JH5-JH7 domains are located in the N-terminal and contain FERM, which is essential for binding of the JAK kinase to the intra cytoplasmic portion of the cytokine receptor [7].

RATIONALE FOR TARGETING JAK 2 IN MPN'S

The discovery of the BCR – ABL 1 inhibitor, Imatinib and its success in the therapy of CML used as cancer medicine in the era of kinase inhibitor [8]. These drugs target kinases that are abnormally activated in cancer cells, with the objective of blocking cellular proliferation and inducing apoptosis. The JAK 2 V617F mutation generates a constitutively active TK, and so there is a rationale for developing JAK 2 inhibitors for treating patient with MF and other MPNs. The ATP is the source of phosphate groups utilized by TK for phosphorylating protein targets and most TK inhibitors in current development act by competing with ATP.

The JAK 2 V617F mutation occurs outside of the TK domain, it is likely that most JAK 2 inhibitors will target both the mutated and wild type kinase. Inhibition of wild type JAK 2 will lead to myelosuppression, because JAK 2 is an important mediator of hematopoietic growth factor signaling. JAK 2 inhibitors might function in patients with both mutated and unmutated MPNS. If JAK2 inhibitors are used in the treatment of PV,ET [9] observed Suppression of erythropoiesis and thrombopoiesis as side effects. In MF because of JAK2 inhibitors in the normalization of proinflammatory cytokines [10] leading to weight gain, improvement in fatigue, and reduction of spleen size. There is great therapeutic benefit for employing these drugs to achieve symptom

control and improve the quality of life of patient with MPNS.

SCAFFOLDS USED AS JAK2 INHIBITORS:

Till now the various scaffolds used in the synthesis of JAK2 inhibitors are mentioned in TABLE-1

JAK 2 INHIBITORS UNDER CLINICAL TRIALS:

CEP-701(Fig-3): It is also known as a lestaurtinib is a TK1 which belongs to the chemical class of indocarbazol of alkaloid. It is a potent FLT3 and JAK2 inhibitor [11].In a preclinalstudy, CEP-701 inhibited both wild type and mutated JAK-2, with a half maximal inhibitory concentration(IC50)of 1nm for wild type JAK-2 .CEP-701 also inhibited growth of JAK-2 V617F-Positive HEL 92 cells xenografted into nude mice .80-100mg twice daily as a liquid formulation. The patients who received liquid formulation have less tolerability than the patients who received capsule formulation. Further results from the second study are awaited in the near future.With the administration of CEP701, transfusion independence, reduction in spleen size with improvement in cytopenias [12.]It is associated with dose related toxicity such as Myelosuppression (anemic 14%, thrombocytopenia 23%) GI toxicity (diarrhea 72%, nausea vomiting 27%).

AZD1480 (Fig-4): It isa pyrazolylpyrimidine compound that selectively and potently inhibits JAK2 [13].This compound block STAT 5 activation, inhibits the cell proliferation and induced apoptosis in the human JAK2V617F positive. Megakaryoblastic SET2 cell line. In a mouse model, AZD-1480 reduced the proliferation of stem cells transferred with the JAK2 mutant proteins.It inhibits the tumor growth, and tumor cells lists had reduced levelsPhospho STAT3. A phase 1 clinical trial is currently underway to evaluate the activity of AZD1480 in patients with MPNS.

R723: It is a potent and highly selective inhibitor of JAK2 [14]. This compound is strongly anti-proliferative against mouse and human cell lines that have the JAK2V617F mutation , constitutive STAT5 phosphorylation in JAK2V617F positive cells was inhibited 10-20 fold more potent than IL-2 induced STAT5 phosphorylation . It is a strong compound effecting on EPO receptor signalling, in vivo currently the compound is at a preclinical development stage .

XL019 (Fig-5): It is a potent , reversible and selective inhibitor of both wild type(and mutated JAK2 and shows good selectivity for JAK2 in vitro biochemical assays.It was evaluated in patients MFand standard therapy resistant PV.Given orally once daily or three times weekly. Tolerated dose is 25 and 50mg/kgNon- hematological adverse effects such as fumigation, peripheral neuropathy , neurotoxicity, confusion and balance disorders, leukocytosis , decrease of circulating blasts, improvement of anemia's , priorities and poor appetite were reported [15].

INCB018424 (Fig-6): It is a potent JAK inhibitor targeting JAK1, JAK2,and TYK2. It inhibits constitutive and IL-6 stimulated phosphorylation of STAT3 and reduces production of proinflammatory cytokines in all MF patients regardless of their JAK2 mutation status. MF is characterized by high levels of circulating CD34+cells which correlate with the stage of disease. INCB018424 treatment resulted in reduced circulating CD34+cells.The MF is characterized by high levels of circulating CD34+cells.Treat with this drug reduced the CD34+cells.The starting dose of 25mg PO bid was demonstrated to be maximum tolerated dose (MTD) . It also improved hyper catabolism-

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associated hypocholesterolaemia and pathologically decreased serum /eptin,presumable due to inhibition of both JAK1 and JAK2.,also resulted in rapid reduction of splenomegaly.The drug is also in Phase2 trials in psoriasis where a topical version is used.

SB1518(Fig-7): It is a JAK2 inhibitor that has activity against both wild type and mutated JAK2,being selective against JAK1 and JAK3.It is a potent ,selective and an orally active drug.It inhibits proliferation of Baf/3 cells transferred with the erythropoietin receptor (EPOR) and the JAK2 V617F mutation and decreased phosphorylation of JAK2 and STAT5.Due to this mutated JAK2 led to normalization of evaluated WBC count , reduction of GFP-labelled BAF/3 cells in peripheral blood ,improvement in cytopenia and hepatosplenomegaly ,reduction of phosphorylated –STAT5 in involved organs and increased survival.Given 100-600mg orally once daily in 28-day cycles.It is associated with some side effects like splenomegaly,abdominal pain,diarrhoea,nausea and thrombocytopenia.Due to these side effects recommended dose for phase2 clinical trials is 400mg daily once.

TG101348(Fig-8): It is an orally available, potent JAK2 inhibitor (ic50=30nm) with greater selectivity against (JAK1) (IC50=105NM) and JAK3 (IC50=996NM).It inhibits the proliferation and induce apoptosis of JAK2 V617F positive HEL cells and Ba/f3 cells transduced with JAK V617f. It was well tolerated in patients with MF when the drug was taken orally once a day .The MTD was declared to be 680 mgs /day.This led to an improvement in symptoms like pruritis,night sweats,fatigue and early satiety.The most frequent non hematological toxicities were nausea/vomiting (68% of patients) and diarrhoea (54% of patients) anorexia.No neurologic toxicities were observed. thrombocytopenia was seen in 25% of patients; neutropenia was seen in 11 %, anaemia less than 50% decrease in spleen size was seen in virtually all patient and half of these patients demonstrated complete dissolution of leukocytosis .No consistent change in levels of plasma. Cytokines including EPO, TPO, TNF, a, IFN, y, IL-6 AND IL6- IL8 were seen in the patients treated with this drug.

CYT387(Fig-9): It is an amino pyrimidine compound is an ATP competitive small molecule that potently inhibits JAK1 and JAK2 . Inhibits proliferation of cell lines that depend on signalling by JAK kinases for proliferation, including Ba/f3 cells engineered to express both EPOR and JAK2 V617F Inhibition of proliferation was accompanied by apoptosis and decreased JAK2 , ERK1/2 and STAT 5 phosphorylation in EPOR – JAK2V617F positive Ba/f3 cells. CYT387 selectively suppressed the invitro growth erythroid colonies harboring JAK2V617F from PV patients.Therapy results in erythrocytosis , leukocytosis ,and bone marrow fibrosis improved hemoglobin levels , normalized WBC counts and reduced spleen size.A reduction but not elimination of the JAK2 disease burden ,with partial normalization of progenitor cell distribution and differentiation by this compound was also observed in the model.A phase 1 clinical trials with CYT387 in patients with MF is currently underway and results will be presented in the near future.

THE CURRENT STATUS OF DRUGS USED AS JAK2 INHIBITORS:

The current status of JAK2 inhibitors are given in TABLE-2

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Discussion

The development of JAK-2 inhibitors has started the targeted therapies for patients with Ph-negativeMPNS. Even though these drugs are not seen to eradicate the malignant clone,there is still great benefit to begained from the use of these compounds. Improvements in systemic symptoms and splenomegaly can significantly impact on the quality of life of patients with MF, which is a significant clinical benefit.

JAK-2 inhibitors are a novel class of agents with promising results for treating patients with MF,PV and ET. Further studies are needed to better understand and define their role in the treatment of Ph-negative MPNS.

Future studies addressing these questions would help in developing an effective combination therapy based on current JAK-2 inhibitors as well as the second and third generation of JAK-2 inhibitors.

- Determine the mechanism of action of these drugs and which cells they are targeting. Neoplastic cells, normal cells (or) both.
- Discover biomarkers predictive of response to JAK-2 inhibitors.
- Evaluate the impact of JAK-2 inhibitors on survival and leukemic transformation of ph-negative MPNS.

Table 1: Scaffolds used as Jak2 Inhibitors

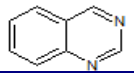
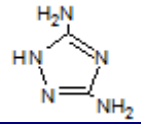
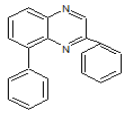
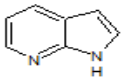
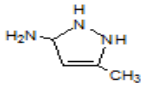
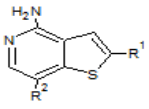
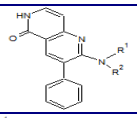
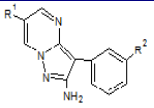
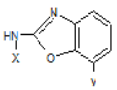
NAME	STRUCTURE
Quinazoline derivatives	
2,4-diamino triazole	
2,8-diaryl quinoxalines	
3,4-ring fused 7-azaindoles	
Pyrazol-3-ylamino pyrazines	
Thieno pyridines	
Napthrindone	
2-Amino pyrazolo(1,5-a)Pyrimidines	
2-Amino-aryl-7-aryl benzoxazoles	

Table 2: Current Jak2 inhibitors under clinical trials

NAME	PHASE OF CLINICAL TRIAL
CEP-701/ Lestaurnib	I/II
AZD-1480	I
R723	Pre-Clinical
XL019	I
INCB-18424	III
SB1518	I
TG101348	II
CYT387	I

Figure 1

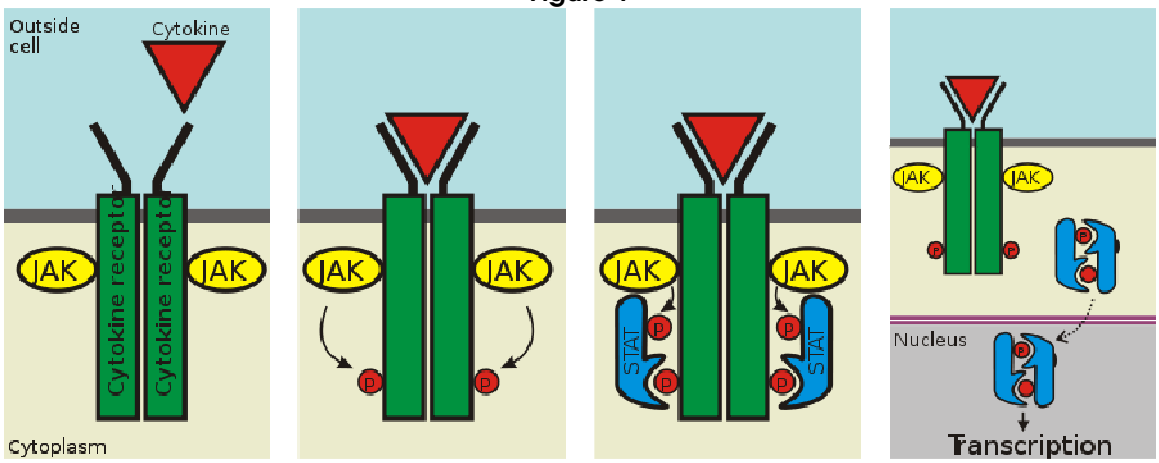
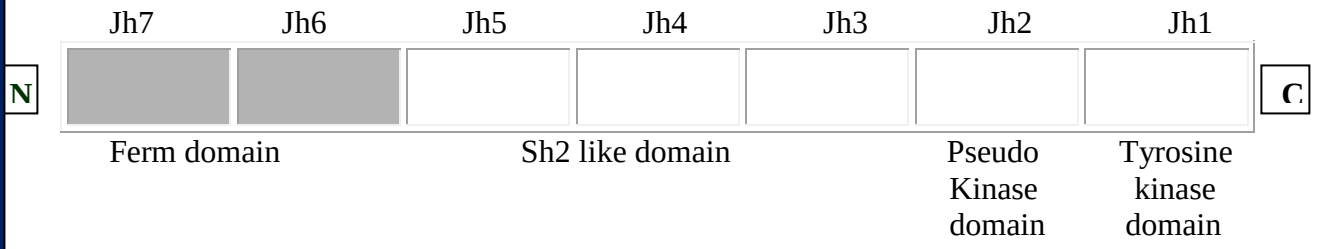


Figure 2



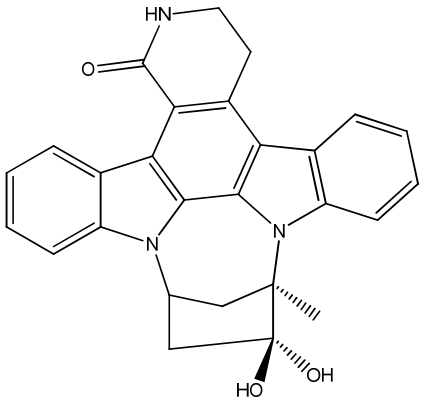


FIGURE 3: CEP701/Lestaurnib

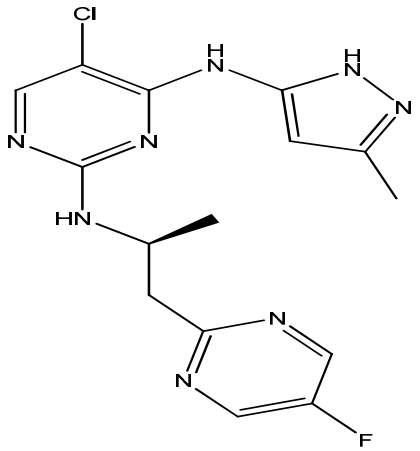


FIGURE 4: AZD1480

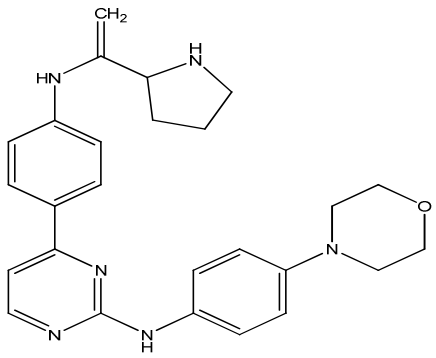


FIGURE 5: XL019

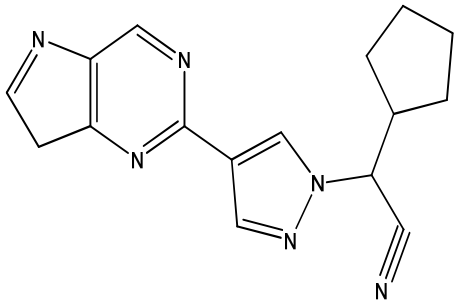


FIGURE 6: INCB-18424

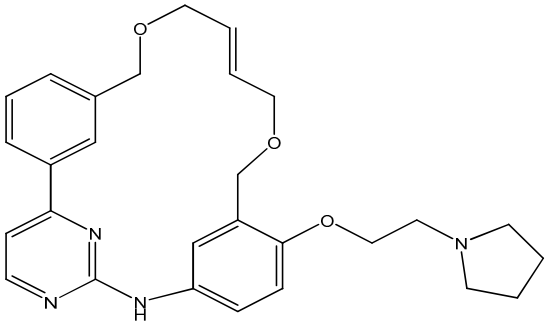


FIGURE 7: SB1518

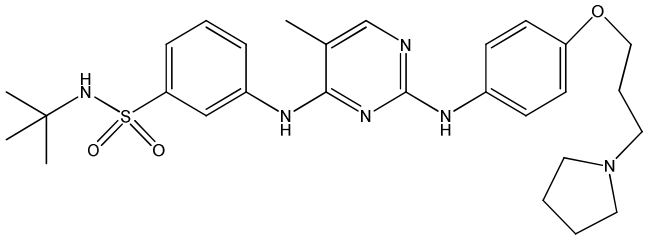


FIGURE 8: TG101348

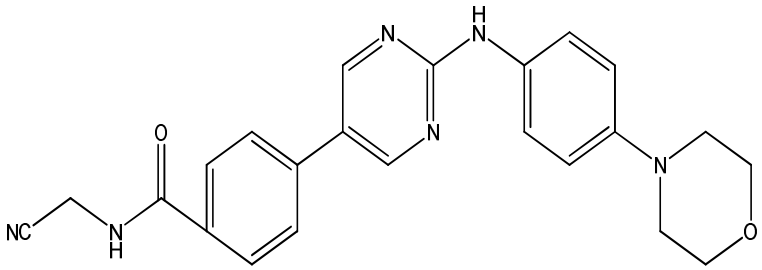


FIGURE 9: CYT387

BIBILIOGRAPHY:

1) Dameshek W. Some speculations on the myeloproliferative syndromes. Blood 1951; 6: 372-5.
2) Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. Blood 2010; 116: 988-92.
3) Leonard WJ, O'Shea JJ, Jaks and STATS; Biological Implications Anner Rev Immunol. 1998; 16: 293-322.
4) Yu H, Jove R, The STATS of Cancer- new molecular targets come of age. Nat Rev Cancer; 2004; 4(2): 97-105.
5) Giordanetto F, Kroemer RT; prediction of the structure of human Janus Kinase 2 (JAK 2) Comprising JAK homology domains 1 through 7, protein Eng: 2002; 15(9): 727-737.
6) Wilks AF; Harpur AG; Kurban RR, Ralph SJ, Zurcher G, Ziemiecki A. Two novel protein tyrosine Kinases, each with a second phosphotransferase- related catalytic domain define a new class of protein Kinase. mol .cell biology, 1991; 11(4); 2057-2065.
7) Radtke S, Haan S, Jorissen A; et al. The Jak 1 SH 2 domain does not fulfill a classical SH2 function in JAK/STAT signaling but plays a structural role for receptor interaction and up-regulation of receptor surface expression J Biol Chem. 2005; 280(27): 25760-25768.

8) Krause DS, Van Etten RA. Tyrosine Kinases as targets for cancer therapy. N Engl J Med. 2005; 353(2): 172-187.

9) Vannucchi AM. How do JAK2- inhibitors work in myelofibrosis: an alternative hypothesis. Leuk Res. 2009; 33(12): 1581-1583.

10) Vannucchi AM, Migliaccio AR, Paoletti F, Chagraoui H, Wendling F. Pathogenesis of myelofibrosis with myeloid metaplasia: lessons from mouse models of the disease. Semin Oncol. 2005; 32(4): 365-372.

11) Hexner EO, Serdikoff C, Jan M, et al. Lestaurtinib (CEP 701) is a JAK 2 inhibitor that suppresses JAK 2/ STAT 5 signaling and the proliferation of primary erythroid cells from patients with MPD. Blood 2008; 111: 5663-71.

12) Santos FP, Kantarjian HM, Jain N, et al. Phase 2 study of CEP-701, an orally available JAK 2 inhibitor, in patients with primary (or) post-polycythaemia Vera/ essential thrombocythemia myelofibrosis. Blood 2010; 115(6); 1131-1136.

13) Hedvat M, Huszar D, Herrmann A, et al. The JAK 2 inhibitor AZD 1480 potently blocks Stat 3 signaling and oncogenesis in solid tumors. Cancer cell 2009; 16: 487-97.

14) Markovtsov V, Tonkin E, Fang S, Liu C, Gelman M, Lang W, et al. In vitro and in vivo inhibition of JAK 2 signaling by potent and selective JAK 2 inhibitor.ASH Annu Meet Abstr. 2008; 112(11): 3721.

15) Pardanani AD, Gotlib J, Jamieson C,Cortes J, Talpaz M, stone RM et al . ASH Annu meet Abstr 2008; 112(11)-97.

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