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Strategic therapeutic approaches to overcome emerging dual SRC/ABL kinase inhibitors resistances in chronic phase Ph positive chronic myeloid leukemia

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ABSTRACT

Chronic myeloid leukemia (CML) is a haematopoietic neoplasm with clinically distinct phases and BCR⁄ABL1 oncogene. Imatinib mesylate, a potent inhibitor of BCR-ABL was highly effective in CML but later in-vitro derived cell line with resistance namely BCR-ABL duplication point mutation, P loop mutation, T315I mutation, C helix, SH2 domain, activation loop, C terminal lobe, SRC family kinase activation led to development of Nilotinib. Although it has potential drug targets as BCR-ABL kinase, KIT, PDGFR but has no role in overcoming in Src family kinase. It prompted strategic rational drug design of Dual Src Family Kinase/Abl Inhibitor Dasatinib, active against 15 clinically significant Imatinib resistant BCR-ABL mutations but inactive against T315I mutation. The propensity of Ph^+ CML to develop novel mechanism of resistance led designing of rational therapeutic approaches to eradicate minutest residual diseases along with long term resistance risk.

Key words: Chronic myeloid leukemia, Resistances, BCR-ABL, Tyrosine kinase inhibitor, Mutation

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INTRODUCTION

Chronic myeloid leukemia (CML) is a haematopoietic neoplasm characterized by cytokine-independent myeloproliferation, multi-step evolution with clinically distinct phases of the disease and the BCR⁄ABL1 oncogene.[1] Almost 95% of patients with chronic myeloid leukemia (CML) show positive Philadelphia chromosome 22q, which results from a fusion reciprocal translocation among chromosomes 9 and 22.[2] The production of fusion protein, BCR-ABL through the activation of downstream signaling pathways causes constitutively cell transformation. BCR-ABL1, 210 kDa sized cytoplasmic fusion gene oncoprotein contribute critically to the manifestation and progression of CML.[3,4] BCR-ABL is clinically classified into chronic phase, accelerated phase and aggressive leukemic phase called blast phase.[5-7] Before the Imatinib approval by US FDA in 2001 there was no specific targeted drug therapy to change the natural progression to CML, Although cytotoxic drugs such as Busulfan, Hydroxy Urea and Interferon (Inf)-alpha were used.[8] However there was no long lasting response for the patients on such cytotoxic chemo therapy and relapse was observed in most patients.[9,10]

EVOLUTION OF IMATINIB

Imatinib mesylate, a potent inhibitor of BCR-ABL was the first major breakthrough in target cell therapy and has shown highly effective cytogenetic response in clinical trials.[11,12] In a phase II clinical trial the patients in chronic who were not responding to interferon-alpha 95% of the patients achieved complete haematological response and 60% achieved cytogenetic response.[13] Another phase III trial in newly diagnose diseases a superior haematological response and cytogenetic response was observed with Imatinib compare to Interferon (Figure 1).

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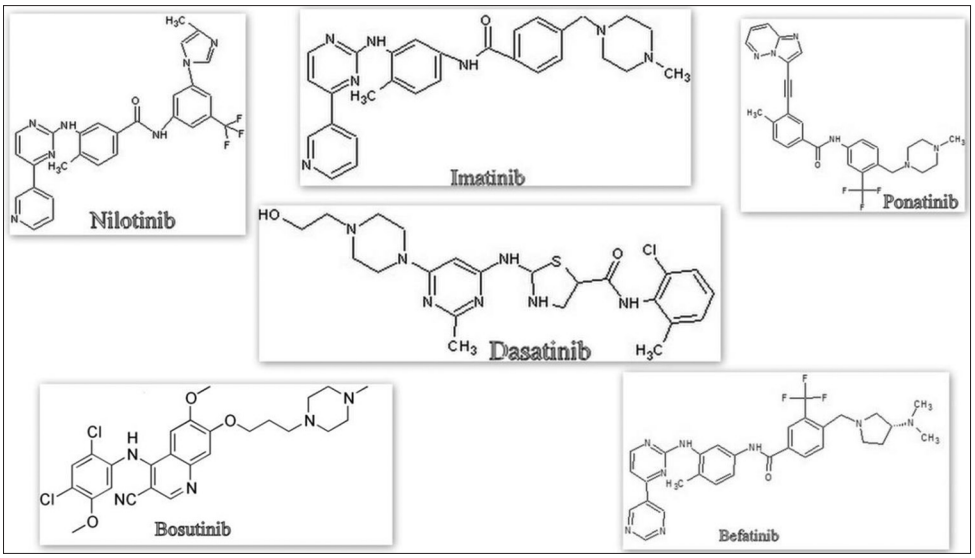

Figure 1: Structures of Bcr-Abl tyrosine-kinase inhibitors

EMERGENCE OF IMATINIB RESISTANCE

Soon after the introduction of Imatinib investigators began to describe in-vitro derived cell line with resistance to the drug. The therapeutic response assessed with Imatinib is based upon meeting hematologic, cytogenetic, molecular mile stones established by National Comprehensive Cancer Network (NCCN).[14,15] Patients that fail to achieve defined responses at predefined chronological time points are described as primarily resistant to therapy and those loosing previously obtained mile stones in diseases are termed secondarily resistant.[16] The patients with suspected treatment failure and thus suspected resistance, all diagnostic test including a complete re staging and re-biopsy of Bone Marrow (histology, morphology, chromosome analysis, FISH), BCR-ABL1 mRNA level and screening for BCR-ABL mutation should be performed and karyogram should be reported on the percentage of Ph+ cell and for additional chromosome abnormalities.[17-19] Predominantly Imatinib resistance was clinically classified as BCR-ABL dependent and independent mechanism. The dependent mechanism is further categorized as BCR-ABL duplication leading to higher expression of pathogen, BCR-ABL mutation manifesting as point mutation, P loop mutation, T315I mutation, C helix, SH2 domain, subtract binding site, activation loop, C terminal lobe by amplification or overexpression of independent pathways eg. SRC family kinase activation. Alternative mechanisms include over expression of P-glycoprotein efflux pump deregulation of SRC family of tyrosine kinases (SFK) activity and activation of other pathways. Further low expression, activity or polymorphism of organic cation transporter1 significantly produces intra cellular level of Imatinib due to restricted drug import.[20-22]

ADVENT OF 2ND GENERATION TYROSINE KINASE INHIBITOR: NILOTINIB

Although escalation of Imatinib does shown to overcome primary resistance but the short acting response, resistance and intolerance led to choice of alternative drug with improve potency and ability to prevent the emergence of drug resistance clones acting in different pathway improving response rate and potential increase in survival. Nilotinib, a second generation phenyl amino pyrimidine derivative structurally similar to Imatinib was found 10 to30 folds more potent than Imatinib in inhibiting activity of BCR-ABL kinase in proliferation of Ph expressing cells.[23] It effectively inhibit the auto phosphorylation of BCR-ABL tyrosine 177 involved in CML pathogenesis. It binds to the inactive conformational of the ABL kinase domain mostly through the lipoprotein interaction by making 4 hydrogen bonds involving the Pyridyl-N and the back bone NH of Met-318, the Anilino-NH and the side chain OH of Thr-315, the Amido-NH and side chain carboxylate of Glu-286 and the Amido carboxyl with back bone NH of the ASP 381.[24,25] Nilotinib is effective against most mutation associated with Imatinib but remains resistant to T315I mutant as a consequence of loss of a H bond interaction between threonine-O and aniline-NH on Nilotinib and a steric clash between the Isoleucine-methyl group and 2-methyl phenyl group of nilotinib[23] (Figure 1).

Nilotinib appears to target the inactive ABL domain yet it has demonstrated activity against multiple Imatinib resistance mutations. Organic Cation transporter (OCT1) has no role in cellular import of Nilotinib although it does appear to be a substrate for Pgp.[26] Nilotinib has potential drug targets as BCR-ABL kinase, KIT, PDGFR

but has no role in overcoming in Src family kinase (SFK). Nilotinib resistant cell lines have also demonstrated Pgp overexpression, Lyn kinase activation and Abl amplification thereby remains sensitive only to Dasatinib.[23]

DEVELOPMENT OF DUAL SRC FAMILY KINASE/ ABL INHIBITOR: DASATINIB

The extensive understanding of mechanism of Imatinib resistance has prompted the search for alternate BCR inhibitors which can act as dual Src family kinase and ABL inhibitor (Figure 1). Strategic rational drug design led to Dasatinib, a novel orally available small molecule multi targeted kinase inhibitor that potentially inhibits BCR-ABL and Src family and is 325 fold more potent than Imatinib against cell expressing wild type BCR-ABL.[27] This multi kinase inhibitor active against ABL mutation and many other tyrosine kinase family which are Imatinib resistant, such as platelet derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), KIT and Src family kinase.[28] Dasatinib binds to Abl with less stringent conformational requirements than Imatinib and there by an increased potency is exhibits but reduces selectivity compare to Imatinib.[29]

An x-ray crystal structure of human ABL kinase domain (residues 225 to 512) complex with Dasatinib at 2.4 Å resolution encompasses the cardinal structural features of an activated protein kinase separated in two lobes (Figure 2). The smaller NH2 terminal lobe is composed of I prominent helix α-c and five stranded β sheet. A single poly peptide strand acting as a hinged connects the two lobes which can rotate with respect to one another on ATP binding. The highly flexible P-loop connecting β1 and β2 strands, contains a glycine rich sequence motif (GXGXφG) where φ is tyrosine and phenyl alanine caps the site of phosphate transfer under

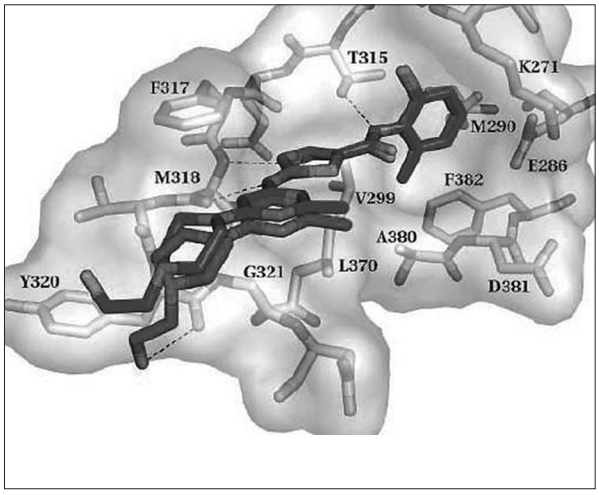

Figure 2: Dasatinib & Target Mutant sites in the ATP-binding site

which lies ATP binding site lies two lobes with deep cleft. In Abl kinase this residue is TYR[253] prominently 3 hydrogen bond recognized between Dasatinib and Abl. 3 nitrogen of amino thiazo ring of Dasatinib and amide nitrogen of Met[318] are bonded by are hydrogen bond, 2 amino hydrogen of Dasatinib is bonded with hydrogen to the carbonyl oxygen of Met[318]. The third hydrogen bond is formed between amide nitrogen of Dasatinib and side hydroxyl oxygen of Hhr[315]. Dasatinib sits in ATP sites enclosed the by two lobes, with amino thiazo moiety of molecule occupying the site. The ATP unoccupied hydrophobic pocket near Thr[315] is probed by 2-choloro 6 methyl phenyl ring of Dasatinib orthogonal to thiazole carboxamide group. The two hetero aromatic CH-O=C interaction with hinged region back bone notable C4 thiazo carbon with carbonyl oxygen of glu[316] and C5 pyrimidine carbon with the carbonyl oxygen of Met[318]. Vander wall interaction of substituted pyrimidine occupying a hydrophobic cleft created by Leu[248] and Gly[321] and that of the 2-choloro 6 methyl phenyl ring occupies a hydrophobic pocket composed of Thr[315] methyl group, Met[290], Val[299], Ile[313] and Ala[380]. The exposed COOH terminal portion of hinged region contacts to piprazine group via Vander wall contacts. The activation ensures activating catalytic machinery by virtue of active conformation; also it provides a binding platform for peptide substrate interactions. The active conformation is stabilized on active loop phosphorylation simultaneously destabilizing inhibitory conformation. Alpha carbon back bone structural alignment of kinase domain of ABL and Src family member LCK adopt a conformation to activate LCK.

Tyr[393] of activation loop of wild type ABL kinase aligns well with phosphorylated Tyr[394] of LCK. The kinase conserved DFG motif, Asp[381]-Phe[382], Gly[383] marks NH2 terminal portion of activation loop. The catalytic base Asp363 interacts with the substrate at its hydroxyl group and Asn[368] forms hydrogen bonding that orients Asp[363]. These residues in ABL-Dasatinib structure super imposed well with activated LCK structure. A conserved Lys271-Glu286 salt bridge orients glycine side chain for interacting ATP phosphatase and maintains an active kinase conformation. There by affirming binding of Dasatinib to activated ABL kinase[30] (Figure 2).

Side chain of Q252 contribute to the distorted P loop conformation by packing up against Y253 which leads to Imatinib interaction and hydrogen bonds with N322, Y253 does not interact with Dasatinib but the hydroxyl of Y253 hydrogen bonds with back bone of R367, an interaction that must not be critical due to Dasatinib against Y253f mutant E255 hydrogen bonds to its own back bone in Dasatinib bound form whereas in Imatinib bound form, it hydrogen bonds with the hydroxyl of Y257, stabilizing Imatinib bound conformation of the p loop.

Q252 points into solvent in the Dasatinib bound form of Abl demonstrating Dasatinib activity against Q252H/R mutations. M244 proximally interacts with the P loop of both Dasatinib and Imatinib bound forms of Abl and change in the identity of this residue causes changes in its interaction with the P loop potentially destabilizing specific inactive Abl conformation which is indispensible for Imatinib binding. H396 conformation which resides in activation loop shows no discernable interaction with protein or inhibitor which explains why H396R mutation doesn't affect Dasatinib activity; on the other hand it is involved in hydrogen bond with Imatinib bound form which stabilizes the inactive conformation of activation loop. The position and conformation of other COOH terminal lobe mutation sites like M351, E355, F359 and F486 are similar between the Dasatinib and Imatinib bond forms of Abl. Dasatinib doesn't reside near F395 residue which explicates its potency and activity against BCR-ABL with mutation in this site.[30]

ABL-Bcr-Abl (Abelson) kinase; SFK- Src family kinase; KIT, CD117; TEC- Tec protein kinase;STE20,-serine/threonine 20 kinase; CAMK2G-calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma kinase; PDGFR-platelet-derived growth factor receptor; FGFR1-fibroblast growth factor receptor 1; IGF1R-insulin like growth factor receptor; JAK-Janus kinase; FLT3-fms-like tyrosine kinasereceptor-3; RET-rearranged during transfection kinase; TRK-tropomyosin-receptor-kinase; MCL-1-myeloid cell leukemia sequence 1; TIE2-tyrosine kinase with immunoglobulin-like and EGF-like domains 2; KDR-kinase insert domain receptor A gene.

COMPARISON OF DASATINIB COMPLEX WITH IMATINIB COMPLEX

Dasatinib has grater potency against BCR-ABL a striking structural difference exist between Abl-Dasatinib complex and ABL-Imatinib complex.(Figure 3) The activation loop in Imatinib Abl complex folds back towards the ATP binding site forming interaction with both the inhibitor and the P loop there by causing an inhibitory kinase conformation. Phe^{382} of DFG motif in Imatinib bound form orients to the ATP binding site, π–stacks with pyrimidine ring of Imatinib leading to an edge to face interaction with $nTyr^{253}$ of the P loop. On the other hand Phe^{382} is sealed in hydrophobic pocket in Dasatinib bound form there by halting the ATP access in contrast to conformation with activated kinases. Although the Dasatinib and Imatinib have overlapping central cores then rest structure is in opposite direction. N-2-hydroxy ethyl papridin group of Dasatinib for which Imatinib lacks counterpart extends out toward solvent exposed protein. In contrast to benzamidin branch of Imatinib Dasatinib does not protrude towards Phe^{382} occupied hydrophobic pocket which illustrate Dasatinib ability to bound in the active conformation. Another significant difference lies in orientation of P loop. An edge to face aromatic interaction between Tyr^{253} of P loop bent towards pyridine and pyrimidine loop of Imatinib. Contrariwise weak electron density is found over comparative extended structure of P loop in Dasatinib bond complex. A hydrogen molecule bonds hydroxy moiety of Tyr^{253} to the back bone carbonyl of Arg^{367} in Abl-Dasatinib complex.

Dasatinib is inhibitor of Src family kinase as it can overcome the resistance due to Src family kinase activation. Further it doesn't bind to BSR-ABL with same stringent conformational requirement it can inhibit a; BCR-ABL kinase domain mutant except T315I. It is also not a substrate of multi drug P-glycoprotein efflux pump and may be therapeutically active in Imatinib and Nilotinib resistant patients. Its specific mode of binding to Abl may lead to new vulnerable sites that could converse new types of drug resistance. Mutation have been found on Phe317 which is the potential vulnerable site for the drug[29] (Figure 2).

Dasatinib is the first dual BCR-ABL kinase inhibitor and Src family kinase inhibitor which efficiently overcome Imatinib resistant Abl mutations. Dasatinib was found to be active against 15 clinically significant Imatinib resistant BCR-ABL mutation comprising 12 altered amino acid locations (T315I, Y253F/H, M244V, H396R, G250E, Q252H/R, E255K/V, F486S, M351T, F359V, E355G and F317L) (Figure 3).

Early recognition of mutant clones offers clinical benefits. Nine mutations associated with the substitution of 6 amino acid residues F317L (TTC3TTG, TTC3TTA, TTC3CTC), V299L (GTG3CTG, GTG3TTG), M244V (ATG3GTG), E355G (ATG3GTG), F311I (TTC3ATC) and F359V (TTC3GTC) are vastly pertinent with respect to resistance to second generation TKIs.[32,33]

RATIONAL DRUG DESIGNING: TARGETED THERAPEUTIC APPROACHES IN OVERCOMING T315I

The frequency of T315I mutation in BCR ABL1 mutated Chronic Myeloid Leukemia is 20.2%.There only two mutation which were clearly resistant to Dasatinib and retained kinase activity on presence of micromolar concentration of Dasatinib. An approach translating amino acids location in mutant in crystal structure of ABL kinase in Dasatinib reveals remarkably significant insights

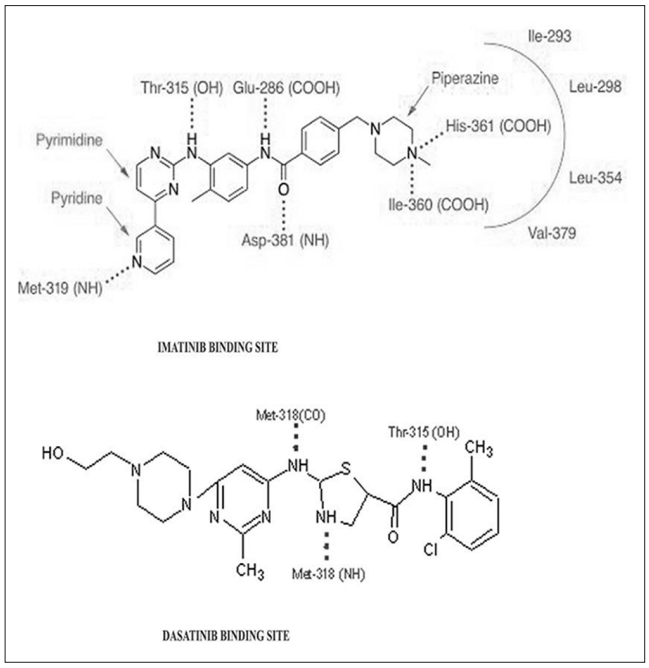

Figure 3: Binding Site of Imatinib & Dasatinib

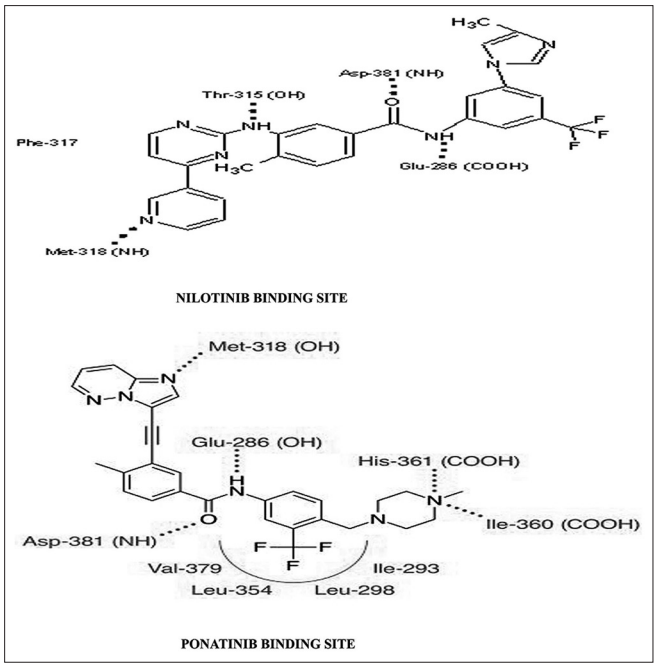

Figure 4: Binding Site of Nilotinib & Ponatinib

regarding the mutations. A Van der wall contact between 2 chloro 6 methyl phenyl group of Dasatinib and side chain methyl group of amino acid location in Abl kinase crystal structure.

It is postulated that T315I mutation is most probably due to loss of a hydrogen bond that involves Dasatinib with the side chain of T315 which is critical contact point for Dasatinib. Another reason for T315 resistance can be due to the increase steric bulk in this pocket. There by it is suggested that the apparent ability of Dasatinib to acknowledge multiple states of the enzyme lead to a greater Abl binding affinity of Dasatinib over Imatinib. Hence a critical interaction between Dasatinib and Thr315 of the hinged region of the fusion complex causes loss of activity against T315I mutation. The higher rate of genomic instability may foster the development overtime of multiple mutation within the same or indifferent BCR-ABL positive sub clones, which are selected or deselected in accordance to the resistance to the inhibitor employed and the spectrum of sensitivity.[34] The T315 is observed in 15% of Ph^+ leukemic patients who are Imatinib positive.[35] To create a specific panel of assays to monitor Nilotinib and Dasatinib resistance the genotyping efforts should be restricted to a small number of limited candidate mutation that confer resistance to a particular drug. Nicolini reported a decrease progression free survival and overall survival for the patients harboring T315I alteration and P loop mutations like G250E, Y253F-H and E255/V (Figure 4).

All though this resistance seems to be troublesome but it can be considered as a downfall of targeted therapy in cml. In the three major retrospective studies exploring the incidence of Abl Kd mutation in Imatinib patients conducted to compare the frequency of T315I mutations and P loop mutation reported that incidence of T315i was 4%, 11% and 19% respectively whereas incidence of P loop mutation was much higher being 28%, 46% and 39% respectively.[35] The T315I mutation as is evidently more prevalent in chronic phase CML.Nevertheless now a day's vast majority of the patients are early chronic phase cml who receive Imatinib at first line therapy.

DISCUSSION

A strategic therapeutic approach is to design the targeted agents which can act with a different mode of action for example the drugs that target the BCR-ABL or BCR-ABL downstream signal transducer alone or in combination with another BCR-ABL kinase inhibitor (Table 1).

Recently histone-deacetylase inhibitor Panobinostat (LBH589) which depletes BCR-ABL and induce growth arrest and causes death of cells expressing t315i BCR-ABL, when administered alone and highly effective when administered with Nilotinib.[26] Another histone-deacetylase inhibitor Vorinostat also called as Suberoylanilide Hydroxamic acid (SAHA) when given alone or in combination with Dasatinib has shown promising results in several malignant conditions.[31] Many trials are assessing combination of Vorinostat with conventional agents to overcome various resistances in CML patients. Panobinostat (LBH589)

Table 1: Emerging novel small molecule multi-kinase inhibitors in chronic myeloid leukemia and their potential targets

Initial name	Official name	Sponsors	Major targets/potential drug targets	Inhibits growth of CML cells bearing BCR/ABL1 T315I	Histologist under investigation
AMN107	Nilotinib	Novartis	ABL, KIT, PDGFR,	No	CML Ph+, lymphoma
BMS354825	Dasatinib	BMS	Abl, Src, Lyn, Btk, Kit, PDGFR,.	No	CML Ph+
INNO-406	Bafetinib	CytRx	Abl, Lyn, wtKit,	No	CML
PHA-739358	Danusertib	Nerviano Medical Sciences	Abl, AuK, Ret, Trk-A Aurora A&B, FGFR1, RET, TRK.	Yes	CML, myeloma, prostate
KW-2449		Kyowa Hakko Kirin Pharma	Abl, AuK Aurora A, FGFR1, FLT3	Yes	CML, AML
SKI-606	Bosutinib	Pfizer, Inc	ABL, CAMK2G SFK, STE20, TEC		Ph+ CML, breast cancer
AT9283		Astex Therapeutics	Abl, AuK, JAK2, JAK3, ABL, Aurora A&B, FLT3, JAK2, JAK3	Yes	CML, AML, ALL, MDS, Myelo fibrosis, NHL, solid cancers
XL228		Exelixis	Abl, AuK, Src, IGF1R Aurora A, FGFR1-3, Phase I	Yes	CML, Ph+ ALL, lymphoma, myeloma, solid tumors
DCC-2036	Rebastinib	Deciphera Pharmaceuticals	Abl, FLT3, KDR SFK, TIE2	Yes	CML, Ph+ ALL
MK-0457	Tozasertib	Merck	ABL, Aurora A&B, FLT3, JAK2	Yes	CML, ALL, MDS

and Vorinostat have illustrated attenuation of ATP binding and Chaperone function HSP90 by inhibiting histone deacetylase 6 and inducing acetylation of heat shock protein 90 (HSP90) which causes proteasomal degradation, polyubiquitylation and depletion of HSP90-client proteins, including BCR ABL and downstream effectors C-RAF and AKT. Both histone-deacetylase have demonstrated the ability to induce apoptosis in human leukemia cells via accumulation of pro-apoptotic proteins (Bax, Bim) coupled with depletion of anti-apoptotic factors (Bcl2, Bcl-xl, surviving) which enhance the effects of BCR ABL inhibitors.

Another a dual Abl/Lyn kinase inhibitor Bafetinib is reported to be 25 to 55 folds potent than Imatinib in vitro and at least 10 fold as effective as Imatinib mesylate in suppressing the growth of BCR ABL bearing tumors. It has also demonstrated activity in 12 of 13 Imatinib resistance cell lines. Another molecule undergoing phase II clinical trial Tozasertib is pan aurora inhibitor mostly against aurora A with Kiapp of 0.6 nM, less potent toward aurora B/aurora C and 110 fold more selective for aurora A than 55 other kinases. It induces toxicity with Ic50 of approximately 300 nM and exhibits an AURB like inhibitory phenotype of G2/M arrest, apoptosis in BaF3 cells transfected by Abl or FLT-3 mutant and wild type kinases and endoreduplication. Histone H3 phosphorylation is revoked subsequent to Tozasertib treatment.

F317L is another significant mutation that turns out to be a problematic mutant in Dasatinib resistance. F317 was observed to make off-center aromatic π stacking with pyrimidine and thiazo ring of Dasatinib which explains slight loss in activity of Dasatinib against F317L mutation.[30] G250E mutation reduces the flexibility of P loop and inhibit P loop to adopt Imatinib induce conformation whereas Dasatinib exhibits an improve activity against G250 mutation which arises due to low priority of P loop interaction of Dasatinib.[30]

Ponatinib is a multi targeted tyrosine kinase inhibitor targets Abl, fibro blast growth factor receptor 1 (FGFR1), fms-like tyrosine kinase receptor-3(FLT3), vascular endothelial growth factor receptor, KIT and potent inhibitor of T315I mutation and indicated in Ph^+ CML, acute lymphoblastic leukemia and other advanced haematological malignances. X-ray crystallographic analysis of Ponatinib in T315I BCR-ABL mutated kinase demonstrates that imidazole pyridazine core rest in adenine pocket of enzyme (Figure 1). The methyl phenyl group occupies a hydrophobic group behind t315, the ethylene linkage forms a favorable Vander wall interaction with amino acids and the trifloro methyl group binds to pocket induced by the active conformation kinase, in the hinged region 5 hydrogen bonds are generated with the back bone of Met318 along with the side chain Glu286 and protonated methyl piprazine with the back bone carbonyl atoms of Ile360 and His361.

CONCLUSION

With many novel drug molecule availability of second and third generation tyrosine kinase inhibitors and stem cell targeting drugs promise a brighter outlook of success in CML patients. The propensity of Ph^+ CML to develop novel mechanism of resistance offers a formidable challenge and has stimulated the research in numerous dynamic arenas like designing rational therapeutic approaches to eradicate the minutest residual diseases so

that risk of late term resistance is eliminated. Alternatively primary therapeutic strategies can be further explored to abate primary resistance. Additional novel therapies combined with established therapies can be investigated to treat primary and secondary resistance.

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Authors Contribution:
RR – Designed the review; developed the protocol; **AS –** Collaborated in the design of the review and double checked eligibility and quality; **DK –** Identified selected, extracted and entered data; **SD –** Analyzed the data, wrote the first draft of the manuscript and updated the review & applied eligibility criteria, extracted data; **DP –** Reviewed the draft and final versions of the manuscript, critically appraised the studies to be included; **SR –** reviewed the draft and final versions of the manuscript

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