Document heading doi: 10.21276/apjhs.2019.6.1.9 **Research Article**

Therapeutic approaches to enhance the BCR-ABL tyrosine kinase inhibitors efficacy in chronic myeloid leukemia

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Abstract

Chronic Myeloid Leukemia (CML) is a well-known myeloproliferative disease characterized by the presence of the Philadelphia chromosome and its oncogenic product, BCR-ABL, a constitutively expressed tyrosine kinase that is present in the majority of the patients. The treatment of CML was transformed by the introduction of imatinib mesylate commercially known as Gleevec; a BCR-ABL tyrosine kinase inhibitor (TKI) which dramatically increased the survival rate and quality life of the patients. Despite having high efficacy majority of the patients have developed resistance to imatinib. To overcome this problem, new treatment options are required to treat CML patients. In this regard, the fragment-based drug discovery is a novel and generic methodology which uses the combination of targeted chemotherapeutic drugs with the suitable fragments from the screened library. These ligand-efficient individual fragments examine the chemical space of the target transporter protein, covering a relatively large area due to their small sizes, may provide a way to future medicine to treat CML patients.

Keywords: BCR-ABL, Chronic Myeloid Leukemia, Maybridge Rule of 3 Fragment Library, Philadelphia Chromosome, Tyrosine Kinase Inhibitor.

Introduction

The genetic basis of cancers is commonly seen in hematological malignancies due to the presence of chromosomal abnormalities. That hematological disorder includes the pre-leukemias, myeloproliferative disorders and acute leukemias. Chronic Myeloid Leukemia (CML) with its well-defined genetic characterization has given an opportunity for both molecular understanding of cancer progression and advancement of tools to study them. CML is characterized by the increased proliferation of hematopoietic stem cells [1-3]. CML is genetically distinguished by the presence of the reciprocal translocation t (9;22), (q34; q11); the Abelson proto-oncogene (abl) on chromosome 9 is translocated to the Breakpoint Cluster Region (bcr) on chromosome 22, forming a fusion gene in which ABL-related tyrosine kinase activity is constitutively activated [2, 4, 5].

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Diagnosis of CML

The BCR-ABL fusion protein facilitates the growth and development of CML through interaction with numerous downstream signaling pathways, resulting in changed cellular adhesion, mitogenic signaling activation, and apoptotic inhibition leads to the alteration of hematopoietic stem cells [6]. The defective DNA repair is also associated with BCR-ABL mediated signaling, resulting in further chromosomal aberrations and mutations, which is the primary cause of violent nature of advanced phase of CML [7]. CML is the highly studied neoplasm [8] and it accounts for approximately 15% of all new cases of leukemia, affecting about 1-2:100,000 adults per year. This disorder affects mostly males (1.4/ 1.3 %) with a median age of 57-60 years [9-11]. The data provided by Indian Cooperative Oncology Network (2010) obtained from cancer research institutes across India on the basis of clinical examination of CML patients have shown the difference in the incidence of CML cases i.e., 16.6% at Gujarat Cancer and Research Institute (GCRI), Gujarat, to 70% at Indira Gandhi Institute of Medical Sciences (IGIMS), Regional Cancer Centre (RCC), Patna, Bihar, India

[12]. Since, the generation of tyrosine kinase inhibitors (TKIs), the survival rate and quality of life of CML patients have potentially increased.Despite the potentially high efficacy of imatinib, not all but approximately 33% of patients have weak response to imatinib [13, 14]. Current studies have revealed that resistance to imatinib may develop from some mechanisms. One of the main reasons for the treatment failure is the inadequate response due to the point mutation in the ABL kinase domain, leading to a structuralchange in the domain and overexpression of BCR-ABL [15-17] (Figure 1).The only known risk factor that cause damage to the human body in younger people is exposure to dose dependent radioactive radiation which cause malignancies, particularly leukemia[18]. About 40% of CML patients are asymptomatic, and diagnosis is established during routine blood tests [19]. The typical symptoms of the patients are fatigue, weight loss, night sweats and abdominal discomfort due to splenomegaly [20]. The natural history of CML can be progressed through three distinct clinical stages termed chronic phase (CP), accelerated phase (AP) and blast crisis (BC) [21, 22].

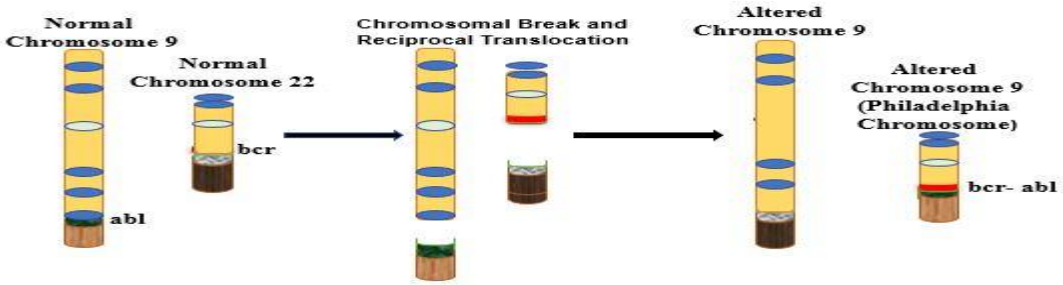

Figure 1: A schematic image of a reciprocal translocation between chromosomes 9 and 22. Thetranslocationbetween the two chromosomes is characterized by Philadelphia chromosome in which ABL-related tyrosine kinase activity is constitutively activated.

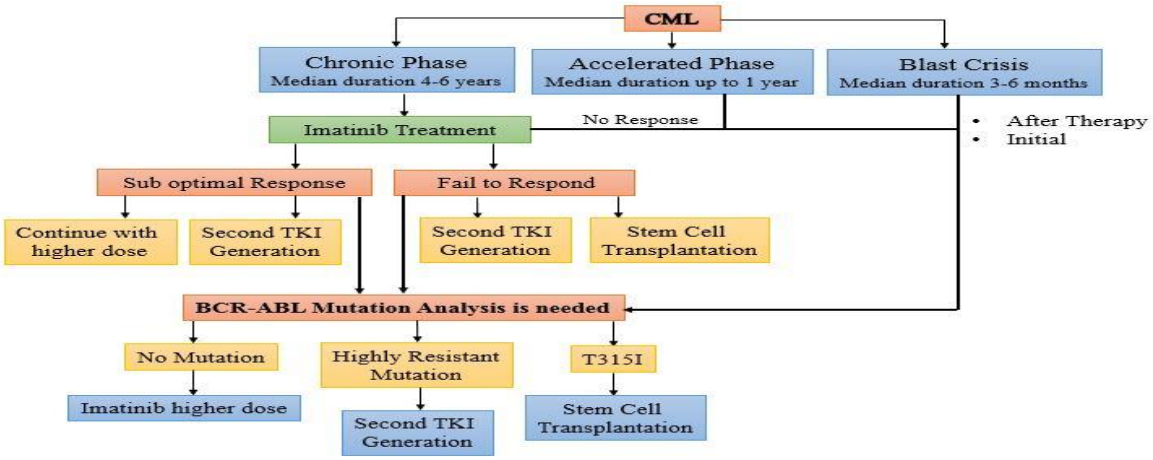

Figure 2: The clinical procedure for CML therapy is dividedinto chronic, accelerated and blast phase. The response of imatinib treatment has been illustrated in the figure. For each response either the continuation of therapy with imatinib or next generation, TKIs will be assessed.

The therapeutic response can be evaluated by the absence of measurable pH^+ related translocations in the bone marrow or peripheral blood [23]. The cytogenetic examination has a restricted role in the detection and follow-up of minimal residual disease (MRD),and it has a crucial role in the early recognition of inevitablehematological decline [24]. The occurrence of any residual leukemic cells evading the detection sensitivity of conventional diagnostic approaches is termed MRD [25]. Numerous molecular techniques are being used to detect the *BCR-ABL* gene. Fluorescent in situ hybridization (FISH) is a technique which allows quick evaluation of several hundred cells in a time

regulated manner. FISHis naturally performed by co-hybridization of a *BCR* and *ABL* probe to denatured metaphase chromosomes or interphase nuclei. Southern blot analysis has limited sensitivity and cannot be used to evaluate minimal residual disease. It is widely used in Ph-negative patients to detect Ph-negative, *BCR-ABL*-positive CML. Detection and quantification BCR-ABL protein may be performed by Western blot analysis also. It allows the detection of the three BCR-ABL protein isoforms [26]. PCR is one of the most sensitive molecular techniques which is highly suitable for the evaluation of minimal residual disease [27, 28]. The broad implementation of strategies for stratified medicine MRD is useful, as the therapies lead by quantitative molecular measurements are likely to become more usual [3]. Bringing all these points together, it is clear that most of the patients have undetectable amounts of BCR-ABL and still continue to undergo a rapid decline with the use of imatinib and other TKIs that efficiently improved patient survival rate [29].

Treatment options for CML

Since 1850, many treatment options were recognized for CML disease. Treatment choices in CML patients are based on the patient's age and clinical phase of the disease [30]. Treatment choices categorized in three classes: potentially curative therapy, palliative and targeted therapy and combination therapy-personalized medicine (Figure 3).Busulfan was used as the initial agent to deliver effective hematologic control in patients with CML, but its use should be discouraged outside the setting of preparative regimens for allogeneic SCT [31]. Allogeneic SCT is associated with significantly poor survival, worse outcome after allogeneic SCT, and highlysevere side effects including delayed myelosuppression and organ damage [32, 33]. Hydroxyurea has 50–80% hematological response and is an outstanding debulking agent that permits the fast control of the blood count [34]. But, it has rare cytogenetic responses and does not appear to change the natural history of CML. However, it should not be considered as the final therapy for CML. The other palliative therapies include 6-mercaptopurine, 6-thioguanine, cytarabine, melphalan, other chemotherapies.

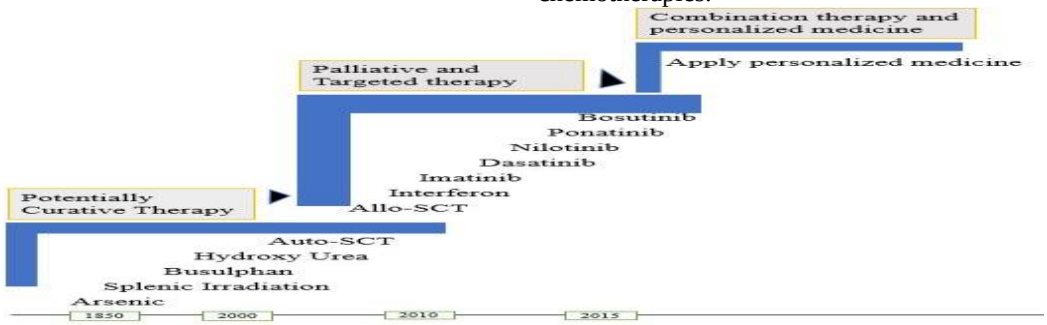

Fig 3:The picturedepicts three different therapeutic classes of CML which have broadly categorized into potentially curative therapy, palliative and targeted therapy and new combination therapy and personalized medicine

The newly synthesized anticancer drugs are not broadly cytotoxic but target proteins, as they are crucial for the growth and development of cancer cells. Such treatments options are often mentioned as targeted therapy and are commonly used along with traditional chemotherapeutic agents as follows:

i. **Alkylating agents:** The oldest group of chemotherapeutics currently in use. Mainly derived from mustard gas used in the war field, and can alkylate various molecules, including proteins, RNA and DNA.

ii. **Anti-metabolites:** These resemble nucleosides and delay DNA and RNA synthesis by either blocking the enzymes required for synthesis.

iii. **Anti-microtubule agents:** These are plant-derived chemicals that block cell division by inhibiting microtubule function.

iv. **Topoisomerase inhibitors:** Topoisomerase inhibitors can affect the activity of two enzymes: topoisomerase I and topoisomerase II.

v. **Cytotoxic antibiotics:** These antibiotics are a diverse group of drugs that have numerous mechanisms of action.

vi. **Tyrosine Kinase Inhibitors (TKIs):**It is a type of pharmaceutical drug which hinders tyrosine kinases. Tyrosine kinases are enzymes which are responsible for the activation of many proteins by signal transduction cascades. The activation of proteins takes place by adding a phosphate group to it (phosphorylation).

Table 1: Classification of chemotherapeutic agents in classes and their respective sub-types with the examples used for the treatment of various cancers

S. No.	Class	Subtype	Example	Uses
1	Alkylating agents	Nitrogen mustards	Mechlorethamine, Cyclophosphamide, Melphalan, Chlorambucil, Ifosfamide, Busulfan	Breast cancer, Lung cancer, Lymphocytic leukemia, Myelogenous leukemia
		Nitrosoureas	N-Nitroso-N-methylurea, Carmustine, lomustine, Streptozotocin	Hodgkin's disease, Myelogenous leukemia
		Tetrazines	Dacarbazine, Mitozolomide, Temozolomide	Hodgkin's disease, Myelogenous leukemia
		Aziridines	Thiotepa, Mytomycin, Diaziquone	Hodgkin's disease, Germ cell tumor
		Cisplatin and derivatives	Cisplatin, Carboplatin, Oxaliplatin	Germ cell tumor, Stomach cancer Bladder cancer Colorectal cancer
		Nonclassical alkylating agents	Procarbazine, Hexamethylmelamine.	Hodgkin's disease
2	Anti-metabolites	Anti-folates	Methotrexate, Pemetrexed	Breast cancer, Bladder cancer, Lymphocytic leukemia
		Fluoropyri midines	Fluorouracil, Capecitabine	Breast cancer, Stomach cancer, Colorectal cancer
		Deoxy-nucleoside analogues	Cytarabine, Gemcitabine, Decitabine, Vidaza, Fludarabine, Nelarabine, Cladribine, Clofarabine, Pentostatin	Stomach cancer, Lymphocytic leukemia
		Thiopurines	Thioguanine, Mercaptopurine	Lymphocytic leukemia
3	Anti-microtubule agents	Vinca alkaloids	Natural: Vincristine, Vinblastine Synthetic: Vinorelbine, Vindesine, Vinflunine	Hodgkin's disease, Bladder cancer Lung cancer
		Taxanes	Paclitaxel, Docetaxel	Tumor
		Podophyllotoxin	Etoposide, Teniposide	Germ cell tumor
4	Cytotoxic antibiotics	Anthracyclines	Doxorubicin, Daunorubicin, Pirarubicin, Aclarubicin, Mitoxantrone	Breast cancer, Bladder cancer, Lymphocytic leukemia
		Derivatives	Epirubicin, Idarubicin	Stomach cancer
		Others	Actinomycin, Bleomycin, Plicamycin, Mitomycin	Hodgkin's disease, Germ cell tumor

5	Topoisomerase inhibitors	Topoisomerase I	Camptothecin: Irinotecan, Topotecan	Metastatic colon or rectal cancer
		Topoisomerase II	Poisons: Etoposide, Doxorubicin, Mitoxantrone, Teniposide	Hodgkin's disease, Lung cancer
			Catalytic inhibitors: Novobiocin, Merbarone, Aclarubicin	Breast cancer, Tumor
6	Tyrosine Kinase Inhibitors	PGDFR	Imatinib, Nilotinib, Dasatinib, Bosutinib, Thiadiazole Derivatives	Colorectal cancer, Lymphocytic leukemia, Myelogenous leukemia

BCR-ABL Tyrosine Kinase Inhibitors therapy: Tyrosine kinases are a different class of enzymes that catalyze the transfer of a phosphate group from adenosine triphosphate (ATP) to target proteins that play a vital role in cell signal cascade that regulate cell proliferation, differentiation, anti-apoptotic signal transduction andprogrammed cell death [35]. The increasing research output has confirmed that imatinib exerts major immunomodulatory effects on lymphoid cells such as antigen-presenting cells and dendritic cells [36].Current research in the field of molecular targeted therapieshas transformed cancer treatment. The application of TKI therapy could be considered as a model for the successful treatment of cancer [37]. In the United States, five TKIs have been approved for the treatment of CML including imatinib, dasatinib, nilotinib, bosutinib and ponatinib [38, 39]. High-throughput *in vitro* analysis for TKIs confers 2-phenylaminopyrimidine formerly known as a signal transduction inhibitor 571 (STI 571) as a primary compound was optimized for its activity forparticular kinases on CML cell lines which is popularly known as Imatinib or Imatinib mesylate [40]. Food and Drug Administration (FDA; 2001) approved the replacement of IFN-α with Imatinib mesylate, a first TKI for CML as a standard treatment [2]. Imatinib also showeffect on other molecules of the metabolism and receptors for cell signaling, for example, BCR-ABL, ABL, ARG, PDGFR- α / β, C-kit receptor, and c-FMS receptors etc [41]. Clinical evidence indicates that Imatinib mesylate acts as an effective and long lasting TKI therapy for CML patients and it should be monitored carefully to evaluate their response to treatment and the detection of early decline. It includes three different kinds of responses; hematological response (HR), cytogenetic response (CR) and molecular responses (MR) [42-44]. A complete hematologic response (CHR) can be defined as the normalization of blood counts and spleen size. Measure the blood counts every two weeks and then testing for every three months till CHR is achieved. Cytogenetic response is the most extensively used method to observe the response in CML patients. The cytogenetic response can be determined by the decrease in the number ofPh-positivemetaphasic cells by the use of both bone marrow aspiration and cytogenetic evaluation systems. The complete absence of Ph-positivemetaphasic cells leads to a complete cytogenetic response (CCR). A molecular response is determined by lowering the amount of BCR-ABL chimeric mRNA. The complete absence of detectable BCR-ABL chimeric mRNA as measured by reverse transcriptase polymerase chain reaction (RT-PCR) is referred to as complete molecular response (CMR). All tyrosine kinase inhibitors being easily absorbed in the gastrointestinal tract which makes them highly suitable for the treatment. In addition, not only first TKI generation medicines like imatinib but other TKI generations also inhibit several different signal transduction pathways, which are listed below in the table-2.In cancer, mutant kinases frequently act as oncogenes that promote tumor cell survival, growth or replication by causing aberrations in proliferation, apoptotic resistance, angiogenesis and adhesion through interference with β-integrin signaling and multiple downstream pathways during metastasis [45, 46]. There is significant evidence that malignant transformation of hematopoietic cells by BCR-ABL protein depends on its TK activity [3]. The inactivationof the BCR-ABL signal transduction pathways and prevention of disease development to treat CML is promoted by TKI molecule which acts as a "smart drug" [47]. TKIs decrease the number of malignant cells by competitive inhibition at the ATP-binding site within BCR-ABL protein,which then leads to closed or inactive configuration and inhibition of the enzyme activity on substrate proteins [48, 49]. ATP prevents tyrosine phosphorylation and downstream signaling, resulting in arrested cellular growth and apoptosis [50]. The process of competitive inhibition of imatinib can be seen in the given figure.

Table 2: The TKI generations with their related targets and binding sites, with approval year

TKI Generations		Targets	Binding Site/ Inhibitor type	Approval Year
First Generation	Imatinib	Inactive BCR-ABL, PDGFR, ARG, KIT, VEGF, DDR1	ATP Binding site/ ATP Competitive	2001
Second Generation	Dasatinib	ARG, TEK, BTK, Active & Inactive BCR-ABL, SRC, DDR1, EPHA2, PDGFR	ATP Binding site/ ATP Competitive	2006
	Nilotinib	ARG, KIT, VEGFR, Inactive BCR-ABL, DDR1, NQO2, PDGFR,	ATP Binding site/ ATP Competitive	2007
Third Generation	Bosutinib	BCR-ABL, SRC, TEK, CAMK2G, C-Kit, PDGFR	ATP Binding site/ ATP Competitive	2012
	Ponatinib	All mutated forms of BCR-ABL, T351I, FGFR, TIE2, VEGFR	ATP Binding site/ ATP Competitive	2012

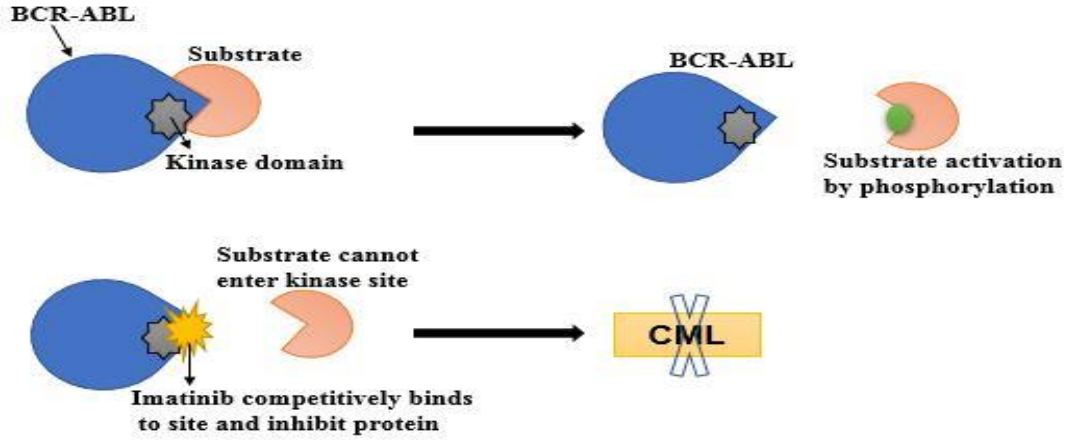

Figure 4: **Schematic representation of the mechanism of imatinib action which acts as a competitive inhibitor at the BCR-ABL protein ATP-binding site. The imatinib blocks the binding of ATP to the BCR-ABL tyrosine kinase and inhibit constitutive pathways leading to an inactive configuration and the inhibition of TK activity**

The overall survival of CML patients has dramatically increased with the use of TKIs. While during the introduction period of imatinib the five-year overall survival rate was 24%, which increased steadily to 39% (1966-1998) and 56.8% (1999-2006) and the overall survival was estimated to 85% [2, 51]. On the other hand, lack of adherence to TKIs inevitably results in disease progression and treatment resistance [52]. Some non-ATP competitive inhibitors such as ONO12380 [53], the Aurora kinase inhibitor MK-0457 (VX-680) [54], and the p-38 inhibitors BIRB-796, SGX-70430 [55] are in clinical trial to overcome imatinib, nilotinib, or dasatinib resistance in CML patients.Although the clinical consequence of these new target drugs remains to be explained, it may be possible that these drugs play a critical role in the efficacy and safety of these drugs.The growing evidence indicating that TKIs exerts significant

immunomodulatory side-effects include nausea, diarrhea, vomiting, muscle pain and fatigue. Almost 30% of individuals using TKIs show itchy skin rashes including myelosuppression [43,56]. Overall, TKIs have changed CML from a life-threatening disease (10-20% mortality/ year) to a chronic disease that can be managed by oral medicines (1-2% mortality/year) [19].

The mechanism of resistance to TKI Treatment

Evidence from clinical trials and *in vitro* analysis has shownthat cells treated with TKIs tend to nullify the inhibitory effects of these agents [57]. The rate of imatinib resistance within four years was reported up to 20%, increasing to 70-90% in accelerated phase/ blastic phase in CML patients [58-60]. Indeed, chromosomal abnormalities like trisomy in chromosome number 8 and translocation of oncogenes occur, and due to these abnormalities, TKI resistance occurs that results to impaired treatment ([46, 61]. Based on the clinical trials of the disease, resistance to treatment can be sub-divided into primary and secondary types. When the frontline therapy has not achieved the expected response is called Primary resistance [44] whereas secondary resistance develops after a targeted response to the frontline therapy was completed but subsequently lost [47]. Moreover, the primary resistance can be sub-divided into either primary hematologic resistance or cytogenetic resistance. Additionally, for secondary resistance, the following criteria are in practice, i.e., loss of complete hematologic remission, loss of complete cytogenetic response, loss of major molecular remission, detection of kinase mutations with a known failure of sensitivity to imatinib, and clonal evolution [62]. Almost about 33% CML patients have shown that imatinib treatment is not much effective due to primary/secondary resistances or due to intolerance after six years evaluation of the IRIS study [63]. According to many pre-clinical and clinical studies, from a mechanistic perspective, the emergence of resistance to treatment observed in some CML patients can be categorized into BCR-ABL-dependent and BCR-ABL independent mechanisms. BCR-ABL-dependent mechanisms include BCR-ABL mutations and amplification, along with impaired signaling pathways. BCR-ABL-independent mechanisms include drug efflux, mediated by ATP-binding cassette (ABC) Transporters, deficient base excision repair (BER) for chromosomal abnormalities. Overall, understanding the underlying causes of resistance is a crucial step towards battling CML [64]. BCR-ABL independent mechanisms are characterized by deregulations in drug transport [61, 65]. On the other hand, BCR-ABL-dependent mechanisms are characterized by the emergence of mutations in several structural sub-units related to tyrosine kinase domain, and can be divided into four groups: 1. mutations caused by direct binding of TKI, 2. mutations in the ATP binding site, 3. mutations in the activation loop, 4. mutations in the catalytic domain [66]. However, not all mutations cause clinical resistance to imatinib. The most common mutations which together account for about 2/3rd of all mutations include T315I, E255D/K/R/V, Y253F/H, G250A/E (P- or glycine-rich loop), H396P, M351T and F359C/L/V/R (C-lobe). T315I mutation is the muchcommon type (about 15–20%) and induces resistance to nearly all clinically available TKIs, except ponatinib. Current studies in CML patients treated with imatinib revealed mutations in regions also exterior of the kinase domain [67]. Thus, the clinical significance of these mutations for almost all CML patients is currently unclear.

Strategy to enhance the TKI efficacy

With the growing need to modernize the treatment process, screening of fragment libraries has become an essential tool for this program. The fragment-based drug discovery is a novel and generic methodology which uses the combination of chemotherapeutic drugs with fragments from the Maybridge Rule of 3 Fragment Library (MBF) to further direct the nucleoside analog drugs towards their transporters.These ligand-efficient fragments examine the chemical space of the target transporter protein, covering a relatively large area due to their small sizes. This may lead to greater transporter binding, which in turn increases the intracellular transportation of drug and consequently its drug efficacy. However, the success rates are often low, and several hits fail to develop into optimization [68, 69]. The understanding aboutfragment-based screening to get accurate 'hits' is rapidly developing by accelerating the drug discovery process. A general approach for hit identification is high-throughput screening (HTS) [70]. The Maybridge library constructs via choosing thousands of fragments from the Maybridge collection of more than 70,000 compounds are screened to assess their biological property against a target.The Maybridge Ro3 Fragment Libraries are pharmacophorically-rich compounds. Fragment-based screening has a benefit that a few hundred to a few thousands of compounds screened because of their lower complexity and have a higher possibility of matching to a target protein binding site [71]. The affinity of the compound is one of the first criteria taken in consideration for hit selection and optimization during subsequent stages of drug discovery. Moreover, affinity is often optimized through the introduction of lipophilic groups (cLogP),

as these contribute favorably to the hydrophobic effect without the need for specific interactions with the target. Additionally, molecular size (M_r) should also be taken with an affinity to prevent misleading in alone [72]. However, 'Lipinski's Rule of 5' [73] is used to maximize the probability of surviving development of an oral drug based on the number of rotatable bonds [74]. Similarly, Congreve and his colleagues (2003) proposed a 'Rule of 3' (Ro3)[75] for fragment library which is based upon a structural study of fragments that bind to a number of kinases and protease targets. The fragment hits with the following physicochemical properties allow an excellent opportunity for the development of leads with superior ADME attributes.

Table3: The table illustrates the criteria for fragment- like (Rule of Three) and drug-like (Rule of Five) molecules for the development of fragment hits for drug discovery

Type of Compounds	Fragment-like	Drug-like
Rule	Rule of Three* [75]	Rule of Five [73]
Thresholds Molecular Weight (Mr)	≤300	≤500
Lipophilicity (cLogP)	≤3	≤5
H-bond donor	≤3	≤5
H-bond acceptor	≤3	≤5
*The number of rotatable bonds ≤ 3 and polar surface area ≤ 60 $(A^0)^2$ as additional criteria.		

A group of researchers have documented a Maybridge compoundthat targetInositol-3-phosphate synthase (INO1) and it has been genetically authenticatedas a drug target for *Trypanosoma brucei*, the causative agent of African sleeping sickness. The compound that interacts with *T. brucei*INO1 was employed to screen about670 compounds from May Ro3 fragment library and checked for their trypanocide and cytotoxic activities [76]. Unfortunately, many of these compounds are cytotoxic against mammalian cells, and therefore they are unlikely to proceed as a lead compound. However, the biological activities of associated compounds such as 2-amino-phenol or 269e have anti-microbial activity[76]. Therefore, this phenomenon is reflected in the general trend towards the generation of desired compounds during the hit optimization process. It will be more interesting to watch the future direction of further developments in this field.

Conclusion

The diagnosis of CML patients has significantly enhanced over the last couple of years. Whereas the average survival rates was ranging from 4-6 years during the different clinical stages of CML. The revolutionary development of imatinib has signified one of the major breakthroughsin the treatment of CML patients. However, imatinib and other TKIs efficiently improved patient survival rate, but their complete molecular remission (CMR) has been achieved by a small number of CML patients only. Therefore, the discovery of new compounds that can inhibit BCR-ABL is vital to curing the disease. Additionally, it has been evident from the result of *T. brucei* that combination of May Ro3 fragment library with the drug has the potential to cure the disease. Similarly, May Ro3 fragment library with TKIs may able to interfere with specific signaling pathways like PI-3-kinase and proteasome inhibitors of tumor cells and has a significant effect on membrane transporters to cure the disease. Therefore, it would likely to be an classical model in changing our therapeutic thinking and approach, not only in CML but other malignancies as well.

Abbreviations

ARG**:** abl related gene, BCR-ABL**:** Break point cluster region-Abelson proto-oncogene, BTK**:** Bruton's tyrosine kinase, CAMK2G**:** Calcium/calmodulin-dependent protein kinase type II gamma chain, C-Kit**:** tyrosine-protein kinase Kit, **DDR1**:Discoidin domain receptor, EPHA2**:**Ephrin type-A receptor 2 precursor, E255D/K/R/V: Glutamic acid (E) to Aspartic acid (D)/ Lysine (K)/ Arginine (R)/ Valine (V) mutation at position 255, F359C/L/V/R: Phenylalanine (F) to Cysteine (C)/ Leucine (F)/ Valine (V)/ Arginine

mutation at position 396, FGFR**:Fibroblast growth factor receptor,** G250A/E: Glycine (G) to Alanine (A)/ Glutamic acid (E) mutation at position 250, H396P: Histidine (H) to Proline (P) mutation at position 396, M351T: Methionine (M) to Threonine (T) mutation at position 396, NQO2**:** NAD(P)H dehydrogenase, quinone 2, PDGFR**:**Platelet-derived growth factor receptor, SRC**:** Src family kinase, STI571**:** Signal transduction inhibitor 571, TEK**:** TEK receptor tyrosine kinase, TIE2**:**Angiopoietin receptor, T315I**:** Threonine (T) to Isoleucine (I) mutation at position 315, VEGFR**:**Vascular endothelial growth factor receptor, Y253F/H: Tyrosine (Y) to Phenylalanine (F)/ Histidine (H)mutation at position 253.

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References

1. Deininger, M. W. N. Optimizing therapy of chronic myeloid leukemia. Experimental Hematology 2007;35(4):144-154.
2. Karimiani, E. G., Marriage, F., Merritt, A. J., Burthem, J., Byers, R. J. and Day, P. J. Single-cell analysis of K562 cells: an imatinib-resistant subpopulation is adherent and has upregulated expression of BCR-ABL mRNA and protein. Experimental Hematology 2013;42(3):183-191.
3. Verma, M., Karimiani, E. G., Byers, R. J, Rehman, S., Westerhoff, H. V. and Day, P. J. Mathematical modelling of miRNA mediated BCR.ABL protein regulation in chronic myeloid leukaemia vis-a-vis therapeutic strategies. Integrative Biology (Camb) 2013;5(3):543-554.
4. Markose, P., Chendamarai, E., Balasubramanian, P., Velayudhan, S., Srivastava, V. and Mathews, V. Spectrum of BCR-ABL kinase domain mutations in patients with chronic myeloid leukemia from India with suspected resistance to imatinib-mutations are rare and have different distributions. Leukemia and Lymphoma 2009;50(12):2092-2095.
5. Wieczorek, A. and Uharek, L. Management of Chronic Myeloid Leukemia Patients Resistant to Tyrosine Kinase Inhibitors Treatment. Biomark Insights 2015;10(3):49-54.
6. Sawyers, C. L. 3 Signal transduction pathways involved in BCR-ABL transformation. Bailliere's Clinical Haematology 1997;10(2):223-231.
7. Calabretta, B. and Perrotti, D. The biology of CML blast crisis. Blood2004;103(11): 4010-4022.
8. Caldemeyer, L., Dugan, M., Edwards, J. and Akard, L. Long-term side effects of tyrosine kinase inhibitors in chronic myeloid leukemia. Current Hematologic Malignancy Reports 2016;11(2):71-79.
9. Chen, R. and Chen, B. The role of dasatinib in the management of chronic myeloid leukemia. Drug Design, Development and Therapy 2015;9:773.
10. Hoglund, M., Sandin, F. and Simonsson, B. Epidemiology of chronic myeloid leukaemia: an update. Annals of Hematology 2015;94(2):241-247.
11. Trivedi, D., Landsman-Blumberg, P., Darkow, T., Smith, D., McMorrow, D. and Mullins C. D. Adherence and persistence among chronic myeloid leukemia patients during second-line tyrosine kinase inhibitor treatment. Journal of Managed Care and Specialty Pharmacy 2014;20(10):1006-1015.
12. Singhal, M. K., Sengar, M. and Nair, R. Summary of the published Indian data on chronic myeloid leukemia. South Asian Journal of Cancer 2016;5(3):162-164.
13. Alvarado, Y., Kantarjian, H., O'brien, S., Faderl, S., Borthakur, G., Burger, J., Wierda, W., Garcia-Manero, G., Shan, J. and Cortes, J. Significance of suboptimal response to imatinib, as defined by the European Leukemia Net, in the long-term outcome of patients with early chronic myeloid leukemia in chronic phase. Cancer: Interdisciplinary International Journal of the American Cancer Society 2009;115(16):3709-3718.
14. Druker, B. J., Guilhot, F., O'brien, S. G., Gathmann, I., Kantarjian, H., Gattermann, N., Deininger, M. W., Silver, R. T., Goldman, J. M., Stone, R. M. and Cervantes, F. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. New England Journal of Medicine 2006;355(23):2408-2417.
15. Apperley, J. F. Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. The Lancet Oncology 2007;8(11):1018-1029.
16. Elias, M. H., Baba, A. A., Azlan, H., Rosline, H., Sim, G. A., Padmini, M., Fadilah, S. A. W. and Ankathil, R. BCR-ABL kinase domain mutations, including 2 novel mutations in imatinib resistant Malaysian chronic myeloid leukemia patients—Frequency and clinical outcome. Leukemia Research 2014;38(4):454-459.
17. Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N. and Sawyers, C. L. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001; 293(5531), 876-880.
18. Tsushima, H., Iwanaga, M. and Miyazaki, Y. Late effect of atomic bomb radiation on myeloid disorders: leukemia and myelodysplastic syndromes. International Journal of Hematology 2012;95(3):232-238.

19. Bayraktar, S. and Goodman M. Detection of BCR-ABL Positive Cells in an Asymptomatic Patient: A Case Report and Literature Review. Case Reports in Medicine 2010;1-3. doi:10.1155/2010 /939706.

20. Breed, C. D. Diagnosis, treatment, and nursing care of patients with chronic leukemia. In Seminars in Oncology Nursing 2003;19(2):109-117.

21. Chereda, B. and Melo, J. V. Natural course and biology of CML. Annals of Hematology 2015;94(2):107-121.

22. Meng, Z. and Li, Y. H. One of the Mechanisms in Blastic Transformation of Chronic Myeloid Leukemia: Epigenetics Abnormality. 2016;24(1): 250-253.

23. Intermesoli, T., Castagnetti, F., Soverini, S., Bussini, A., Spinelli, O. and Gnani, A. Durable molecular response despite F317L and E255K mutations: Successful treatment of chronic myeloid leukemia with sequential imatinib, nilotinib and dasatinib. Leukemia Research 2012;36(1):10-11.

24. Pavlovsky, C., Giere, I., Moiraghi, B., Pavlovsky, M. A., Aranguren, P. N. and Garcia, J. Molecular monitoring of imatinib in chronic myeloid leukemia patients in complete cytogenetic remission: does achievement of a stable major molecular response at any time point identify a privileged group of patients? A multicenter experience in Argentina and Uruguay. Clinical Lymphoma Myeloma and Leukemia 2011; 11(3):280-285.

25. Yin, J. A. L., O'Brien, M. A., Hills, R. K., Daly, S. B., Wheatley, K. and Burnett, A. K. Minimum residual disease monitoring by RT-qPCR in core-binding factor AML allows risk-stratification and predicts relapse: results of the UK MRC AML-15 trial. Blood 2012 Oct 4;120(14):2826-35

26. Guo, J. Q., Wang, J. Y. and Arlinghaus, R. B. Detection of BCR-ABL proteins in blood cells of benign phase chronic myelogenous leukemia patients. Cancer Research ; 1991; 51(11) :3048-3051.

27. Cross, N. C., Melo, J. V., Feng, L. and Goldman, J. M. An optimized multiplex polymerase chain reaction (PCR) for detection of BCR-ABL fusion mRNAs in haematological disorders. Leukemia 1994;8(1):186-189.

28. Coustan -Smith, E., Song, G., Clark, C., Key, L., Liu, P., Mehrpooya, M., Stow, P., Su, X., Shurtleff, S., Pui, C. H. and Downing, J. R. New markers for minimal residual disease detection in acute lymphoblastic leukemia. Blood, 2011;9;117(23): 6267-76

29. Melo, J. V. and Ross, D. M. Minimal residual disease and discontinuation of therapy in chronic myeloid leukemia: can we aim at a cure? American Society of Hematology Education Programe Book 2011(1):136-142.

30. Goldman, J. M. and Druker, B. J. Chronic myeloid leukemia: current treatment options. Blood 2001; 98(7):2039-2042.

31. Clift, R. A., Buckner, C. D., Thomas, E. D., Bensinger, W. I., Bowden, R., Bryant, E., Deeg, H. J., Doney, K. C., Fisher, L. D. and Hansen, J. A. Marrow transplantation for chronic myeloid leukemia: a randomized study comparing cyclophosphamide and total body irradiation with busulfan and cyclophosphamide. Blood 1994; 84(6):2036-2043.

32. Goldman, J. M., Szydlo, R., Horowitz, M. M., Gale, R. P., Ash, R. C., Atkinson, K., Dicke, K. A., Gluckman, E., Herzig, R. H. and Marmont, A. Choice of pretransplant treatment and timing of transplants for chronic myelogenous leukemia in chronic phase. Blood 1993; 82(7):2235-2238.

33. Hehlmann, R., Heimpel, H., Hasford, J. T., Kolb, H. J., Pralle, H., Hossfeld, D. K., Queisser, W., Loffler, H., Hochhaus, A. and Heinze, B. Randomized comparison of interferon-alpha with busulfan and hydroxyurea in chronic myelogenous leukemia. The German CML Study Group Blood, 1994; 84(12):4064-4077.

34. Kennedy, B. J. Hydroxyurea therapy in chronic myelogenous leukemia. Cancer 1972;29(4):1052-1056.

35. Yokoo, M., Kubota, Y., Tabe, Y. and Kimura, S. Comparative study of the anti-leukemic effects of imatinib mesylate, Glivec tablet and its generic formulation, OHK9511. Biological and Pharmaceutical Bulletin 2015;38(3):411-416.

36. O'brien, S. G., Guilhot, F., Larson, R. A., Gathmann, I., Baccarani, M., Cervantes, F., Cornelissen, J. J., Fischer, T., Hochhaus, A., Hughes, T. and Lechner, K. (2003). Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. New England Journal of Medicine, 2003;348(11):994-1004.

37. Trivedi, R., Mithal, A. and Chattopadhyay, N. Recent updates on the calcium-sensing receptor as a drug target. Current Medicinal Chemistry 2008;15(2):178-186.

38. Okabe, S., Tauchi, T., Katagiri, S., Tanaka, Y. and Ohyashiki, K. Combination of the ABL kinase inhibitor imatinib with the Janus kinase 2 inhibitor TG101348 for targeting residual BCR-ABL-positive cells. Journal of Hematology and Oncology 2014;7(1):37.

39. Burchert, A., Saussele, S., Eigendorff, E., Muller, M. C., Sohlbach, K., Inselmann, S. and Hoffmann, J. Interferon alpha 2 maintenance therapy may enable high rates of treatment discontinuation in chronic myeloid leukemia.Leukemia, 2015; 29(6):1331.

40. Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., Zimmermann, J. and Lydon, N. B. Effects of a selective inhibitor of the

Abl tyrosine kinase on the growth of Bcr–Abl positive cells. Nature Medicine1996;2(5):561.

41. Kantarjian, H. M., Cortes, J., La Rosee, P. and Hochhaus, A. Optimizing therapy for patients with chronic myelogenous leukemia in chronic phase. Cancer: Interdisciplinary International Journal of the American Cancer Society 2010;116(6):1419-1430.

42. Huang, X., Patel, S., Ahmed, N., Seiter, K. and Liu, D. Severe toxicity of skin rash, fever and diarrhea associated with imatinib: case report and review of skin toxicities associated with tyrosine kinase inhibitors. Drug Design, Development and Therapy 2008;2:215-219.

43. Weisberg, E., Manley, P., Mestan, J., Cowan-Jacob, S., Ray, A. and Griffin, J. AMN107 (nilotinib): a novel and selective inhibitor of BCR-ABL. British Journal of Cancer 2006;94(12):1765-1769.

44. Watkins, D. B., Hughes, T. P. and White, D. L. OCT1 and imatinib transport in CML: is it clinically relevant? Leukemia 2015;29(10):1960-1969.

45. Bhamidipati, P. K., Kantarjian, H., Cortes, J., Cornelison, A. M. and Jabbour, E. Management of imatinib-resistant patients with chronic myeloid leukemia. Therapeutic Advances in Hematology 2013;4(2):103-117.

46. Horvat, I., Antolic, M. R., Zadro, R., Sertic, D. and Labar, B. Clinical significance of T315I ABL kinase domain mutation detection in patients resistant to imatinib mesylate therapy. Biochemia Medica 2010;20(1):75-81.

47. Cojbasic, I., Macukanovic-Golubovic, L., Mihailovic, D., Vucic, M. and Lukic, S. Improved prediction of clinical outcome in chronic myeloid leukemia. International Joiurnal of Hematology 2015; 101(2):173-183.

48. Assouline, S. and Lipton, J. H. Monitoring response and resistance to treatment in chronic myeloid leukemia. Current Oncology 2011;18(2): e71-e83.

49. Stone, R. M. Optimizing treatment of chronic myeloid leukemia: a rational approach. Oncologist 2004;9(3):259-270.

50. Zhang, H., Chang, G., Wang, J., Lin, Y., Ma, L. and Pang, T. CUEDC2 sensitizes chronic myeloid leukaemic cells to imatinib treatment. Leukemia Research 2013;37(11):1583-1591.

51. Dusetzina, S. B., Winn, A. N., Abel, G. A., Huskamp, H. A. and Keating, N. L. Cost sharing and adherence to tyrosine kinase inhibitors for patients with chronic myeloid leukemia. Journal of Clinical Oncology 2014;32(4):306-311.

52. Jemal, A., Siegel, R., Xu, J., and Ward, E. Cancer statistics, 2010. CA: a Cancer Journal for Clinicians 2010;60(5):277-300.

53. Gumireddy, K., Baker, S. J., Cosenza, S. C., John, P., Kang, A. D., Robell, K. A., Reddy, M. R. and Reddy, E. P. A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. Proceedings of the National Academy of Sciences 2005;102(6):1992-1997.

54. Giles, F. J., Cortes, J., Jones, D., Bergstrom, D., Kantarjian, H. and Freedman, S. J. MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. Blood 2007;109(2):500-502.

55. Quintas -Cardama, A., Kantarjian, H. and Cortes, J. Flying under the radar: the new wave of BCR–ABL inhibitors. Nature Reviews Drug Discovery 2007;6(10):834.

56. Cashman, J. D., Eaves, C. J., Sarris, A. H. and Eaves, A. C. MCP-1, not MIP-1alpha, is the endogenous chemokine that cooperates with TGF-beta to inhibit the cycling of primitive normal but not leukemic (CML) progenitors in long-term human marrow cultures. Blood 1998;92(7):2338-2344.

57. Sierra, J. R., Cepero, V. and Giordano, S. Molecular mechanisms of acquired resistance to tyrosine kinase targeted therapy. Molecular cancer 2010;9(1):75.

58. Chahardouli, B., Zaker, F., Mousavi, S. A., Kazemi, A., Ostadali, M., Nadali, F., Rostami, S., Alimoghaddam, K. and Ghavamzade, A. Evaluation of T315I mutation frequency in chronic myeloid leukemia patients after imatinib resistance. Hematology 2013;18(3):158-162.

59. Cortes, J., Jabbour, E., Kantarjian, H., Yin, C. C., Shan J. and O'Brien, S. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. Blood 2007;110(12):4005-4011.

60. Redaelli, S., Piazza, R., Rostagno, R., Magistroni, V., Perini, P., Marega M. and Boschelli, F. Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib resistant BCR/ABL mutants. Journal of Clinical Oncology 2009;27(3):469-471.

61. Shimada, K., Tomita, A., Minami, Y., Abe, A., Hind, C. K., Kiyoi, H., Cragg, M. S. and Naoe, T. CML cells expressing the TEL/MDS1/EVI1 fusion are resistant to imatinib-induced apoptosis through inhibition of BAD, but are resensitized with ABT-737. Experimental Hematology 2012;40(9):724-737.

62. Baccarani, M., Deininger, M. W., Rosti, G., Hochhaus, A., Soverini, S., Apperley, J. F., Cervantes, F., Clark, R. E., Cortes, J. E., Guilhot, F. and Hjorth-Hansen, H. European Leukemia Net recommendations for the management of chronic myeloid leukemia. Blood, Aug 2013;122(6):872-84.

63. Hochhaus, A., O'brien, S. G., Guilhot, F., Druker, B. J., Branford, S., Foroni, L., Goldman, J. M., Muller, M. C., Radich, J. P., Rudoltz, M. and Mone, M. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia 2009; 23(6):1054.

64. Balabanov, S., Braig, M. and Brummendorf, T. H. Current aspectsofresistance. against tyrosine kinase inhibitors in chronic myelogenous leukemia. Drug Discovery Today Technologies 2014;11:89-99.
65. Nicolini, F. E., Mauro, M. J., Martinelli, G., Kim, D. W., Soverini, S., Müller, M. C., Hochhaus, A., Cortes, J., Chuah, C., Dufva, I. H. and Apperley, J. F.Epidemiologic study on survival of chronic myeloid leukemia and Ph+ acute lymphoblastic leukemia patients with BCR-ABL T315I mutation. Blood 2009;114(26):5271-5278.
66. Diamond, J. M. and Melo, J. V. Mechanisms of resistance to BCR-ABL kinase inhibitors. Leukemia & Lymphoma 2011;52(Sup1):12–22.
67. Sherbenou, D. W., Hantschel, O., Kaupe, I., Willis, S., Bumm, T., Turaga, L. P., Lange, T., Dao, K. H., Press, R. D., Druker, B. J. and Superti-Furga, G. BCR-ABL SH3-SH2 domain mutations in chronic myeloid leukemia patients on imatinib. Blood 2010;116:3278-3285.
68. Mestres, J. and Veeneman, G.H. Identification of "latent hits" in compound screening collections. Journal of Medicinal Chemistry 2003;46(16);3441–3444.
69. Gribbon, P. and Andreas, S. High throughput drug discovery: what can we expect from HTS? Drug Discovery Today 2005;1(10):17–22.
70. Goodnow Jr, R. A. Hit and lead identification: integrated technology-based approaches. Drug Discovery Today: Technologies 2006;3(4):367-375.
71. Hann, M. M., Leach, A. R. and Harper, G. Molecular complexity and its impact on the probability of finding leads for drug discovery. Journal of Chemical Information and Computer Sciences 2001;41(3):856-864.
72. Keseru, G. M. and Makara, G. M. The influence of lead discovery strategies on the properties of drug candidates. Nature Reviews Drug Discovery2009;8(3):203.
73. Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 1997;23(1-3):3-25.
74. Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W. and Kopple, K. D. Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry 2002;45(12):2615-2623.
75. Congreve, M., Carr, R., Murray, C. and Jhoti, H. A 'rule of three' for fragment-based lead discovery? Drug discovery today2003;19(8):876-877.
76. Major, L. L. and Smith, T. K. Screening the MayBridge rule of 3 fragment library for compounds that interact with the Trypanosoma bruceimyo-inositol-3-phosphate synthase and/or show trypanocidal activity. Molecular Biology International, 2011:389364.doi: 10.4061/2011/389364

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