REVIEW

Tyrosine Kinase Inhibitors in Ph+ Chronic Myeloid Leukemia Therapy: a Review

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Abstract

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative hematopoietic stem cell disorder. Deregulated BCR-ABL fusion tyrosine kinase activity is the main cause of CML disease pathogenesis, making BCR-ABL an ideal target for inhibition. Current tyrosine kinase inhibitors (TKIs) designed to inhibit BCR-ABL oncoprotein activity, have completely transformed the prognosis of CML. Interruption of TKI treatment leads to minimal residual disease reside (MRD), thought to reside in TKI-insensitive leukaemia stem cells which remain a potential reservoir for disease relapse. This highlights the need to develop new therapeutic strategies for CML either as small molecule master TKIs or phytopharmaceuticals derived from nature to achieve chronic molecular remission. This review outlines the past, present and future therapeutic approaches for CML including coverage of relevant mechanisms, whether ABL dependent or independent, and epigenetic factors responsible for developing resistance against TKIs. Appearance of mutant clones along the course of therapy either pre-existing or induced due to therapy is still a challenge for the clinician. A proposed in-vitro model of generating colony forming units from CML stem cells derived from diagnostic samples seems to be achievable in the era of high throughput technology which can take care of single cell genomic profiling.

Keywords: Tyrosine kinase inhibitors - targeted therapy - minimal residual disease - colony forming units

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Introduction

Chronic myeloid leukemia (CML) is the utmost widely studied human malignancy, first discovered by two pathologists Drs. Rudolf Virchow and John Hughes Bennett, in 1845 (Nowell, 1960; Deininger, 2000). It accounts for ~20% of all leukemias with annual incidence of 1.6 cases per 100,000 adults, with a male-to-female ratio of 1.4/1.3 and median age of approximately 45- 55 years (Cortes, 2004; Rohrabacher, 2009). Pathogenetically, it is a clonal myeloproliferative hematopoietic stem cell disorder characterized by excessive accumulation of clonal myeloid precursor cells in primitive hematopoietic tissues.

It was the first genetically analyzed human malignancy in which a consistent Chromosomal translocation t(9;22) accounted which created Bcr-Abl fusion gene (Gishizky et al., 1993; Nowell and Hungerford, 1960; Kantarjian et al., 2006; Quintas and Cortes, 2009; Cortes et al., 2012). The generated hybrid Bcr-Abl fusion gene display very strong and constitutive tyrosine kinase activity leading to activation of downstream signaling pathway.

As Bcr-Abl is causative of disease progression, considered as an attractive target for inhibition of CML (Sawyers, 1999; Hehlmann et al., 2007; Chen et al., 2010; Goldman, 2010; Zhu et al., 2014). However, substantial heterogenic observation reported throughout the trend of the diseases. Clinically, a multistep diseases advances in three distinct series of cascade from indolent chronic phase (CP) to inevitable intermediate accelerated phase (AP) to fatal blastic phase (BP) (Faderl et.al., 1999; Calabretta and Perrott, 2004). Advent of TKI has changed the paradigm of understanding the intricate mechanism of CML biology. Contemporary efforts are emphasizing on adjusting dose and design better compound than TKIs to escape the mutational problems.

However, striking disputes to manage disease is unable to eradicate persistence minimal residual disease (MRD) despite of Bcr-Abl inhibition. The underlying cause of disease persistence is unidentified and it is uncertain whether mechanisms central to leukemia stem cell survival are dependent or independent of Bcr-Abl activity. Thorough understanding of the mechanism of resistance is an essential step towards managing the disease. In this article we have summarized the rationale of FDA approved TKIs, their limitations, success and possible future combinatorial and new strategies and identification of new targets to eradicate persistent Ph+ CML cells from a biological perspective.

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Evolution of CML Therapy

Conventional Chemotherapeutic treatment Options: a Pre-Imatinib Era

In 19th century CML was primarily managed with many arsenic agents such as Fowler's solutions & splenic radiation till 1950s (Galton, 1953; Goldman, 2010). In 1953 these therapeutic strategies were replace by introduction of Conventional chemotherapies such as busulfan (1954), Hydroxyurea (1970), interferon-alpha (1980). A major disadvantage of these therapeutic strategies is to only limit the progression of myeloid tissue thus couldn't prevent the disease progression and onset to the transformation of blastic crisis was observed within 5-6 years. However, many patients frquently encountered with increased marrow and hepato toxicity. Thus, allogenic hematopoitic stem cell transplantation (HSCT) is the only curative option in majority of patients. However, allo -HSCT is not affordable and not appropriate due to scarcity of HLA matched donor availability and risk of developing chronic graft-versus-host-disease (cGVHD) in lower match (Galton, 1953; Biggs, 1993; Kantarjian et al., 2002; Kantarjian et al., 2003).

Discovery of TKIs as Front Line Therapy: Era of Targeted Therapy

Imatinib

Intricate molecular mechanisms of Bcr-Abl fusion paved way to researchers to direct towards developing compound which specifically target and blocked ABL tyrosine kinase activity. In the late 1980, utilizing an innovative and high throughput screening techniques, a scientist at Ciba- Geigy (Now Novartis, Basel, Switzerland), discovered lead compound, first class of tyrosine kinase inhibitor 2-phenylaminopyrimidine, known as (ST1-571) or GlivecTM (Buchdunger et al., 2000; Heinrich et al., 2000; Deininger et al., 2005). Compound was primarily introduced as PDGFR inhibitor but found to be potent ABL kinase inhibitor due to its capability to directly competing ATP binding sites of kinase domain (KD) (Figure 1A). It also has promising effect on other tyrosine kinases e.g. c-kit, ARG (Druker et al., 1996; Deininger et al., 1997; Okuda et al., 2001). Due to its tremendous efficacy in Phase I & II clinical trials with more than 90% complete hematological response (CHR) and 30-40% complete cytogenetic responses (CCyr), received FDA approval in 2001 for BCR-ABL Ph+ CML patients (Talpaz et al., 2002). In Phase III large randomized 'IRIS' (International Randomized study of Interferon v ST1571) trial, efficacy of Imatinib evaluated against standard IFN +low dose Cytarabine in newly diagnosed CP patients. However, long term 8 years follow up represented some drawbacks. Only 55% patients remained on Imatinib arm of study, representing therapy failure, due to discontinuation and toxicity in remarkable proportion of patients (Hughes et al., 2010;O'Brien et al., 2003). Though, exceptionally advantageous effect of Imatinib, primary and secondary resistance are always major cause of concern, in advanced phase and relapse cases (Hochhaus et al., 2002; Mello and Chuah, 2007). In such scenario shifting to second generation TKI is the only preferred alternative which may achieve response or improved clinical outcomes. Numerous clinical data (DASSION, ENESTnd, BELA) proven efficacy of second generation TKIs versus Imatinib as first line treatment in CML patients.

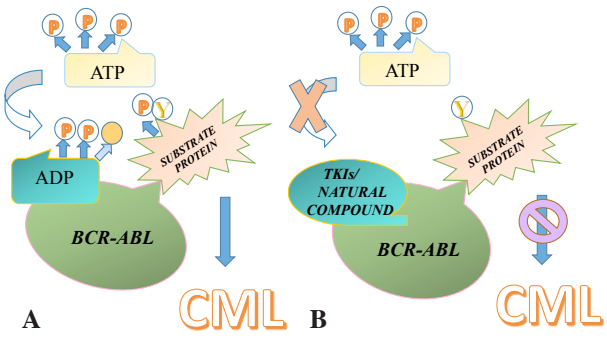

Figure 1. BCR-ABL Mechanisms. A) Binding of ATP to bcr-abl oncoprotein Phosphorylate tyrosine residue of substrate protein leading to progression of CML. B) Competitive binding of TKIs/natural compound to ATP binding pocket of kinase domain prevents phosphorylation of substrate protein leads to inhibition of CML

Second & Third Generation TKIs

Dasatinib & Bosutinib -dual ABL-SRC kinase inhibitor approved by FDA in 2007 & 2012, respectively as a front line therapy for Imatinib resistance patients (Lombardo et al., 2004; Tokarski et al., 2006; Puttini et al., 2006; Gambacorti et al., 2007). It is structurally unrelated to Imatinib and able to bind both active and intermediate confirmation of abl kinase domain, respectively. Nilotinib - a structural analogue approved by FDA in 2007 able to bind in inactive form of kinase domain (Shah et al., 2004; O'Hare et al., 2005; Weisberg et al., 2005). In in vitro conditions Dasatinib (325 fold) Nilotinib- (20-30 fold) and Bosutinib (>200 fold) shown more potency and efficacy than Imatinib with wild type Bcr-Abl with IC_{50} of 0.8μM & 0.25 μM & 13nM, respectively (Talpaz et al., 2006; Hochhaus et al., 2008; Cortes et al., 2011). STARTs trials confirms superiority of Dasatinib over Imatinib in CP, AP & BC phase patients (Cortes et al., 2008; Shah et al., 2008; Apperley et al., 2009; Saglio et al., 2010).

A four arm randomized Phase III dose optimization trial affirm scheduled dose of Dasatinib in Imatinib intolerance CP, AP & BC patients are 100mg, 70 mg and 140 mg once daily, respectively. (Kantarjian et al., 2010). Another, Phase III DASSION and ENESTnd studies were directed to assess efficacy and comparison of the use of Dasatinib & Nilotinib v 400mg of Imatinib in newly diagnosed CML-CP patients. It was demonstrated that Dasatinib & nilotinib are far superior in terms of achieving faster CCyR, MMR and lower progression rates than 400mg of Imatinib (Kantarjian et al., 2009; Kantarjian et al., 2011) A multinational phase III 'BELA' trial addressed to demonstrate the efficacy & response of Bosutinib as upfront therapy than Imatinib in untreated CML CP patients (Cortes et al., 2012; Gambacorti et al., 2014). Though 2nd generation TKIs have improve the quality of life and lower the disease progression

and also shows significant inhibition against number of kinase domain mutations however, occurrence of most lethal mutation "T315I" is major cause of concern (Kantarjian et al., 2006; Puttini et al., 2006; Gambacorti et al., 2007; Giles et al., 2008). Apart from this patients continually encountered with some hematological and non-hematological toxicity during the course of study. Third generation A True pan Bcr-Abl inhibitor Ponatinib specifically designed to inhibit fatal mutation T315I. Phase I & II early phase study of ponatinib was design to treat resistance cases with documented mutation story of T315I and F317L mutations. A complete cytogenetic response was observed in pretreated patients and including those with T315I positive CML (O'Hare et al., 2009; Cortes et al., 2012; Cortes et al., 2013).

Disease Persistence & Resistance

However, biggest obstacle of targeted therapies are that residual diseases arise with different mechanisms leading to disease persistence and resistance. Several Bcr-Abl dependent and independent mechanisms may contributes to resistance includes over expression of Bcr-Abl gene amplification, Sequestration of Imatinib by plasma protein binding (alpha-1 AGP), over expression of ABC transporter (P-gp), decreased intracellular organic-cation transporter (hOCT1), clonal evolutions, activation of Bcr-Abl downstream signaling pathway, quiescent stem cells and pathway activation. However, role of other nuclear and post transcriptional events e.g. aberrant expression of microRNA and epigenetic modification are still unknown mechanisms as they may potential contributors to CML stem cell survival after long term TK therapy.

Bcr-Abl Dependent Mechanisms:

Kinase Domain Mutations (KDM)

Potentially the most frequent clinically relevant mechanisms that change Imatinib sensitivity are mutations within the ABL kinase that hampered the conformational change of kinase domain either directly interfere with Imatinib binding or establishing a distinct conformation to which Imatinib is unable to bind in its inactive form. More than 100 different point mutations involving different amino acid substitution have been reported in different loops of kinase domain (Figure 2). Of the reported subsets G250E, Y253H, E255K/V, V299L, T315I, F317L/I, F359V/I/C, H396R, E450G/V, E459K) are unaffected to all TKIs (Branford et al., 2006; Soverini et al., 2005; Soverini et al., 2006; Gorre et al., 2001; Redaelli et al.,2009).

However, certain compound mutations are lethal because of their ability to confer cross-resistance to all available TKIs, in contrast to mutations in different clones that are individually susceptible to one or more TKIs. Although more than one or multiple Bcr-Abl1 mutations have been reported in many studies but it is still unidentified which fraction of these signify compound mutation (Jamshid et al., 2013). But 3rd generation TKIs capably effective to circumvent most of mutations along with lethal "T315I" mutations but remains susceptible to certain compound mutations. It is still an open debate whether KDM represent the primary cause of resistance or whether they may simply be an indicator of underlying cytogenetic or genomic instability?

Bcr-Abl Gene Amplification

Association of Bcr-Abl gene amplification with Bcr-Abl kinase activity was reported by Le Coutre, Mahon and colleagues. They first in-vitro experimentally confirmed the elevated Abl kinase activity is due to genetic duplication of Bcr-Abl gene in resistance cells (Mahon et al., 2000). Nevertheless, Gorre et al. (2001) successfully demonstrated association of kinase activity & genetic duplication of the Bcr- Abl gene in Imatinib resistance CML patients. This may be advocated that quiescent imatinib insensitive CD34+ cells acquired with residual subclones and point mutations expressing high amount of Bcr-Abl level. These resistant mutant subclones develop mutations much faster in of Bcr- Abl overexpressing cells. Yet a majority of the patients fail to clinically demonstrate abl amplification as a primary mode of treatment failure (le Coutre et al., 2000; Gorre et al., 2001).

Bcr-Abl Independent Mechanisms

Several drug efflux and influx mediators are involved in the mechanisms of resistance to Imatinib. Imatinib as well as other tyrosine kinase inhibitors are substrate of well described Pgp efflux transporter. The role of ABCB1 (Pgp) as a possible mechanism of resistance to Imatinib has been suggested but its role is still unclear and its overexpression has not been reported directly in patients with resistance to Imatinib (Illmer et al., 2004; Galimberti et al., 2005) Uptake transporter hOCT1has also been proposed as an important factor regulating intracellular Imatinib availability and anticipated its role in Imatinib resistance but its expression correlation to patient's survival is still unknown (White et al., 2008; White et al., 2010). Several studies have reported variability of Imatinib concentrations between patients treated with similar doses of drug. Gambacorti-Passerini and colleagues hypothesized that excessive binding of Imatinib to AGP could interfere with its therapeutic effect (Jorgensen et al., 2002; Gambacorti et al., 2000; le Coutre et al., 2002) Jorgensen et al. (2002) were not able to demonstrate binding of Imatinib to AGP Thus, the role of AGP binding

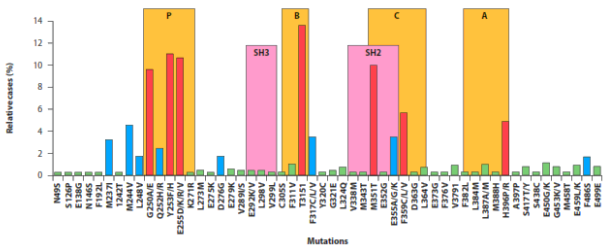

Figure 2. Relative Frequency of Kinase Domain Mutations in Different Regions. Different loops are indicated as P-loops or ATP binding site (P), Imatinib binding site (B), catalytic site (C), activation loop (A). (Adapted from book T.P. Hughes et al., 2014. "Handbook of Chronic Myeloid Leukemia")

in Imatinib resistance remained controversial and AGP as cause of Imatinib resistance is now generally accepted as an unlikely mechanism.

Epigenetics

The underlying epigenetic changes of DNA methylation in CML is not fully implicited. Aberrant DNA methylation of multiple genes characterizes at advanced stages of CML and the disease when resistant to Imatinib. The methylation status of promotor region of several tumor-suppressor gene's (TSGs), with result ranging from rare or no hypermethylation (e.g., SFRP1, RASSF1A) to hypermethylation at progression (e.g., CALCA, CDKN2B, EBF2, ESR, HIC1, TFAP2A, ATG16L2 and PDLIM4, DAPK, ER) has been associated with poor response to Imatinib treatment and progression to CML (Lubbert et al., 2000, Katerina et al., 2013).

However, these studies conducted on random choice of genes surveyed and experimented in other malignancies. It has been observed that out of all frequently methylated genes, mainly four genes PDLIM4, TFAP2A, TFAP2E and EBF2 have showed a significant increase in methylation in CML-Blast Crisis as compared to CML chronic phase. Most frequently methylated ABL1 proximal promoter hypermethylation was observed in CP BM derived colonies as compared to normal BM. However, in advance stage it shows allele specific de novo methylation in progenitor colonies. Though promising role of ABL1 hyper methylation and its significance is still creating many discrepancy in CML (Sun et al., 2001).

MicroRNAs

MiRNAs are dynamic regulatory molecules could functions as either tumor suppressors or oncogenes, may represent as potential drug target and biomarker in CML disease progression. Unfortunately, no information is available regarding their involvement in the response to chemotherapy and resistance mechanisms thus, they could not act as complete biomarker spectrum (Marina and Harvey, 2011). In CML, several microRNAs have been deregulated were rapidly restored under Imatinib therapy.

Out of all miR-150 and miR- 17-92 cluster are well characterized in CML whereas, contribution of other miRNAs in disease progression is still controversial. Many miRNAs also been predicted through bioinformatics analysis including s (miR-19a, miR- 19b, miR-17, miR-20, miR-92a, miR-106a, miR-221, miR- 222, miR-126, miR-146a, miR-181a, miR-181b, let7c, miR- 155). Predicted miRNA may be useful for identification of exact target contributed in CML survival.

However, many high throughput studies identified differential expression of many microRNAs e.g miR-129,. miR-191, mir -199, mir- 520, and mir328, miR-103, miR-150, miR-451, miR-144 as possible predictors for clinical resistance at different phases of CML (Flamant et al., 2010; San et al., 2009; Hershkovitz et al., 2012; Katerina, 2012). However, mir-203 directly regulate bcr-abl activity. Overall, these findings represent that use of single miRNA may be ineffective, combinatorial miRNA expression profiles and identification of exact target or other strategies are needed to improve diagnosis, monitoring disease progression, and drug response.

High Throughput Era

Advent of targeted therapies has transformed the clinical management and prognostic settings of CML. However, disease still unveils a noticeable clinical and biological heterogenecity. Thus, exact characterization and prediction of response to tyrosine kinase inhibitor therapy are still warranted. However, BCR-ABL1 mutation analysis is advocated to facilitate choice of suitable treatment in Ph^+ CML who are resistance to TKI therapy. Still, detection of low level compound & polyclonal mutations at appropriate stage anticipate critical guidance for subsequent therapy selection. Inappropriate therapeutic strategy may lead to treatment failure with clonal expansion of the resistant mutant. There are range of conventional and sequencing based techniques are currently available for identification and quantification of mutations in kinase domain. Though, direct sequencing and other methods have several potential disadvantages due to relatively low sensitivity, difficulty to precisely quantitate the mutated subclone and the lack of informatively regarding the clonal composition when multiple mutations are present.

Next-generation sequencing (NGS) affords a higher level of sensitivity (1-5%) to sight clinically relevant ABL1 mutations that are not currently observed by sanger sequencing (Angelo et al., 2013; Kastner et al., 2014). So in near future, conventional sequencing technique will probably be replaced by NGS, which has high-throughput, high-sensitivity and quantitative amplicon deep sequencing among its applications. Apart from mutation spectrum numerous microarray based gene expression profiling and proteomics studies have disclosed genes which may be over or under expressed in imatinib-resistant cells. Many of these encodes proteins involved in many adhesion/cytoskeleton, DNA repair and signal transduction and/ or transcriptional regulatory mechanisms which could unravel disease progression independently of BCR-ABL kinase activity.

However, there is as yet no conclusive functional evidence that any of these candidate proteins identified by profiling studies are in actually responsible for clinical resistance in CML patients. This type of profiling may be more useful in identifying patterns of gene expression that may be predictive of poor response (primary resistance) to imatinib. (Pizzatti et al., 2006; Fontana et al., 2007; Colavita et al., 2010).

Quiescence CML Stem Cells

Although the development of point mutations is generally accepted to be the most common feature associated with acquired resistance to Imatinib, question arises as to why Imatinib does not eradicate all leukemic cells even in the best responses. So, lastly attention has been focused on CML stem cells that remains resistant to TKIs by virtue of its quiescent or dormant state (Bing et

al., 2010; Jiang et al., 2005).

Understanding how CML stem cells escape the effects of TKIs is crucial for disease eradication. TKIs are available to control mature progeny of CML cells but do not to completely eradicate disease, as the CML stem cells evade the treatment. However, many in vitro and in vivo studies explain that these cells survive in spite of nearly complete inhibition of BCR-ABL kinase activity by the TKIs, suggesting that their primary resistance is BCR-ABL kinase-independent and possibly a pool of highly resistance population repopulate leukemic clone even in best molecular responses (La et al., 2003; Copland et al., 2006).

Several groups have tested various hypotheses, but the mechanism of resistance remains elusive and being intensively investigated in search of genes and protein expressed independently of bcr-abl activity. So it is obvious that stemness of Bcr-Abl-expressing stem cells are maintained by a complex molecular network involving Bcr-Abl and its interaction with other downstream signaling pathways includes Wnt/β-catenin, Alox-5, Jak/STAT, Pten pathways etc. These pathways would be specifically involved in the survival regulation of LSCs but not normal stem cell counterparts. Thus, identification and inhibition of key bcr-abl downstream signaling molecules/ pathways will offer effective therapeutic strategies aiming to eradicate leukemic stem cells (LSCs) (Donato et al., 2003; Hu et al., 2006).

Table 1. Non- Tyrosine kinase inhibitors effective against "T315I" mutations

No	Compound Name	Inhibitors
1.	XL228	Protein Kinase
2.	Rebastinib (DCC-2036)	Allosteric
3.	GNF-2	Allosteric
4.	Bafetinib (INNO-406)	Bcr-Abl/Lyn Kinase
5.	Flavopiridol	CDK
6.	VX- 680, MK-0457	Aurora kinase
7.	PHA-739358, XL228, AT9283	Aurora kinase
8.	Geldanamycin analougue 17-AAG	HSP90
9.	HHT + imatinib	Plant alkaloid
10.	Omacetaxin	Plant alkaloid derivative
11.	SAHA+ Nilotinib	HDAC
12.	PP2A	Activators FTY720
13.	Bortezomib	Proteosome
14.	Azacitidine, Decitabine+Imatinib Fazarabine, DHAC	DNMT

Beyond Tyrosine Kinase Inhibitors

Though the prominent success of imatinib against bcr-abl, resistance due to lethal "T315I" mutation is major challenge. Many agents that do not function as kinase inhibitors are also tested in BCR/ABL-dependent leukemia therapy and various most promising strategies are in development to circumvent problem. These agents includes non- ATP competitive inhibitors, "switch pocket" inhibitors, protein kinase inhibitors, allosteric inhibitors, proteasome inhibitors & HSP90 inhibitors. Dual bcr-abl/Lyn kinase and aurora kinase inhibitor are also well promising strategies (Nimmanapalli et al., 2001; Yu et al., 2003; Nimmanapalli et al., 2003; Fiskus et al., 2006; Cheetham et al., 2007; Gorre et al., 2010; Zhang et al., 2010; Chan et al., 2011). Other strategies being studied include a synergistic combination of TKIs with HDAC inhibitors, RAC GTPases inhibitors and plant alkaloid inhibitors are also warrant attention (Table 1).

Natural Compounds as an Anticancer Agent

In CML, recurrence, resistance and side-effects due to chemotherapeutic agents reduce the clinical efficacy of therapeutic regime. Thus, there is constant need to develop a combination or synergistic anticancer therapy that can reduce the adverse effects and also increase the therapeutic efficacy of anti-cancer agents. Anti-cancer agents discovered from natural products always remain as an important source of new drug leads and new chemical entities in treatment of cancer due to their unprecedented and unpredictable properties. Several anticancer agents derived from plants and their derivatives have been proven to be effective for cancer prevention and therapeutics including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide (Lucas et al., 2010; Pan et al., 2012). Several groups have evidenced the anti-cancer and protective effect of many plant derived compounds in CML treatment. A Cephalotaxus alkaloids, Homoharringtonine, effectively induced apoptosis in CML hematopoietic progenitor cells either alone or in combination with IFN-α and cytosine arabinoside (Ara-c) in Imatinib resistance and non- responder patients (Visani et al., 1997).

In addition, many plant derived myelosupressive inhibitors Gossypol, Gallic acid, alpha-bisabolol, emodin, reseveratrol, silibinin, curcumin and Omacetaxin respectively, appeared to synergize the effect of TKI while inducing apoptosis at different concentration in CML cell lines or Bcr-Abl Ph+ resistance CML stem cells (Ahmed et al., 2001; Meng et al., 2007; Yang et al., 2007; Puissant et al., 2008; Markus and Maciej, 2008; Bonifacio et al., 2012; Chandramohan et al., 2012; Can et al., 2012; Geylani et al., 2012). Recently, Wu et al. (2003) reported that C817, a novel derivative of curcumin suppressed the growth of both Imatinib sensitive and resistance CML cell including wild-type K562, K562/G01, 32D-T315I, 32D-Q252H, and 32D-Y253F cells at low IC_{50} values. Furthermore, significant inhibition of CFU and LTC-ICs implicates that C817 could eradiate human myeloid leukemia progenitor/ stem cells (Wu et al., 2008). Moreover, Rakshit et al. also

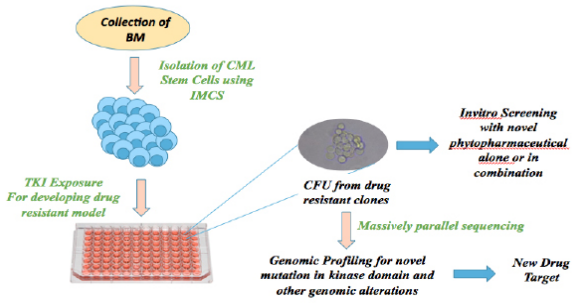

Figure 3. Proposed In-vitro Model for Identification and Characterization of CML Stem Cells

reported role of ROS in chlorogenic acid induced cell death in CML cell line as well as primary CML cells. Zhang et al. (2008) demonstrated that beta-phenylethyl isothiocyanate (PEITC) exerted the cytotoxic effect in Glivec-resistant CML cells by regulating redox signaling.

Collectively, all these data represented the potentiality of natural compound in synergy with TKI in CML treatment and strengthen the hope of applying these combination in clinical setting for better management of Ph+ CML patients (Figure 1B). Publication from our group on enhancement cytotoxic effect of cytatrabine in synergism with hesperidin & silibinin in AML favours this claim (Desai et al., 2015).

Concluding Remarks

The race of TKI is unending due to biological variability of disease and emerging technological advancement in novel mutation detection by next generation sequencing. Each mutation detected may not be clinically actionable, therefore these mutants need to be evaluated using sequence and structure based tools including molecular dynamic simulation prior to establishing its role in disease pathogenesis. Search for newer small molecule inhibitor against new drug target establishing using high throughput technologies is continuous process supported by medicinal chemist and synthetic chemist. The Proposed model depicts an in -vitro approach to established drug resistant model for identification of new drug target and screening with phytopharmaceutical on existing target to assess synergism which inturn may provide better management of CML (Figure 3).

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