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PHARMACOGENETICS AND PHARMACOGENOMICS OF TARGETED THERAPEUTICS IN CHRONIC MYELOID LEUKEMIA

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Abstract

The advent of targeted therapeutics has greatly improved outcomes of chronic myeloid leukemia (CML) patients. Despite increased efficacy and better clinical responses over cytotoxic chemotherapies, many patients receiving targeted drugs exhibit a poor initial response, develop drug resistance, or undergo relapse after initial success. This inter-individual variation in response has heightened the interest in studying pharmacogenetics and pharmacogenomics (PGx) of cancer drugs. In this review, we discuss the influence of various germline and somatic factors on targeted drug response in CML. Specifically, we examine the role of genetic variants in drug metabolism genes, i.e. *CYP3A* family genes, and drug transporters, i.e. *ABC* and *SLC* family genes. Additionally, we focus on acquired somatic variations in *BCR-ABL1*, and the potential role played by additional downstream signaling pathways, in conferring resistance to targeted drugs in CML. This review highlights the importance of PGx of targeted therapeutics and its potential application to improving treatment decisions and patient outcomes.

1. INTRODUCTION

Anti-cancer treatment strategies have evolved in recent decades to reflect the new technologies and treatment options that have been developed in response to the wide variation and heterogeneity of patient outcomes with similar clinical and pathological characteristics. In many cases, patients treated with the same therapeutic regimens exhibit significant differences in drug response and survival outcomes. Thus, traditional treatment decision-making strategies that rely only on the histopathological and clinical factors of disease or environmental factors like age and sex are not fully effective for all patients [1, 2]. With the emergence of human genome sequencing technologies and high-throughput genetic analysis, it has become abundantly clear that an individual's genetic makeup can affect response to drugs, thus giving rise to increased interest in closely examining genetic profiles in cancer patient care.

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COMPLIANCE WITH ETHICAL STANDARDS

CONFLICTS OF INTEREST

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This heightened interest has resulted in an increase in studies focusing on the role of pharmacogenetics and pharmacogenomics (often jointly abbreviated as PGx) in treating cancers [3, 4]. PGx can be defined as the study of genetic factors that can influence a patient's response to drugs. Here, genetic factors can refer to single nucleotide polymorphisms (SNPs), haplotypes or other heritable mutations, copy number variations (CNVs) and chromosomal alterations. Before the advent of genome-wide approaches, treatment approaches relied on studying candidate genetic variants important in drug response, which assumes the importance certain genes hold in drug sensitivity [3]. Examining and identifying both somatic and germline genetic factors that influence drug sensitivity is important to provide prognostic information, predict treatment outcomes, and improve drug efficacy and safety for individual patients [1]. PGx approaches incorporating a more comprehensive understanding of the genetic and molecular processes may also lead to the identification of new molecularly targeted agents that are safer and more effective for cancer patient care. Thus, by personalizing therapy tailored to an individual patient's genotype, anti-cancer treatment strategies can maximize efficacy and minimize toxicity in patients [4].

Due to the nonspecific effects with a narrow therapeutic index of cytotoxic chemotherapeutic agents and resulting in serious toxicities, the relevance of PGx approaches was considered highly significant for such regimens. On the other hand, because targeted therapies act upon cancer-cell specific genes or proteins, there has been less emphasis on investigating PGx of targeted therapeutics. However, the importance of PGx of targeted therapeutics becomes clear in cases where patients either do not respond, develop resistance, or react adversely to drugs. One example of this phenomenon includes chronic myeloid leukemia (CML) patients with the BCR-ABL1 fusion protein who do not respond to the tyrosine kinase inhibitor (TKI) imatinib and require consideration of additional genetic factors in selecting appropriate drugs. Studying the PGx of targeted therapies is especially important when considering that, in the case of CML, up to one third of patients treated with imatinib may not demonstrate complete cytogenetic response or become imatinib-resistant [5].

1.1 Overview of CML biology and treatment

CML is a myeloproliferative disorder characterized by the presence of a reciprocal translocation between chromosomes 9 and 22, also known as the Philadelphia chromosome (Ph). This translocation results in the fusion of Abelson murine leukemia viral oncogene homolog 1 (*ABL1*) gene from chromosome 9 with the breakpoint cluster region (*BCR*) gene on chromosome 22, thus forming the *BCR-ABL1* fusion gene [6]. As a constitutively active tyrosine kinase, the fusion protein-product of this gene activates a number of oncogenic signaling pathways, including PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, and JAK/STAT [7]. The annual incidence rate of CML is 1–2 cases per 100,000 and accounts for 10% of newly diagnosed cases of leukemia in the United States [8]. The progression of CML is structured by phases, with 85% of patients diagnosed in the benign chronic phase (CP) [9]. Without successful treatment during CP, the disease can progress to the accelerated phase (AP), and to the often-fatal blast crisis (BC) phase. Prognosis varies with the phase of CML, age, and therapeutic response [10].

Prior to the advent of targeted therapy for CP-CML, patients were treated with busulfan and hydroxyurea chemotherapy, producing complete hematological response (CHR: 12,000 white cells/ μ l blood) in 50–80% of patients. Complete cytogenetic remissions (CCyR: no detectable Ph⁺ cells) were rare [11, 12]. The subsequent introduction of human cytokine interferon- α therapy produced higher rates of complete hematologic and cytogenetic responses [13]. However, the BCR-ABL1 inhibitor imatinib mesylate, which was approved by the US FDA in 2001, outperformed all previous therapeutics achieving CHR in 95%, and CCyR in 76% of the patients in the IRIS trial for CML treatment [14, 15]. Since the introduction of imatinib as frontline therapy for CP-CML, the 5-year survival rates have dramatically increased from 31% in the early 1990's to 66% in the years between 2006 to 2012 [16].

As noted above, about 25% of the patients in the IRIS trial were resistant to imatinib, while an additional 20–25% of the patients achieving CHR eventually developed resistance with increased risk of disease progression [17]. In the 8-years follow-up study of the original IRIS trial, only 55% of the patients remained on imatinib with 16% of withdrawals attributed to unsatisfactory treatment outcome [18]. A common mechanism of resistance to imatinib was point mutations in the kinase domain of *BCR-ABL1* oncogene. To overcome this, second-generation TKIs, including dasatinib and nilotinib, were developed with several-fold higher affinity to BCR-ABL1 than imatinib. These drugs significantly improved patient outcomes in imatinib-resistant CML [19, 20]. In addition, the second generation TKIs were superior in achieving major molecular response (MMR: *BCR-ABL1* RNA level 0.1% on the International Scale) and CCyR by 12-month in newly diagnosed CP-CML patients as compared to imatinib and were approved as frontline treatment options [21, 22]. However, only about 50% of the resistant cases can be explained on the basis of *BCR-ABL1* point mutations [23]. In addition, a number of patients develop drug intolerance or cytotoxicity, experiencing both physical and mental side effects (physical functioning, role limitations because of physical problems, bodily pain, general health perceptions, vitality, social functioning, role limitations because of emotional problems, and mental health; patient-reported) [24], highlighting the importance PGx of targeted therapies.

In this review, we will discuss the role of patient germline genetics and acquired somatic alterations that may influence response to targeted TKIs. We discuss the role of genetic determinants that influence processes such as enzymatic drug metabolism, cellular influx and efflux, in addition to the role of mutations in genes directly targeted by the TKIs. We further discuss the role of alternative downstream pathways that circumvent the effects of BCR-ABL1 inhibition and could influence response to TKIs.

2 GERMLINE GENETIC DETERMINANTS OF DRUG RESPONSE

Targeted TKIs for CML are administered orally, and their bioavailability is dependent on gastrointestinal absorption, first-pass metabolism, and cellular influx and efflux. Thus, variations in the genes regulating metabolism and transport can impact pharmacokinetics and pharmacodynamics of TKIs in CML patients. A figure illustrating the enzymes involved in pharmacokinetics and pharmacodynamics of imatinib can be found online at <https://www.pharmgkb.org/pathway/PA164713427>

2.1 Drug metabolism genes

2.1.1 Cytochrome P450—The metabolism of TKIs primarily occurs in the cytochrome P450 complex. This complex is comprised of a family of hemoproteins that catalyze reactions relating to drug metabolism, as well as the synthesis of cholesterol, steroids, and other lipids. The cytochrome P450 3A4 (*CYP3A4*) is the main enzyme responsible for the first-pass metabolism of TKIs, while other enzymes including *CYP3A5*, *CYP2C8*, and *CYP2D6* are also involved to a lesser extent.

Imatinib is converted by the *CYP3A* enzymes [25] into its main metabolite N-desmethyl imatinib, which is pharmacologically active but 3–4 times less cytotoxic than imatinib [26]. The importance of *CYP3A* enzymes was highlighted in a study that showed CML patients achieving a complete molecular response (CMR; no detectable *BCR-ABL1* transcript using qPCR) demonstrated higher *CYP3A4* and *CYP3A5* enzymatic activity than patients with partial molecular responses [27]. However, recent pharmacokinetic studies suggest that the predominant role of *CYP3A4* may be taken over by *CYP2C8* over the course of imatinib treatment, driven by time-dependent auto-inhibition of *CYP3A4* activity by imatinib [28]. Thus, the inhibition of *CYP3A4* activity over time may elevate the role of other cytochrome P450 enzymes in imatinib metabolism. An example of such compensatory effect comes from a study that examined the influence of the *CYP3A5*3* polymorphisms on the clinical outcome and steady-state trough plasma concentration of imatinib in 110 Nigerian CML patients [29]. This study reported that the GG genotype in *CYP3A5*3* was associated significantly with higher trough plasma concentrations, but not associated with clinical outcomes. Another study investigated the influence of the *CYP2B6* 15631G>T polymorphisms on imatinib response in 48 CML patients in Morocco [30]. This study reported that CHR loss was higher in patients carrying the *CYP2B6* 15631GG/TT genotype when compared with 15631GT, while CCyR was higher in the 15631GG/GT genotype compared with 15631TT. In addition, primary cytogenetic resistance was higher in patients with the 15631GG/TT genotype compared with 15631GT carriers, whereas side effects were more common in patients with the 15631GG genotypes compared with GT/TT carriers.

CYP3A4 is also the primary isoform responsible for the metabolism of nilotinib [31] and dasatinib [32]. Dasatinib functions as a competitive inhibitor of *CYP3A4* and *CYP2C8* [33, 34], whereas nilotinib has inhibitory activity against *CYP3A4*, *CYP2C8*, *CYP2C9* and *CYP2D6* [35]. While the role of polymorphisms in these genes has not been extensively studied in CML patients receiving dasatinib or nilotinib, several studies have focused on drug-drug interactions with these TKIs, considering most CML patients take TKIs throughout their lives along with other drugs (reviewed in [36]).

These findings provide preliminary evidence that along with *CYP3A4* activity, identifying polymorphisms in additional P450 enzymes could help predict imatinib therapeutic response. However, these reported variants may be population-specific and must be profiled in larger CML cohorts to assess clinical relevance.

2.1.2 UGT1A1—Another aspect of TKI metabolism involves glucuronidation of the active metabolite into inactive compounds for elimination. This reaction is catalyzed by the uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) enzyme, which transforms small

lipophilic molecules, including TKIs, into water-soluble metabolites for excretion. UGT1A1 also glucuronidates bilirubin in humans, and the (TA)₇ polymorphism in the TATAA element of the promoter (*UGT1A1**28), downregulating its expression, has been strongly linked with hyperbilirubinemia [37]. A pharmacogenetic analysis of the TA-repeat polymorphism demonstrated an association between the (TA)₇/(TA)₇ genotype and the risk of hyperbilirubinemia in imatinib-resistant or intolerant CML patients treated with nilotinib [38]. This study also suggested that since UGT1A1 did not glucuronidate nilotinib, potent inhibition of UGT1A1 activity by nilotinib, combined with the (TA)₇ polymorphism, was the most probable reason for increased rate of hyperbilirubinemia in CML patients. A separate study investigated the influence of *UGT1A1* polymorphisms and nilotinib trough plasma concentrations on nilotinib-induced hyperbilirubinemia in Japanese CML patients [39]. A higher proportion of patients with hyperbilirubinemia carried the *UGT1A1* *6/*6 and *6/*28 genotypes when compared to the other genotypes (allele *6 represents *UGT1A1* 211G>A). Additional case studies of Japanese CML patients reported that in addition to grade 2 hyperbilirubinemia, some patients with the *6/*6 or *6/*28 genotype had other severe toxicities including QT interval prolongation, elevated lipase levels, anemia, and hepatic cyst hemorrhage [40]. These findings indicate the benefit of identifying the patients' risk for increased hyperbilirubinemia and severe toxicity through prospective *UGT1A1* genotyping prior to nilotinib therapy.

Table 1 provides a summary of PGx variants of drug metabolism genes associated with CML targeted drugs.

2.2 Drug transport genes

The intracellular concentration of TKIs depends on influx and efflux of the drugs via transmembrane transporter proteins and can determine the efficiency of BCR-ABL1 inhibition. The multidrug resistance ATP-binding cassette (MDR-ABC) family proteins ABCB1 (also known as MDR1 or P-GP) and ABCG2 (also known as BCRP2) are key mediators of active drug efflux. The role of MDR-ABC proteins in multidrug resistance has been widely studied in several cancer types and polymorphisms in these gene serve as predictive markers for drug response [41]. However, the precise mechanism of MDR-ABC transporter mediated resistance in CML patients is contentious.

Clinical studies have established that *ABCB1* expression levels are elevated in advanced stages of CML [42], and that high *ABCB1* expression is associated with lower rates of MMR and imatinib resistance [43, 44]. At the molecular level, *in vitro* studies with the K562 cell line demonstrated that an increase in ABCB1-mediated drug efflux is a potential mechanism of resistance to imatinib [45, 46]. However, the results of *in vitro* studies with other TKIs are far from unambiguous. One study reported that ABCB1 only induced resistance against dasatinib and not nilotinib, while ABCG2 induced resistance against both drugs [47]. Another study reported that nilotinib resistance was only associated with ABCB1 activity and not ABCG2 [48], while a different study showed that nilotinib resistance was indeed correlated with both ABCB1 and ABCG2 protein expression [49]. A large amount of variation observed across *in vitro* studies could be attributed to differences in drug concentrations tested, duration of the experiment, and end-point measure of drug efficacy.

Nevertheless, consistent clinical correlations observed between MDR-ABC protein activity and imatinib resistance have prompted studies to investigate the role of commonly inherited variants of *ABCB1* and *ABCG2*.

2.2.1 ABCB1—Gurney et al. investigated the association between the three most common *ABCB1* variants, 1236T>C, 2677G>T/A and 3435C>T, and imatinib clearance in 8 CML and 14 gastrointestinal stromal tumor (GIST) patients [50]. They reported that the rate of reduction in imatinib clearance was significantly lower in patients with the TT genotype at all three loci. Subsequently, Dulucq et al. evaluated the clinical impact of *ABCB1* variants on imatinib response in a cohort of 90 CML patients in France [51]. In this study, patients with the 1236TT or 2677TT/TA genotype achieved significantly higher rates of MMR. However, another study conducted by Deenik et al. with 46 CML patients in the Netherlands found that the 1236CT/TT, 3435TT, and 2677TT genotypes were actually associated with reduced rates of MMR and CMR [52], in agreement with the initial report suggesting a lower reduction in imatinib clearance rate in patients with TT genotypes [50]. In response, Dulucq et al. expanded their analysis to a larger cohort of 557 patients and reported that the 2677 G allele was indeed associated with higher MMR rates [53], now in agreement with results reported by Deeniki et al [52].

Evidently, the conclusions drawn in these reports were affected by the sample size of the patient cohorts. To address this issue, meta-analyses of a number of other trials that evaluated the associations between *ABCB1* variants and imatinib response were performed. One meta-analysis covering 1826 patients across 12 studies reported significant associations between 2677 G, 3435 T, and worse response to imatinib in all populations studied, whereas 1236 CC predicted better response specifically in Asian populations [54]. The result in Asian populations was corroborated in a separate meta-analysis, confirming the association between 1236T>C locus with imatinib response in this demographic [55].

At the molecular level, *in vitro* studies so far do not agree whether these polymorphisms indeed play a role in TKI response, with one study suggesting no role [56], while another reporting substantial and statistically significant influence on the activity of all CML TKIs [57]. Nevertheless, researchers agree that expression levels of *ABCB1* are strong predictors of imatinib responsiveness [43, 44] and could be monitored as early response markers in CML patients receiving imatinib [58].

2.2.2 ABCG2—Imatinib, nilotinib, and dasatinib have been shown to be high-affinity substrates of ABCG2, another member of the MDR-ABC family of efflux proteins [59–61]. *In vitro* studies with *ABCG2* overexpression in K562 cells show elevated resistance to imatinib [62], nilotinib [63], and dasatinib [47]. Interestingly, the third-generation TKI ponatinib was not affected by either *ABCB1* or *ABCG2* expression at clinically relevant concentrations [47], while all TKIs could inhibit *ABCG2* activity at high concentrations [47, 62]. While multiple *in vitro* studies support the role of *ABCG2* expression in mediating TKI resistance, few clinical studies have evaluated the role of *ABCG2* expression and variants in CML patients. A study with 188 CML patients in Brazil showed that *ABCG2* expression levels were higher in imatinib-resistant patients as well as in patients that did not achieve MMR [64]. Another study with 215 CML patients in Malaysia evaluated two variants:

ABCG2 34G>A and 421C>A and found that the *ABCG2* diplotype A₃₄A₄₂₁ was significantly correlated with a better imatinib response [65]. A recent meta-analysis compiled data from 14 studies across 2184 patients and also concluded that the 421A variant was significantly associated with higher MMR and CCyR in CML patients [66]. As with *ABCB1*, the correlation between *BCG2* expression and TKI response seems to be less contentious, but establishing the overall importance of genetic variants would greatly benefit from studies in larger cohorts.

2.2.3 SLC22A1—The active intracellular uptake of TKIs is controlled by the expression and activity of influx proteins like the solute carrier family 22 member *SLC22A1* (also known as organic cation transporters or OCT1). The involvement of OCT1 in the transport of imatinib has been somewhat contentious with *in vitro* studies showing OCT1 can bind to imatinib, but changes in *SLC22A1* expression levels may or may not be associated with imatinib response. A critical discrepancy in several studies was attributed to primers designed to quantify *SLC22A1* using qPCR, with several studies using primers that spanned SNPs that affected expression levels [67]. A recent study evaluated the association between *SLC22A1* expression and imatinib uptake in multiple cell lines and mouse models and reported that the uptake of imatinib was not dependent on *SLC22A1* expression [68]. On the other hand, OCT1 activity seems to be a strong predictor of imatinib response at the clinical level. In one study, the activity of OCT1 was reported to be a strong predictor of MMR in a cohort of 56 CML patients from Australia [69]. Additional studies from the UK with 70 patients [70] and Brazil with 88 patients [71] confirmed that *SLC22A1* expression levels were predictive of imatinib response over time.

The role of *SLC22A1* genetic variants has also been studied in small cohorts of CML patients, but no clear consensus has emerged about their effect on imatinib sensitivity. A study in 38 Asian CML patients revealed three *SLC22A1* polymorphisms (rs3798168, rs628031, and IVS7+850C>T) to be significantly associated with imatinib clearance [72]. Another study in 50 CML patients from Italy found *SLC22A1* 480C>G to be significantly associated with imatinib clearance [73], whereas the 401G/A genotype occurring in a small fraction of patients was associated with MMR in a study of 132 CML patients from the UK [74]. However, additional studies have reported no association between *SLC22A1* variants and imatinib response [75]. Thus, the rare occurrence of these genotypes necessitates exercising caution in interpreting results from small-sized studies.

2.1.4 Additional Transporters—In addition to the primary efflux and influx transporters discussed above, various other transmembrane transporters that may introduce variability in TKI response have been evaluated. These include the influx transporters solute carrier organic anion transporter family member 1B3 (*SLCO1B3*), solute carrier organic anion transporter family member 1A2 (*SLCO1A2*, also known as OATP1A2), solute carrier family 22 member 4 (*SLC22A4*; also known as OCTN1) and the efflux transporters ATP-binding cassette subfamily A member 3 (*ABCA3*) and subfamily C member 4 (*ABCC4*). The main motivation for exploring additional transporters comes from conflicting results about the ability of TKIs to bind these transporters *in vitro*. For example, one study reported that

imatinib failed to bind OCT1, and its expression was correlated with expression of other transporters, thereby potentially acting as a proxy biomarker for these proteins [76].

A study with 34 Japanese CML patients found that the rate imatinib clearance was significantly higher in patients with the *SLCO1B3* 334GG, *ABCB1* 3435CC [77], and *SLCO1A2* 361GG genotypes [78]. Another study with 118 CML patients in Brazil reported reduced CMR in patients with the *ABCA3* 4548-91 CC/CA genotypes compared to AA carriers, while *SLCO1B3* 699GG and 344TT genotypes were more prevalent in patients that responded to imatinib [79]. A study with 189 CML patients from Italy found that the presence of the C allele in the *SLC22A4* variant rs1050152 was significantly associated with increased rates of MMR [80].

While it appears that there is surmounting evidence that additional drug influx and efflux genes could determine responsiveness to imatinib, most data were obtained from small, population-specific cohorts, and further clinical and experimental validation would help in supporting their overall influence.

Table 2 provides a summary of PGx variants of drug transport genes associated with CML targeted drugs.

3 SOMATIC DETERMINANTS OF DRUG RESPONSE

One of the most widely studied mechanisms of resistance to imatinib treatment involves point mutations in the tyrosine kinase domain of the *BCR-ABL1* fusion oncogene. However, *BCR-ABL1* point mutations are not detected in all patients with TKI resistance and may explain only about half of the resistant cases [81, 82]. Recent reports suggest that the activation of alternate oncogenic pathways following TKI treatment may be responsible for acquired resistance in a *BCR-ABL1*-independent manner. In this section, we discuss the role of *BCR-ABL1* mutations and the activation of alternate pathways in determining TKI response in CML patients.

3.1 BCR-ABL1

Since the discovery of an association between *BCR-ABL1* kinase domain mutations with imatinib resistance [83], more than 90 point mutations that alter TKI's ability to bind BCR-ABL1 have been identified [82, 84]. As TKIs inhibit BCR-ABL1 activity by binding and inactivating the ATP-binding loop of the ABL1 kinase domain, mutations can confer resistance by altering the conformation of the protein to prevent TKI access, reduce binding affinity, or to weaken hydrogen bonds required for binding or maintain the ATP loop in an active conformation [84].

The first study investigating mechanisms of imatinib resistance identified the T315I mutation arising from a substitution of C to T at base 944 of the *ABL1* gene in about half of the 11 imatinib resistant patients evaluated [85]. The authors confirmed the inability of imatinib to bind the mutated BCR-ABL1 protein using *in vitro* experiments. Subsequently, mutation screening in imatinib-resistant patients identified several additional ABL kinase domain mutations, including E255K, E255V, Y253F, Y253H, F317L, H396P, M351T, along

with T315I [86–88] that conferred varying degrees of resistance [89]. One study evaluated *BCR-ABL1* mutations in 144 CML patients and found that these mutations were strongly associated with resistance in 27 cases [90]. Another study evaluated the mutational profile of 171 patients across all phases of CML who failed imatinib treatment and reported *BCR-ABL1* mutations in about 1 in 3 patients [91]. This study also found that the rate of mutations was significantly higher in patients who attained the BC or AP stage.

The inability of imatinib to bind mutated BCR-ABL1 prompted the development of second-generation TKIs: dasatinib, nilotinib, and bosutinib. The second generation TKIs were able to overcome imatinib resistance arising from point mutations attributable to several-fold higher binding affinities for BCR-ABL1 as compared to imatinib [92]. However, one notable exception was the “gatekeeper” T315I mutation, which conferred resistance to second generation TKIs as well. Phase II trials with the third-generation TKI ponatinib show promising results, with MMR and CCyR observed in patients regardless of T315I status [93].

Longitudinal monitoring of CML patients resistant to TKIs suggests that these point mutations were likely selected during the course of treatment [94], but their presence in pre-treatment CML-CP patients is not well documented. Consequently, the European LeukemiaNet expert panel recommended against monitoring for *BCR-ABL1* mutations prior to treatment or in patients with sustained CCyR. Direct sequencing of *BCR-ABL1* would be most beneficial to patients who exhibit primary or secondary resistance to imatinib or second-generation TKIs [95].

3.2 BCR-ABL1 independent TKI resistance mechanisms

Given the significant proportion of patients resistant to TKIs without evidence of *BCR-ABL1* mutations, studies have shown that pathways that function downstream of BCR-ABL1 could compensate for the loss of BCR-ABL1 activity and may be responsible for acquired resistance to TKI treatment. The main rationale for the existence of such pathways comes from the early investigations that reported the presence of an imatinib-resistant population of CD34⁺ progenitor stem cells in the blood of patients [96]. These CD34⁺ CML stem cells were distinct from normal CD34⁺ cells in their transcriptomic and molecular profile, expressing a number of oncogenic signaling pathways [97]. Furthermore, survival of human CML stem cells was reported to be independent of BCR-ABL1 activity, and imatinib treatment failed to completely clear CD34⁺ CML stem cells [98]. Investigations of therapeutic response revealed that the second-generation TKIs nilotinib and dasatinib were also ineffective in inducing apoptosis in CD34⁺ leukemic cell populations [99, 100]. An emerging theme from these studies seems to indicate that the resistance to TKIs may develop in CML patients despite a complete inhibition of BCR-ABL1 activity. In this section, we outline such acquired somatic alterations that may influence drug response in CML patients.

3.2.1 KIT and HIF1A—The *KIT* proto-oncogene (also known as SCFR, CD117, or c-KIT) encodes a hematopoietic stem cell surface cytokine receptor that is activated in several cancer types and regulates cellular proliferation, differentiation, and migration. CML cells

that are Ph⁺ also show elevated expression of *KIT*, and certain *KIT* polymorphisms may be associated with increased WBC count in CML patients [101]. Molecular studies showed that the TKIs imatinib and nilotinib also directly bind to and inhibit c-KIT activity, but the efficiency of inhibition of CD34⁺ CML cells with elevated *KIT* can vary between drugs [102]. Moreover, complete inhibition of c-KIT seems to be necessary for inducing apoptosis in CD34⁺ cells upon inhibition of BCR-ABL1 [102]. These results suggest c-KIT activation could influence therapeutic response to TKIs. While c-KIT activating mutations have been extensively studied in other diseases such as GIST and AML, reports of c-KIT mutations in CML patients are rare and could be the subject of future studies.

One of the targets activated by c-KIT, hypoxia-inducible factor 1-alpha (*HIF1A*), is a transcription factor that regulates the response to hypoxia. Overexpression of *HIF1A* is associated with several cancer types, with roles in cancer cell metabolism, survival, and invasion. In the absence of *BCR-ABL1* mutations, imatinib-resistant Ph⁺ CML cell populations were shown to have elevated *HIF1A* expression [103]. This study suggested that HIF1A activity could help CML progenitor cells to survive in the hypoxic bone marrow environment. These results were further corroborated in a study with primary CD34⁺ cells isolated from CML patients where the hypoxic environment was shown to protect CML cells despite effective BCR-ABL1 inhibition [104]. Thus, secondary inhibition of HIF1A could enhance the elimination of CML progenitor cells.

3.2.2 Downstream Signaling Pathways—Early investigations into the mechanisms of CML oncogenesis revealed that the PI3K/AKT/mTOR [105, 106], p38/MAPK [107], and STAT5 [108, 109] signaling pathways are activated downstream of the BCR-ABL1 kinase. Subsequent studies showed that these pathways were not only mediating the effects of BCR-ABL1 activation, their complete inhibition was also necessary in order to control growth and proliferation of CML cells.

In a study with 54 untreated and 62 imatinib-resistant CML patients, it was reported that resistant patients had significantly lower levels of BCR-ABL1, CRKL (CRK-like proto-oncogene), and AKT phosphorylation levels [110], suggesting alternative pathways independent of BCR-ABL1 may be activated in resistant populations. In an *in vitro* study, multiple Ph⁺ cell lines that did not carry mutations in the *BCR-ABL1* kinase domain, but were resistant to imatinib, nilotinib, and dasatinib were reported to carry mutations for constitutive activation of the PI3K/AKT pathway [111]. The role of downstream signaling was corroborated in another study that reported activation of the MAPK signaling pathway in CD34⁺ CML cells and found that co-inhibition of both PI3K/AKT and MAPK signaling resulted in a significantly enhanced elimination of CD34⁺ CML progenitor cells [112]. Other studies have reported constitutively active MAPK signaling in CML progenitor cells independent of BCR-ABL1, driven by activating mutations in c-Raf [113].

The involvement of JAK/STAT signaling in TKI resistance has also received attention in recent years, with a report suggesting STAT5 expression levels are upregulated in patients following imatinib treatment [114]. Thus, clonal selection of STAT5 expressing cells could be a mechanism of secondary resistance in Ph⁺ patients who respond to TKIs initially. Multiple recent studies also report that the inhibition of JAK-STAT signaling either via JAK

inhibitors [115], or the suppression of STAT5A and STAT5B, significantly enhances responsiveness to imatinib in Ph+ CML cells and to chemotherapy in imatinib-resistant cells [116, 117].

As shown in multiple reports, pathways downstream of BCR-ABL1 are clearly involved in the persistence of CD34⁺ CML progenitor stem cells. Evidently, insufficient clearance of CML stem cells could result in resistance to TKIs. However, these could be challenging to predict in patients at the time of diagnosis and may require longitudinal genomic profiling of patients to assess the status of oncogenic pathways during the course of treatment.

Table 3 provides an outline of acquired somatic alterations associated with TKI resistance in CML patients.

CONCLUSIONS

The clinical management of CML has improved dramatically since the introduction of BCR-ABL1-targeting TKIs. However, therapeutic resistance and relapse still affect a significant proportion of patients undergoing TKI treatment. As discussed above, several germline variants influence the efficacy of targeted drugs in CML patients, including variants in genes involved in drug transport and metabolism. In addition, somatic variants in drug target genes, as well as a compensatory activation of downstream signaling pathways, can also influence the efficacy of targeted drugs. Despite continual growth in PGx knowledge of targeted therapeutics, CML patients are not routinely profiled for genetic variants to predict treatment efficacy, in part due to most results being reported in small-scale clinical studies. Thus, to obtain reliable clinical biomarkers, the current PGx interactions must be validated in large population studies. Furthermore, to obtain a better understanding of the role of somatic variants in drug resistance or disease relapse, patients receiving targeted therapies should be monitored over time to observe how their cancer genome changes in response to treatment. With a comprehensive understanding of a patient's genetic profile, better treatment decisions can be made in the future, which would ultimately lead to improved treatment outcomes and quality of life.

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KEY POINTS

- Genetic makeup in both host body and cancer tissue affect tyrosine kinase inhibitors (TKIs)' treatment effect in CML.
- Understanding and implementation of pharmacogenomics of targeted therapy can improve treatment outcomes of CML.

TABLE 1

Drug metabolism variants that influence TKI response in CML patients

Genes	Variants	Drug	References
<i>CYP3A5</i>	CYP3A5*3 (GG)	Imatinib	[29]
<i>CYP2B6</i>	15631 (GG/TT)	Imatinib	[30]
<i>UGT1A1</i>	UGT1A1*28 UGT1A1*6	Nilotinib	[38–40]

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TABLE 2

Drug transport variants that influence TKI response in CML patients

Genes	Variants	Drugs	References
<i>ABCB1</i>	1263 (TT), (CT/TT) 2677 (TT/TA), (G) 3435 (TT), (CC)	Imatinib	[50–53]
<i>ABCG2</i>	34 (G>A) 421 (C>A)	Imatinib Dasatinib Nilotinib	[65, 66]
<i>SLC22A1</i>	rs3798168, rs628031 IVS7+850 (C>T) 480 (C>G) 401 (G/A)	Imatinib	[72–74]
<i>SLC22A4</i>	rs1050152	Imatinib	[80]
<i>SLCO1A2</i>	361 (GG)	Imatinib	[78]
<i>SLCO1B3</i>	334 (GG), (TT) 699 (GG)	Imatinib	[77, 79]
<i>ABCA3</i>	4548–91 (CC/CA)	Imatinib	[79]

TABLE 3

Acquired alterations associated with TKI resistance in CML patients

Gene/Pathway	Type of Alteration	Drug	References
<i>BCR-ABL1</i>	Kinase domain point mutations (T315I [†] , Y253H, E255K, E255V, Y253F, Y253H, F317L, H396P, M351T, and others)	Imatinib, Nilotinib, Dasatinib	[85–89] [90–93]
<i>KIT</i>	Activation/elevated expression	Imatinib	[101, 102]
<i>HIF1A</i>	Activation/elevated expression	Imatinib	[103, 104]
PI3K/AKT	Pathway activation	Imatinib, Nilotinib, Dasatinib	[110, 111]
P38/MAPK	Pathway activation	Imatinib	[112, 113]
JAK/STAT	Pathway activation	Imatinib	[114–117]

[†]Note: Ponatinib is equally effective against all kinase domain mutations of *BCR-ABL1*, including T315I.