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Molecular Mechanisms of Resistance to Tyrosine Kinase Inhibitors

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Abstract

Purpose of Review Chronic myeloid leukemia (CML) patients with constitutive activity of BCR-ABL1 oncoprotein frequently derive significant clinical benefits from tyrosine kinase inhibitors (TKIs). Point mutations in the ABL1 kinase domain (KD) are an important mechanism of TKI resistance in CML. In this review, we present molecular mechanisms of TKI resistance paying particular attention to drug resistance which allows for a survival advantage in CML.

Recent Findings Sensitive disease monitoring is a required standard of care for management of CML. Screening of these mutations fail to explain 20–40% of resistant cases where activation of different survival pathways must be the main reason for resistance.

Summary Eliminating TKI resistance appears to be the most successful therapeutic way to decrease leukemic disease burden and potentiate cure. Advances on novel strategies for identifying and confronting drug resistance are rapidly altering management of CML that are resistant to TKI and expanding the landscape of available therapies.

Keywords Chronic myeloid leukemia · Molecular mechanism · Tyrosine kinase inhibitor · Resistance mutations

Introduction

Chronic myeloid leukemia (CML) which is established from a reciprocal translocation between the long arms of chromosomes 9 and 22 is characterized by the presence of the Philadelphia chromosome (Ph+) and presence of BCR-ABL1 oncogenic fusion protein with an activated ABL tyrosine kinase in relatively constant amounts [1, 2]. The incidence of this rare clonal myeloproliferative disease is approximately 1–2 cases/100,000/year [3]. The BCR-ABL1 rearrangement in CML leads to overexpression of ABL1, making it a suitable target for therapy by kinase inhibitors. The advent of tyrosine kinase inhibitors has dramatically altered patient outcomes and improved survival rates to nearly a normal lifespan [4, 5]. In 2001, imatinib mesylate (IM), as a selective tyrosine kinase inhibitor (TKI), was approved for resistant/refractory CML patients, and 2 years later, it was established as a first-line treatment for newly diagnosed patients. Second- and third-generation TKIs have even better response rates, although significant improvements on survival over imatinib have not been clearly demonstrated [6–8]. Therefore, application of hematopoietic cell transplantation in CML patients has declined dramatically [3, 9].

Good-responder patients with the lowest rates of TKI resistance are newly diagnosed CML patients in chronic phase but the poorest responders are patients in blast crisis [10–17]. Overall, CML patients treated with frontline imatinib achieve complete cytogenetic response (CCyR) ~ 70% and ~ 90% at 1 and 5 years, respectively [18]. Response to TKI treatment has been strongly correlated with disease prognosis and is part of the NCCN guidelines for the management of CML. The ability to achieve a major molecular response within 3 to 6 months of initiating TKI treatment (MMR; BCR-ABL1 ≤ 0.1% on the International Scale [IS]) means resistance and progression are unlikely [19].

It is estimated that more than 25% of patients will develop resistance or intolerance to first-line TKI therapy and

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have to change TKIs, at least once during their lifetime. Many of these patients progress to accelerated phase or blast crisis [20•, 21••, 22]. The mechanism of resistance includes point mutations, pumps that control drug bioavailability in terms of drug influx/efflux, and reservoir of leukemic stem cells. Thus, hematologic responders with persistent detectable Ph chromosome in 100% of cells likely have very different resistance mechanisms from those with a three-log reduction in BCR-ABL1 but continue to maintain low-level detectable disease [9]. The best defined mechanisms contributing to TKI resistance are point mutations in the ABL1 kinase domain and BCR-ABL1 genomic amplification [23]. This is termed "intrinsic" resistance. In patients who have minimal residual disease (MRD) after treatment or disease which fail to achieve MMR with TKI treatment, there still may be no detectable mutation in ABL1, meaning the resistance is caused by different mechanisms (termed "extrinsic" resistance), which sustain leukemic cell survival through activating alternative signaling pathways [2, 21••, 24, 25].

Analysis for ABL1 mutation is usually carried out after TKI treatment resistance occurs and in approximately half of these cases ABL1 mutations are detectable leaving the remainder of cases with other mechanisms of resistance [26, 27]. The results of mutation analysis may help clinicians in the selection of other TKIs, since some of ABL1 kinase domain point mutations may cause varying levels of sensitivities to different TKIs.

During the past decade, many attempts have been done to characterize the clinical and biological importance of these mutations to develop novel inhibitors against many BCR-ABL1 mutant forms [28].

Definitions of Response and Resistance How we define disease as resistant and responsive to TKI treatment is critical. Resistance can be classified as hematologic (abnormal peripheral blood counts), cytogenetic (Ph+ chromosome persistency), and molecular (detectable BCR-ABL transcripts by RT-PCR) [29, 30].

According to the European Leukemia Net (ELN) recommendations, both cytogenetic and molecular responses can be categorized as "optimal," "warning," or "failure" and prognoses are dependent on these responses. The life expectancy of optimal responses is similar to that of the general population, but failure is associated with TKI resistance and increased risk of disease progression or death [21••, 31]. For patients in CP, AP, and BC, we have the same definitions and also apply to second-line treatment, when first-line treatment was changed due to intolerance. The assessment of response can be either done by a cytogenetic or molecular test, but if possible, both are recommended [31].

The NCCN and ELN guidelines have divided failure to reach treatment milestones into primary resistance, toxicity, and resistance/progression [31, 32]. Based on ELN criteria, Resistance has been defined using the European LeukemiaNet (ELN) criteria for failure of first-line TKI therapy: less than a complete hematologic response (CHR) and/or no cytogenetic response (CyR; defined as Ph+ bone marrow metaphases > 95%) at 3 months, BCR-ABL1 transcript levels above 10% and/or less than a partial CyR (PCyR; defined as ≤35% Ph+ metaphases) at 6 months, BCR-ABL1 transcript levels above 1% and/or less than a complete CyR (CCyR; defined as no Ph+ metaphases) at 12 months, or loss of a CHR or CCyR or confirmed loss of MMR, mutations, or clonal chromosome abnormalities in Ph− cells at any subsequent time during therapy [31, 33, 34]. According to NCCN guidelines, resistance is the presence of BCR-ABL1 transcript levels with qPCR International Scale (IS) above 10% at ≥6 months and BCR-ABL1 transcript levels with qPCR [IS] above 1% at 15 months [34, 35]. In patients who achieve a major molecular response (MMR; BCR-ABL1 ≤ 0.1% on IS), resistance and progression are unlikely [36]. All patients who obviously respond well, but RT-PCR do not become negative, are considered as resistant as well.

How BCR-ABL TKIs Inhibit CML Cells

Protein tyrosine kinases have important roles in the signaling cascade of a number of cellular activities related to growth, metabolism, apoptosis, and differentiation. BCR-ABL1 TKIs can significantly decrease the proliferation rate of Ph chromosome–positive CML cells by blocking the overactive ABL1 pathway [37].

These TKIs are classified as type I or type II inhibitors, based on whether they recognize an active or inactive kinase conformation respectively [21••, 38]. Type II inhibitors are ATP competitive, display stricter requirements for binding, and reveal more mutational vulnerabilities. This type of TKIs is considered as stabilizers of an inactive enzyme conformation [39]. Type I inhibitors have less selectivity for binding, directly compete for binding with ATP, and have less tendency to mutational escape [21••]. From the list of approved TKIs, dasatinib is categorized as a type I inhibitor; imatinib, nilotinib, and ponatinib are type II inhibitors, and bosutinib displays both features [21••].

Mechanisms of Resistance to Imatinib

Resistance to imatinib by BCR-ABL1 cells was reported as early as 2001, when imatinib was in phase I and II trials [40]. Generally, resistance to imatinib can be subdivided at the mechanistic level, in ABL1 mutation–dependent and ABL1 mutation–independent mechanisms [2, 9, 30]. Both mechanisms may induce overt clinical resistance, but primary resistance is more inclined to be BCR-ABL1-independent while

ABL1 mutation–dependent mechanism is more probable in acquired resistance. Resistance to TKIs is likely a multifactorial process like any other drugs and for appropriate disease management, biological information about basic mechanisms of drug resistance is necessary.

BCR-ABL1-Dependent Resistance Mechanisms

BCR-ABL Mutations

Hotspot mutations in several kinase domains are the dominant mechanism of secondary imatinib resistance but they are neither the only nor even the most frequent (Fig. 1b). At present time, more than 50 different hotspot mutations are known (Table 1). While the presence of the BCR-ABL1 are typically the initial target of most molecular assays, sequencing of targeted hotspots within ABL1 fusion transcript is key to discovering mutations attributing to resistance. According to different research findings, ABL1 mutations are detected in IM resistance CML patients with a frequency between 12 and 63% [29, 40] with similar numbers described in dasatinib and nilotnib [42, 43].

However, the degree of resistance is variable across these mutants in response to different TKIs [3], and the complexity or having multiple mutations changes patient outcome as well as in response the frontline TKI used [42, 43]. The main cause of resistance by mutations is defined by two principles. Mutations might exist de novo and are selected for as they survive frontline treatments or mutations may be induced by TKIs during therapy [25, 44, 45]. The major parts of TKI-resistant ABL1 point mutations restrict flexibility of the enzyme reducing accessibility of the drug binding site [1–4]. T315I, a gatekeeper mutation, was the first mutation indicated in relapsed CML patients [25, 46] and causes the highest level of resistance to the first and second generations of TKIs by replacing the threonine at 315 for isoleucine disabling formation of a hydrogen bond at this position for the TKI to bind to their targets [30]. The most common mutations with imatinib were listed in Table 1 which only 12 positions have been involved in the most compound mutations with probably clinical importance [4, 28, 34, 46–48]. New studies indicate a small but possibly significant indication to identify compound mutations [49, 50].

Nilotinib and dasatinib overcome some of the imatinib-resistant mutants and they have been demonstrated to show

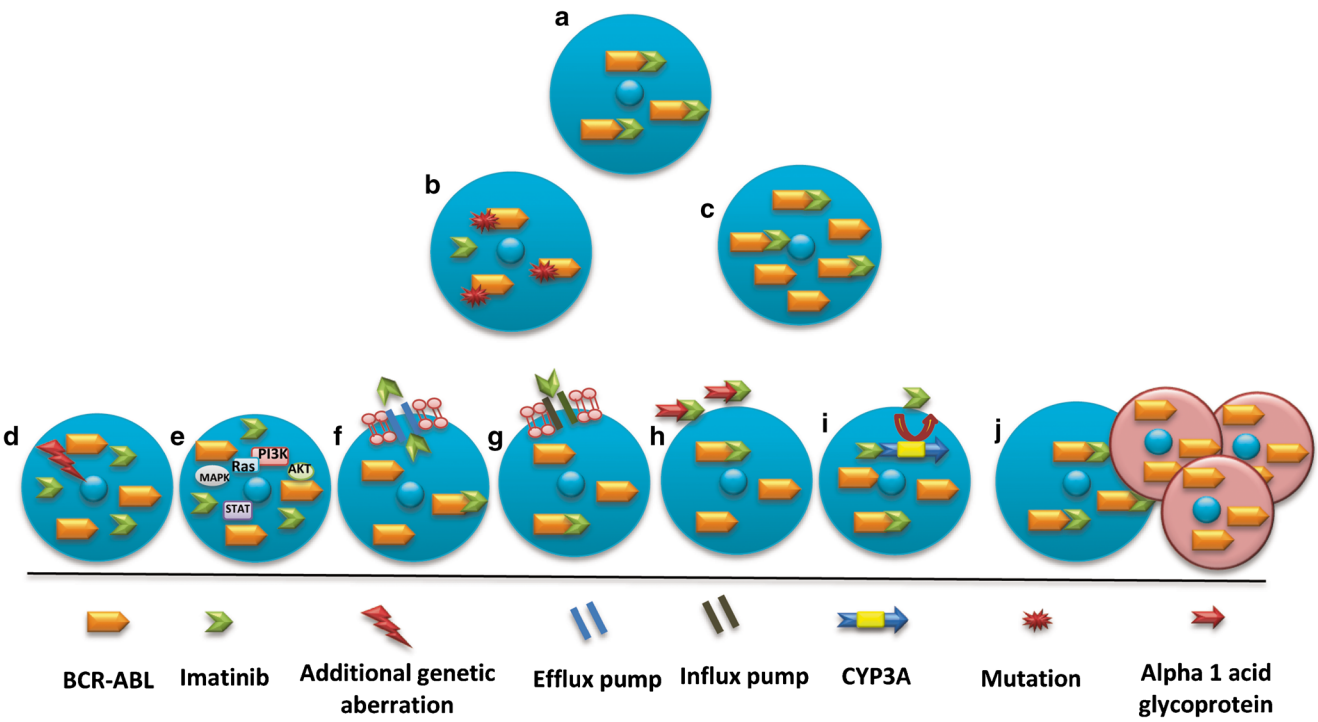

Fig. 1 Mechanisms of resistance towards imatinib. (a) Sufficient amount of imatinib within the cell for inhibition of all BCR-ABL. (b) Mutations within the Abl kinase domain prevent binding of imatinib. c) Overexpression of BCR-AB restores high level of oncogenic signaling and sufficient kinase activity in the presence of a given amount of imatinib. (d) Genetic instability may induce secondary genetic alterations that contribute to clonal evolution and the BCR-ABL-independent resistance mechanism. (e) Different impaired pathways contribute to the BCR-ABL-independent growth and/or survival of the malignant clone. (f) Drug efflux changes intracellular availability of imatinib. (g) Drug influx reduces the intracellular concentrations of imatinib. (h) Increased levels of a1 acid glycoprotein might reduce the amount of unbound imatinib available for inhibition of BCR-ABL within the cell. (i) Metabolization of imatinib by CYP3A confers TKI bioavailability to imatinib. (j) Quiescence CML stem cells have not been removed by imatinib and are insensitive to TKIs

Table 1 Spectrum of mutation resistance in CML for FDA-approved tyrosine kinase inhibitors [21••, 41•]

Resistance	Imatinib	Nilotinib	Dasatinib	Bosutinib	Ponatinib
Highly resistant	L248R	L248R	L248R	L248R	E255V
	E255V	Y253H	T315I	V299L	L238R + F359I
	T315I	E255V	T315A	T315I	
	T315V	T315I	T315V	T315V	
	L248R + F359I	T315V	F317R	F317R	
		F359I	F317V	F317V	
		L248R + F359I	L248R + F359I	L248R + F359I	
Resistant	G250E	G250E	L248V	G250E	L248R
	Y253H	F359V	G250E	E255K	G250E
	E255K		E255K	E255V	Q252H
	F359I		V299L	T315A	E255K
			F317L		F317R
					F359V
					H396R
Moderately resistant	L248V	L248V	Q252H	L248V	M244V
	Y253F	Q252H	Y253H	F317L	L248V
	D276G	Y253F	E255V	F359I	Y253F
	E279K	T315A	F359I	F486S	Y253H
	F317L	F317L	L384M		D276G
	F317R	F317V	F468S		E279K
	F359V	L384M			T315I
	H396P	H396P			T315V
	H396R	H396R			F317V
					F359I
					L384M
					F486S
Sensitive	M244V	M244V	M244V	M244V	E292L
	Q252H	Q252H	E292L	E292L	V299L
	E292L	E292L	Y253F	Q252H	T315A
	V299L	V299L	D276G	Y253F	F317L
	T315A	D276G	E279K	Y253H	M343T
	F317V	E279K	M343T	M343T	M351T
	M343T	F317V	M351T	M351T	H396P
	M351T	M343T	F359V	F359V	
	L384M	M351T	H396P	L384M	
		F486S	H396R	H396Ps	

a smaller spectrum of resistant mutations [51, 52]. Nilotinib tightly binds to inactive ABL1 conformation and dasatinib binds to ABL1 with more flexible conformational compared with imatinib, but they both cannot counteract the resistance inferred by T315I [53–56]. Patients treated with nilotinib most commonly acquire mutations in F359V/C/I, E255K/V, Y253H, or T315I mutations, whereas patients treated with dasatinib more often acquired T315A, V299L, F317L/V/I/C, or T315I mutations. Resistance mutations seen in patients treated with bosutinib have a spectrum similar to dasatinib [57]. Ponatinib, a type II inhibitor, binds ABL1 in a similar conformation to that observed with imatinib, but contrary with imatinib, no hydrogen bond is formed with T315I [21••, 58]; therefore, it is effective against T315I mutation–positive cells [31]. However, compound mutations with two or more mutations including T315I in BCR-ABL1 allele have shown ponatinib failure [41•, 58, 59]. Mutations in imatinib binding site or outside of the kinase domain like disrupted regulatory

domains of BCR-ABL1 are another mechanism of TKI resistance and are crucial for the conformational change from the inactive to active form of ABL1 [25, 60].

KD mutations that are more commonly seen in AP/BP-CML include Q252, Y253, E255, T315, E459, and F486. They are associated with clonal cytogenetic evolution and if they appear during treatment disease are associated with faster progression to AP/BP [9, 34, 46, 61]. Second-generation TKIs are associated with lower rates of blastic transformation [62, 63] and mutation vulnerability than imatinib [64].

Monitoring BCR-ABL1 for Clinical Response to TKIs and the Best Time for ABL1 KD Mutational Analysis

Monitoring of BCR-ABL1 transcript level is recommended in patients with a rising BCR-ABL during therapy and screening of ABL1 KD mutation is recommend in patients when there is failure to first- or second-line TKI treatment to find specific mutations for guidance of other treatment choices [30–32, 65••].

Screening of mutation is also considered reasonable in patients with chronic phase disease that do not achieve treatment milestones, have increasing BCR-ABL1 levels (especially those increasing to MMR), or are in an advanced-phase disease [66]. Mutations can be detected by direct Sanger sequencing [14, 28, 34, 67, 68] or NGS [69, 70••].

Depending on how we define resistance, the clinical phase of CML, and the methodology of BCR-ABL1 detection, 50–90% of patients taking imatininb and experiencing hematologic relapse have KD mutations [29, 71, 72].

Techniques like NGS can be very helpful to screen mutations that are below the limit of detection by direct Sanger sequencing which require approximately 10% of the input ABL1 to show mutations. CML mutations that survive through several lines of therapy, indicating these types of mutations might have important clinical implications and too many mutations, seem not to be easily tolerated [23, 73].

BCR-ABL1 Overexpression

Another mechanism leading to imatininb resistance is BCR-ABL1 overexpression but its clinical importance as a mechanism of resistance is less defined than KD mutations (Fig. 1c). High BCR-ABL1 expression can occur through gene amplification or differential regulation of BCR-ABL1 fusion gene [21••, 71]. Higher levels of BCR-ABL1 oncoprotein restore oncogenic signaling and allow for cell survival despite the presence of TKIs. High expression of BCR-ABL1 is more obvious in advanced-phase disease. These cells are less sensitive to imatinib and develop resistant mutant subclones to TKIs more rapidly in comparison with low-level-expressed BCR-ABL cells in chronic phase [21••]. This may be the reason for much worse responses to IM in patients who are in blast crisis compared with chronic phase patients [9, 25, 74].

BCR-ABL1-Independent Resistance

Multiple alternative signaling pathways have a potent role in BCR-ABL1-independent primary or secondary resistance. These BCR-ABL1-independent resistance mechanisms are an important contributor to MRD, likely due to leukemia stem cell persistence despite deep molecular response to TKI therapy [21••].

The mechanisms of BCR-ABL1-independent resistance are listed below.

Clonal Evolution, Genomic Instability, and Deficient DNA Repair Mechanism

Clonal evolution is another mechanism of IM resistance and is defined as progression after chronic phase and has long been associated with the gain of nonrandom chromosomal abnormalities, like extra Ph chromosome, trisomy 9, trisomy 19, point mutations in the coding sequences of RAS, p53, MYC, or rearrangements of RB and p16, in the karyotype of Ph+ cells [60, 75] (Fig. 1d). Overexpressed BCR-ABL1 fusion protein induces genomic instability in CML stem cells and this instability contributes to accumulation of point mutations in the ABL1 kinase domain and various other molecular and chromosomal aberrations [26, 76, 77]. The emergence of genetic aberrations associated with progression in CML including activation of mutations in GATA binding protein 2 (GATA-2) and partial deletions of RUNX1 and polymyxin resistance protein 16 (PMRD16), as well as expression of RUNX1/PMRD16, is detected in the CML blast phase and may also be involved in disease transformation, and drug resistance [78].

Impaired Alternative Pathways

CML stem cells are not eradicated by TKI likely because they are dependent on more than just BCR-ABL1 activity for survival [75]. This important limitation implies that CML stem cells maintain viability in the presence of TKI via different signaling pathways [2, 79]. Activation of many cellular signaling pathways such as PI3K/AKT, JAK/STAT, Ras/MAPK, and Src plays a role in resistance [75, 80] (Fig. 1e). Activation of alternative pathways can also explain disease recurrence even after discontinuation of therapy despite achieving deep molecular response and persistence disease and MRD positivity in responding cases [21••, 60, 80]. In CML patients, STAT3 activation is another BCR-ABL1 kinase–independent resistance mechanism and represents a major signaling point conferring TKI resistance. Targeting STAT3 in

addition to BCR-ABL1 pathway may help to overcome resistance and eradicate CML stem cells [24].

Drug Efflux and Changes in Intracellular Availability of TKI

Primary resistance in contrast to relapse after initial response has been linked to different expression levels and/or function of the transporter molecules responsible for IM influx/efflux [3] (Fig. 1f). The ATP-binding cassette (ABC) transporters are ATP-dependent multidrug efflux pumps which reduce the concentration of different materials inside the cells by transporting the drug out of the target cell or the cells of the gastrointestinal tract. These transporters include the multidrug-resistant gene product ABCB1 (P-glycoprotein; MDR1), members of the multiple resistant protein ABCC (MRP) family, and the breast cancer–resistant protein ABCG2 (ABCP, BCRP; MXR) which are very potent contributors for resistance to drugs (e.g., imatinib) [30, 81]. Since these drugs may have shared molecular targets but are not necessarily subject to the same transport mechanisms, use of a combination of TKIs may be helpful to overcome the resistance induced by this mechanism [9]. Higher expression of ABCB1 is observed in CML patients in blast crisis compared with CML patients in chronic phase, and MDR-1 upregulation is a strong mediator of decreased sensitivity to chemotherapy in advanced-phase disease.

Drug Influx and Reduction in the Intracellular Concentrations of Imatinib

Role of a1 Acid Glycoprotein Selection of a clone that is partially resistant to imatinib because of mutation or amplification of BCR-ABL1 is possible by diminishing the concentration of the intracellular inhibitor within the cell below values necessary for inhibition of that particular clone because of extrinsic or host factors such as albumin or a1 acid glycoprotein (AGP) plasma levels (Fig. 1h).

It has been suggested that increased expression levels of AGP result in the drug influx reduction into the cell and less unbound available imatinib for inhibition of BCR-ABL1 but there is not enough evidence for AGP being closely connected as a mechanism of resistance in CML [71, 82].

Expression of the Multidrug Resistance Protein (P-Glycoprotein)1 (MDR/P1) Another mechanism of IM resistance is the reduction of intracellular concentrations of TKI by increased drug efflux from the cell (Fig. 1g). Organic cation transporter 1 (OCT-1) is an influx pump for IM that has influence on intracellular drug availability. In patients taking IM, higher activity of OCT-1 is as a predictive marker for improved MMR rates, event-free survival (EFS), and overall survival (OS), whereas cellular uptake of second-generation TKIs (dasatinib and nilotinib) seems to be independent of OCT expression. This supports the observation of superior outcomes with dasatinib or nilotinib and may explain why restricted efficacy of imatinib may be by-passed through switching to another drug [3, 21••, 41•, 83, 84]. Detection of polymorphisms and expression level of ABC transporters are not used routinely in the clinic but their role as a mechanism of TKIs resistance is well studied [9, 21••, 85–88]. Several prospective studies are currently under way to see whether expression level of this influx transporter protein can be used to implicate in treatment decisions.

TKI Bioavailability and Pharmacokinetic Parameters

The cytochrome P450 enzymes metabolize imatinib, dasatinib, and nilotinib, primarily by the CYP3A4 isoform (Fig. 1l), and low trough plasma levels can be potentially explained by insufficient adherence of patients to the therapeutic regimen, other concurrent drugs the patient is taking, variations in the pharmacokinetics of the TKI, and intracellular uptake of the drug [30]. There are considerable variations in CYP3A activity among individuals [30, 89]. This may contribute to variations in level of TKI between CML patients. Interactions from concurrent drugs may inhibit or induce CYP3A4 impact plasma levels of TKI [82]. These drug-drug interactions can negatively influence TKI efficacy and contribute to TKI resistance [21••].

Quiescence CML Stem Cells

CML stem cells are roughly 0.5% of the CD34+ population and they do not need BCR-ABL1 kinase activity for survival. These cells are insensitive to TKIs and resistance/relapse is postulated to mainly originate from these cells (Fig. 1j). This may explain why TKI does not eradicate all leukemic cells even in the best responders. Efforts for combining TKI and drugs targeting these leukemic cells have so far progressed to clinical testing but they are not enough [3]. However, removal of these residual stem cells and identification of signaling pathways essential for CML stem cells, by combining TKI with another agent, would seem the most promising approach to overcome treatment failures [90].

More recently, many other BCR-ABL1-independent factors have been suggested as BCR-ABL1-independent TKI resistance mechanism includes activation of SRC family kinases, activation of compensatory pro-survival/anti-apoptotic pathways, FOXO1, Wnt/β-catenin, Lyn overexpression, the nucleocytoplasmatic transport molecules RAN and XPO1, miR-126, hypoxia-inducible factor 1a, arachidonate 15-lipoxygenase, RAD21 heat shock proteins, ADAR1, MYC, SIRT1, p53, Fap1, AXL tyrosine kinase, PP2A, apoptotic regulators, Cobll1/NF-κB signaling, Hedgehog signaling, epigenetic changes of DNA methylation, MicroRNAs, and IL-2/CD25 signaling [3, 21••, 25, 91]. However, it may be too early

to draw conclusions about whether these findings will translate into clinical significance and help overcome resistance in CMLs to TKIs.

Conclusion

A number of basic and fundamental mechanisms of TKIs resistance have been identified within our short review but the clinical consequences of resistance remain challenging due to heterogeneity of resistance mechanism and that it is likely multifactorial. By understanding these different mechanisms, we can find a common new inhibitor with wide utility to be beneficial for overcoming resistance. Consequently, management of resistant CML now includes use of newer generation TKI and combination therapeutic regimens to overcome resistance mechanisms this leukemia presents thereby increasing life expectancy for our patients. Using techniques with greater sensitivity like NGS allows for detection of early resistance and compound mutations carrying unique resistance profiles; it further enables timely therapy switches and selection of the most appropriate treatments [34, 92].

Compliance with Ethical Standards

Conflict of Interest Dr. Yeung reports grants from OBI Pharmaceuticals and Pfizer outside the submitted work. Dr. Yaghmaie declares no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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