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Intrinsic cellular resistance to BCR::ABL1 inhibitors

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AUTHOR CONTRIBUTIONS

Nataly Cruz-Rodriguez: Conceptualization, literature review, drafting of the manuscript, figure design, and critical revisions. Yulieth-Ximena Llanos-Torres: Literature review, drafting specific sections of the manuscript, tables preparation, and critical revisions. Michael W. Deininger: Conceptualization, supervision, writing the manuscript, critical revisions, and final approval of the manuscript.

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ABSTRACT

The clinical implementation of BCR::ABL1 tyrosine kinase inhibitors (TKIs) for the treatment of chronic myeloid leukemia (CML) represents one of the big successes of mechanism-based cancer therapy. In 2025, survival of patients who start TKI therapy while in the chronic phase is approaching that of age-matched controls. Despite this paradigm shift, significant challenges remain. Some patients still develop overt TKI resistance and progress to bast phase, and the majority continue to harbor residual leukemia and require life-long TKI therapy. Growth and survival signals arising from the microenvironment or from within the leukemia cells confer various degrees of resistance to support a spectrum of leukemic activity ranging from overt acute leukemia in blast phase to persistence of minimal residual disease in patients with a deep molecular response. Here we review cell-intrinsic resistance, covering both reactivation of BCR::ABL1 kinase activity and the less well-defined mechanisms underlying BCR::ABL1-independent TKI resistance. We propose that the pathways used by CML to escape TKI effects reflect the potential and the constraints of BCR::ABL1-driven reprogramming of hematopoietic stem and progenitor cells and that the role of BCR::ABL1 functions other than kinase activity may be underappreciated, providing a rationale for the clinical development of BCR::ABL1 degraders.

INTRODUCTION

Chronic myeloid leukemia (CML) is caused by BCR::ABL1, a constitutively active tyrosine kinase generated as a result of the t(9;22)(q34;q11.2) reciprocal translocation, cytogenetically detected as the Philadelphia chromosome (Ph) (1, 2). In the Western World, most patients are diagnosed in the chronic phase (CP-CML), which is characterized by expansion of myeloid progenitors that maintain terminal differentiation capacity. Without effective treatment, CP-CML invariably progresses to the blast phase (BP-CML), a therapy-resistant acute leukemia of myeloid or lymphoid phenotype. Transformation to BP-CML may occur suddenly or gradually through an intermediary stage historically termed accelerated phase (AP-CML). Although AP criteria were included in earlier classifications, the most recent WHO classification no longer recognizes AP-CML as a distinct disease phase, reflecting continuous debate regarding its diagnostic and biological validity (3, 4). Advanced CML at diagnosis is more common in countries with lower socioeconomic status, which is thought to be the result of delayed diagnosis or, less likely, ethnic differences in disease biology (5). Starting with imatinib, successive generations of tyrosine kinase inhibitors (TKIs) have dramatically improved CML prognosis (6). Today, the survival of patients diagnosed in CP-CML with access to modern health care is close to that of age-matched control populations, a quantum leap compared to the five-year survival in the pre-TKI era (7, 8). However, transformation to BP-CML still occurs in 10-15% of patients, particularly in those with more advanced CML at presentation, inconsistent access to health care, or poor compliance (9). Only one quarter of patients can discontinue TKIs permanently and achieve a state of functional cure termed treatment free remission (TFR) (10). The remainder require lifelong therapy to avoid recurrence of active leukemia, suggesting fully leukemogenic CML stem cells (LSCs) survive despite prolonged exposure to TKIs. In the three decades since the first clinical use of imatinib, an extensive literature on TKI resistance in CML has accumulated. While both intrinsic cell-autonomous and extrinsic microenvironmental mechanisms contribute to resistance, in the present manuscript we focus on cell intrinsic TKI resistance, emphasizing overarching principles and clinical context whenever possible. We structured the paper around the theoretical concept that the patterns of TKI resistance in CML reflect the biological constraints and capabilities of hematopoietic stem and progenitor cells (HSPCs) transformed by BCR::ABL1. We hope that this approach will provide orientation and stimulate future research.

DEFINITIONS

In 2025, CML resistance to therapy is almost synonymous with resistance to TKIs, although one should remember that BP-CML is frequently resistant against multiple modalities, including cytotoxic chemotherapy and hematopoietic stem cell transplantation (HSCT) (11). Mechanistically, TKI resistance may be extrinsic, involving growth and survival factors provided by the microenvironment, or intrinsic, mediated by pathways that originate from within the CML cell. While this concept is logical, distinguishing clearly between extrinsic and intrinsic mechanism is challenging, and clinical resistance may often reflect a combination. This applies both at the single-cell and population level, where multiple cell clones may use different extrinsic or intrinsic strategies to achieve TKI resistance (12, 13). Clinically, resistance can be primary (failure to achieve a desired level of response) or secondary (acquired), occurring after an initial response to TKIs. Lastly, the level of residual leukemia must be considered. In CP-CML, failure to achieve a complete hematologic response is very uncommon, while a substantial proportion of patients fail to achieve a deep molecular response, i.e., a reduction of BCR::ABL1 transcripts by 4-log or more. The term persistence is used to describe a situation where patients harbor low level residual CML but have a very low relapse risk. However, the

rare occurrences of sudden BP in patients even with undetectable *BCR::ABL1* is evidence that no level of residual CML offers absolute protection from transformation to BP (14, 15). Considering how difficult it is to draw the line between resistance and persistence, it may be preferable to conceptualize TKI resistance as a continuum.

REACTIVATION OF BCR:ABL1 KINASE

BCR::ABL1 mutations. In physiological conditions, ABL1 is maintained in an inactive conformation by complex mechanisms that involve binding of myristate to an allosteric pocket at the base of the kinase domain. In this auto-inhibited conformation, the ABL1 SH2 and SH3 domains are packed on the kinase domain, the DFG motif is in an outward position, and the activation loop assumes a closed conformation (16, 17). TKIs separate into several classes according to their binding modes. In the case of ABL1, only type I inhibitors (binding an active conformation), type II inhibitors (binding an inactive conformation) and type IV inhibitors (allosteric, binding to the myristoyl pocket) are presently of practical relevance, but TKIs belonging to other classes may be developed in the future. Point mutations in BCR::ABL1 that impair drug binding are the best-defined mechanism of TKI resistance (18). The spectrum of resistance mutations depends on the type of TKI. Within the ATP-competitive TKIs, the scope of mutations is largest for the first generation (1G) TKI imatinib. In addition to the commonly observed T315I mutation, multiple other mutations have been reported across different studies (Table 1). Although most of these variants are in the kinase domain of BCR::ABL1, some locate to the SH2 domain or the SH1-SH2 linker (19). The 2G TKIs nilotinib, bosutinib and dasatinib exhibit both unique and shared resistance mutations within an overall narrower spectrum of *BCR::ABL1* genotypes. Collectively, they cover all single mutants except T315I, which is sensitive only to the third generation (3G) TKIs ponatinib and olverembatinib. While no single BCR::ABL1 mutant confers absolute resistance to ponatinib, some are less sensitive, most importantly E255V. Clinical resistance to asciminib is associated with mutations not only in the myristoyl (allosteric) pocket, but also in the SH3 domain and in several kinase domain residues already known to confer resistance to ATP-competitive TKIs (20). The sensitivity of BCR::ABL1 mutants to the various TKIs is typically reported in the form of heat maps that report IC_{50} values based on growth inhibition of BaF3 or 32Dcl3 cells expressing the variants. While this provides useful orientation, *in vitro* profiling predicts clinical resistance much more reliably than clinical response. Not all BCR::ABL1 mutations identified in patients have been characterized functionally, and comprehensive validation of predictions based on observed clinical responses is not yet available.

BCR::ABL1 mutations may confer resistance through several mechanisms. Most straightforward is steric hindrance, initially reported for the T315I gatekeeper mutation that creates a bulky substation (18). A more indirect mechanism is conformational rearrangements that stabilize an active kinase conformation, which excludes type II TKIs. For instance ABL1, kinase-domain mutations such as M351T (C-lobe αE helix region) and H396P (activation loop) favor an active kinase conformation that decreases affinity for type II but note type I TKIs (6). In the case of asciminib, mutations that interfere with packing of the SH3 domain on the SH1 domain prevent the assembly of the kinase in the autoinhibited conformation, although asciminib still binds to the myristate pocket. These mutations include M244V, located within the N-lobe ABL1 kinase domain adjacent to the P-loop and F359V in the C-lobe. Rare BCR::ABL1 variants lack ABL1 exon 2 (such as e13a3), which prevents the assembly of an autoinhibited conformation, conferring primary asciminib resistance (20, 21). Several mechanisms may cooperate to achieve high level resistance. In the case of T315I, in addition to creating a steric clash, the mutation disrupts a hydrogen bond with imatinib and stabilizes the

hydrophobic spine of the kinase, favoring an active conformation (22). The ability to leverage more than one mechanism may contribute to the 'success' of T315I in conferring resistance to multiple different TKIs. Another layer of complexity is added by compound mutations, i.e. the presence of two mutations in cis (23). Compound mutations that encompass T315I render BCR::ABL1 resistant to all ATP-competitive TKIs, including ponatinib and, unexpectedly, also asciminib, perhaps by preventing the assembly of the myristate pocket (24). However, T315I-inclusive compound mutants are sensitive to combinations of asciminib and ponatinib (24). Computational simulations suggest that the addition of asciminib reduces Gibbs free energy (ΔG^0) for compound mutant ABL1 to levels equivalent to wild type ABL1 (24). Solving the structure of compound mutant ABL1 in complex with both TKIs will be required to definitively clarify the mechanism. Data for olverembatinib are unavailable in the public domain, but given the similarities between the two molecules, the suspicion is that its activity profile might resemble that of ponatinib (25). The fascinating variety of mutational resistance mechanisms in BCR::ABL1 may reflect the complex mechanism of physiological ABL1 regulation that involves allosteric and ATP binding pockets, offering multiple opportunities to disrupt TKI binding.

Alternative mechanisms of BCR::ABL1 kinase reactivation. Compared to BCR::ABL1 mutations, other mechanism of reactivating the kinase are less well characterized. CML cell lines cultured *in vitro* in the presence of increasing TKI concentrations frequently overexpress BCR::ABL1 through genomic amplification or transcriptional upregulation (26). The high concentrations of BCR::ABL1 protein appear to be poorly tolerated, and upon TKI withdrawal, cells revert to lower levels (26). Possible explanations for this include sequestration of critical signaling molecules by kinase inactive BCR:ABL1, protein aggregation eliciting cellular stress responses, and the energy expense associated with maintaining high levels of BCR::ABL expression. Lastly, an overdose of BCR::ABL1 kinase activity may be detrimental, as reported for B cell acute lymphoblastic leukemia (27). Overexpression of drug efflux pumps can lower intracellular TKI levels, promoting BCR::ABL1 reactivation, and certain polymorphisms in the MDR1 drug transporter are associated with response to imatinib, but there is no evidence that this mechanism can support overt TKI resistance (28). The significance of mechanisms such as increasing expression of MDR1 may be to provide an immediately available bridge until a definitive, genetically encoded mechanism of BCR::ABL1 reactivation is established (29). Understanding how LSCs respond to the very first TKI stress event might reveal how this primordial defense can be overcome.

Activation of mitogen activated protein kinase (MAPK) signaling by mutations in NRAS or KRAS is common in AML patients with resistance to FLT3 or IDH1/2 inhibitors, but very rare in CML (30-32). Why do CML cells strive to reactivate BCR::ABL1 kinase rather than acquiring a different gain of function mutation? One explanation may be that CML LSPCs typically lack mutations in epigenetic regulators that would allow them to tolerate strong mutational MAPK activation rather than undergoing apoptosis or senescence (33-35). Another consideration is balanced signaling output. During malignant transformation of B cells, direct mutational activation of MAPK and JAK/STAT signaling is mutually exclusive, suggesting that fine-tuning signal strength is critical (36). It is tempting to speculate that similar principles apply to CML, but experimental verification is unavailable.

The proportion of patients with clinical TKI resistance who exhibit BCR::ABL1 mutations is lowest in CP-CML and highest in lymphoid BP-CML (37). This may reflect TKI exposure history as well as biological constraints. Advanced phase patients have typically gone through more lines of TKI therapy than CP-CML patients. Additionally, driving advanced CML may require a level of BCR::ABL1 kinase activity that is not achievable by drug efflux of increased BCR::ABL1 expression. An intriguing

observation is that individual CML cases tend to relapse with the same mechanism. Thus, a patient who has developed resistance due to a kinase domain mutation is more likely to fail the next line of TKI treatment through acquisition of a different BCR::ABL1 mutation than through other mechanisms (38, 39). The cause of this intriguing mechanistic fidelity is unknown, but it would be interesting to determine whether the preference for a one of these mechanisms can be linked to specific mutations in additional genes.

BCR::ABL1 scaffold functions. Kinase-independent functions of BCR::ABL1, often referred to as scaffold functions, contribute to leukemogenesis, and they may be even more important when kinase activity is suppressed. $CD34^+$ CML cells exhibit increased proliferation, reduced apoptosis, and aberrant migration and adhesion to BM stroma, a phenotype recapitulated by cord blood $CD34^+$ cells transduced with $p210^{BCR::ABL1}$ (40, 41). TKI treatment of these cells normalizes growth, while abnormal adhesion and migration persist. Consistent with these observations, expression of a kinase inactive BCR::ABL1 mutant ($p210^{BCR::ABL1K1172R}$) has no effect on proliferation, but in part reproduces the migration and adhesion aberrancies (41). The tumor suppressor p27 is downregulated in CML LSPCs (42). Expression is restored by TKIs, but p27 remains partially mis-localized to the cytoplasm, from where it promotes leukemogenesis (43). BCR::ABL1 expression is required for activation of a JAK2/β-catenin survival/self-renewal pathway in LSPCs that involves inhibition of the tumor suppressor phosphatase PP2A. Reproduction of this finding in mouse $Lin^-Sca1^+Kit^+$ (LSK) cells expressing $p210^{BCR::ABL1K1172R}$ indicates that BCR::ABL1 protein rather than kinase activity is required for promoting LSC survival (44). Tyrosine 177 of BCR::ABL1 may be a key mediator of scaffold functions. In untreated CML cells, $BCR::ABL1^{Y177}$ phosphorylation activates signaling through the RAS/MAPK and phosphatidyl inositol 3' kinase (PI3'K) pathways (45, 46). In this situation, $BCR::ABL1^{Y177}$ is autophosphorylated[56]. However, LYN or JAK2 were shown to maintain $pBCR::ABL1^{Y177}$ in TKI-resistant cells treated with TKIs (47-50). Generation of reactive oxygen species (ROS) in CML LSCs is thought to confer genetic instability, promoting progression. ROS remain elevated in LSCs treated with imatinib compared to HSCs, and part of the ROS production is dependent on $pBCR::ABL1^{Y177}$, suggesting persistent $pBCR::ABL1^{Y177}$ may maintain ROS-induced genetic instability (51-53). Although the experimental data supporting the latter phenotype are convincing, recent work on primary CML cells has challenged the notion of a BCR::ABL1-induced mutator phenotype (54). Serum-induced phosphorylation of the adaptor protein SHC promotes its binding to the BCR::ABL1 SH2 domain and activation of RAS/MAPK signaling in a BCR::ABL1 kinase independent manner but is abrogated by BCR:ABL1 degradation (55). Scaffold functions of BCR::ABL1 might also underline a puzzling clinical observation. Kinase inactive $p210^{BCR::ABL1}$ splice forms are present in some CML patients and may be enriched in patients with TKI resistance (56, 57). CML cells carrying these loss of function variants should be eliminated, yet they persist, suggesting the variants provide a net functional gain (19, 58). In aggregate, these data strongly suggest that some signals contributing to TKI resistance originate from the BCR::ABL1 scaffold. Scaffold functions are available immediately upon TKI exposure, perhaps buying LSPCs critical time to activate salvage mechanisms following the shock of first BCR::ABL1 inhibition. This would not be possible with an effective BCR::ABL1 degrader that rapidly eliminates both kinase activity and scaffold functionalities (59).

ACTIVATION OF ALTERNATIVE GROWTH AND SURVIVAL PATHWAYS

Numerous signaling pathways have been implicated in TKI resistance of CML, painting a picture of bewildering complexity. We propose that considering the level of residual leukemia defining resistance may provide useful orientation. One extreme is hematologic resistance with or without

transformation to BP, the opposite persistence of residual disease. While residual leukemia in patients responding to TKIs is thought to reflect survival of LSCs, hematologic resistance involves the progenitor cell compartment. The pathways supporting CML cells despite sustained TKI inhibition of BCR::ABL1 seem to differ depending on the level of resistance and the position of resistant cells in the differentiation hierarchy. The more active the disease, the more the alternative pathways mimic the canonical signaling pathways activated by BCR::ABL1 kinase activity, including MAPK, PI3'K, and JAK/STAT, suggesting progenitor cells attempt to recreate the signaling driven by BCR::ABL1. This mimicry may explain why key features of the CP-CML phenotype are often preserved at relapse, such as myeloid left shift or basophilia. In contrast, many of the pathways implicated in LSC survival are hematopoietic stem cell pathways, indicating that the CML disease process mostly respects the hierarchy of normal hematopoiesis (Figure 1).

Epigenetic reprogramming. Numerous studies have revealed perturbed epigenetic regulation in CML LSPCs. These include aberrant regulation of the polycomb repressive complexes PRC1 and PRC2. In the canonical model, PRC2 (composed of EZH1/2, Suz12 and EED) catalyzes mono-, di-, and trimethylation of H3K27, enforcing a major repressive histone mark. PRC1 binds to chromatin through trimethylated histone 3 lysine 27 (H3K27me3) and ubiquitinates H2AK119 through the action of the RING1A/BRING1B ubiquitin ligases, promoting chromatin compaction and transcriptional repression (60, 61). Several PRC2 components are upregulated in LSCs, and two independent studies reported a critical role for EZH2 in LSC maintenance, consistent with the rarity of inactivating *EZH2* mutations in CML (62, 63). The PRC1 component BMI1 is responsible for ubiquitination of histone H2A K119. BMI1 expression is normally highest in HSCs and declines with differentiation, but its expression is elevated in CP-CML LSCs compared to HSCs, and increases further in BP-CML (34, 64). Epigenetic reprogramming in CML also involves histone deacetylates and DNA methylation, both at diagnosis and at progression (65). Transformation to BP is associated with reduced PRC2 and increased PRC1 activity, and this seems to be independent of which specific additional mutations are present in addition to BCR::ABL1(34). CML cells exhibit increased DNA CpG methylation compared to their normal counterparts, and hypomethylating agents have activity in TKI-resistant CML (66-68). A central question is whether these epigenetic aberrancies are induced primarily by BCR::ABL1 itself or require additional mutations. In CP-CML, where BCR::ABL1 is frequently the only detectable mutation, one must postulate that epigenetic changes are induced by BCR::ABL1 signaling. Whether these changes are reversible following TKI therapy is difficult to study, given the low frequency of LSPCs in TKI responders with residual disease. However, a doxycycline-controlled BCR::ABL1 mouse model provides important clues: LSPCs from leukemic mice exhibit increased DNA methylation compared to mice without BCR::ABL1 expression. While these changes are mostly reversible following remission induced by the addition of doxycycline, some marks persist (69). It would be interesting to determine which epigenetic changes are driven by kinase activity versus scaffold functions. If human CML LSPCs maintained a permanent memory of their BCR::ABL1 exposure that cannot be erased by TKI inhibitors, perhaps this could be accomplished by BCR::ABL1 degraders. An intriguing question is what happens at the epigenetic level in the immediate aftermath of the first TKI-induced BCR::ABL1 inactivation. If a stress-induced epigenetic program helped LSCs escape apoptosis, preemptively blocking this program may contribute to their elimination. Although hypomethylating agents show activity in TKI-resistant CML, they were never tested in combination with TKIs in the frontline, or even as a primer before starting TKIs (70, 71).

Metabolic reprogramming. In contrast to HSCs, LSCs depend on mitochondrial function, particularly oxidative phosphorylation (OXPHOS). It is not well understood which aspects of metabolic reprogramming are dependent on BCR::ABL1 kinase versus scaffold functions, and which are specific to TKI resistance. A recent report showed that BCR::ABL1 increases Myc expression, which suppresses the TXNIP (thioredoxin-interacting protein) transcription factor thereby increasing glucose uptake, glycolysis, and mitochondrial function. In mouse models TXNIP loss accelerates leukemogenesis, imatinib restores TXNIP expression in TKI-sensitive but not resistant CML cells, and TXNIP re-expression re-sensitizes resistant cells to TKIs (72). These findings provide a mechanistic link between BCR::ABL1 signaling and metabolic remodeling.

Targeting mitochondrial metabolism is an attractive strategy to overcome TKI resistance. This can be accomplished by inhibition of mitochondrial translation, as critical respiratory chain proteins are encoded by mitochondrial DNA or by small molecule inhibitors, such as the potent and selective electron transport chain (ETC) complex I inhibitor IACS-010759 (73, 74). Unfortunately, although IACS-010759 showed promising preclinical results, clinical trials had to be discontinued due to lactic acidosis and neurotoxicity, and no efficacy was seen at the doses tolerated (75). The Glasgow group recently reported a high-throughput screen that identified lomerizine, an $Ca2^{+}$ channel blocker approved for migraines, as an inhibitor of isocitrate dehydrogenase and OXPHOS that selectively sensitizes CML LSCs to imatinib (76). Pyruvate anaplerosis is increased in LSCs, facilitated by increased mitochondrial pyruvate carrier 1/2 (MPC1/2) expression and pyruvate carboxylase activity compared to HSCs. Remarkably, this metabolic abnormality is not reversed by TKIs, but is sensitive to the MPC1/2 inhibitor MSDC-0160, which is in clinical development for diabetes (77). Arginine auxotrophy represents yet another selective vulnerability. Unlike leukemia cell lines, $CD34^{+}$ LSPCs do not express significant levels of argininosuccinase 1 (ASS1) and are unable to upregulate ASS1 upon arginine depletion. As a result, they are sensitive to the arginine-depleting enzyme, BCT-100 (78). In xenograft studies, BCT-100 markedly reduced LSC survival, and this effect was not enhanced by imatinib, suggesting arginine dependence does not depend on BCR::ABL1 kinase activity. Finally, BCAT1, a cytosolic aminotransferase for branched-chain amino acids (BCAAs) is induced by the RNA-binding protein Musashi2 during progression to BP (79, 80). Whether this is dependent on BCR::ABL1 activity is unknown. BCAT1 inhibition induces differentiation and impairs BP-CML both *in vitro* and *in vivo*. Collectively, these studies point to extensive metabolic reprogramming in CML that involves both kinase-dependent and kinase-independent mechanisms and contributes to intrinsic resistance to TKIs.

Heterogeneity of CP-CML. With the current resolution of genetic testing, 10 - 15% of CP-CML patients have additional mutations at diagnosis, while in the remainder Ph is the sole detectable genetic abnormality (33, 81, 82). How can we reconcile this with the clinical heterogeneity of CP-CML? One consideration is loss of ABL1 or BCR sequences (or both) flanking the translocation breakpoint at the time of the initial translocation event (83). These deletions are associated with adverse outcome in patients treated with interferon alpha-based regimens, but their negative effect is largely overcome by TKIs (84). Attempts to identify putative tumor suppressors in the commonly deleted region(s) were inconclusive, but recent fine-mapping of BCR::ABL1 breakpoints suggest that we may not yet fully understand the extent of breakpoint-associated heterogeneity[65]. Intriguing work from Jyoti Nangalia's team has revealed that the acquisition of Ph increases HSC growth rates by a large margin, with the highest gains in young patients (54). These data are consistent with the clinical observation that young CML patients often present with aggressive disease and suggest that age-

related host factors modulate CML biology. Single cell RNAseq studies on CML HSPCs have identified two types of differentiation trajectory. Patients with an erythroid-megakaryocyte trajectory tend to have better responses to TKIs and better outcomes than patients with myelomonocytic trajectory (13). Thus, not all HSCs are created equal, and whether BCR::ABL1 gets to drive a Prius or a Porsche may profoundly impact CML biology and TKI response.

Mutations in addition to Ph. Some patients with BP-CML at diagnosis achieve deep and durable responses to TKIs, indicating that the cell clone that lost differentiation capacity behaves like CP-CML. On the other hand, deep responses to the next line of TKI are rare if BP developed during TKI exposure and practically absent in patients lacking BCR::ABL1 mutations, suggesting that BCR::ABL1 kinase independence is partly the result of selective pressure. Recurrent somatic mutations in numerous genes have been identified in BP-CML cells compared to the corresponding CP-CML, but there is no consistent pattern (Table 2) (33-35). A compilation of published data reveals several functional groups of genes. First, mutations in epigenetic regulators and chromatin-remodeling genes such as *ASXL1*, *IDH1/2*, DNMT3A and others can occur in CP-CML but tend to be more frequent in advanced disease. In contrast, mutations affecting the cohesin complex or RNA-splicing factors exhibit no obvious difference between CP-CML and BP-CML. Another set of genes appear mutated at low frequency but exclusively in AP/BP, suggesting a direct role in transformation. Notably, the genes with the clearest association with the BP-CML phenotype are enriched for transcription factors whose disruption is predicted to impair differentiation, including *RUNX1* and *IKZF1*. A smaller subset of genes mutated with increased frequency in BP is associated with RAS/MAPK activation, but this route of resistance appears to be much less common than in other myeloid neoplasms treated with TKIs, e.g., FLT3-ITD^{+} AML. The gene with the highest mutation frequency is *ASXL1.* One explanation for the predominance of ASXL1 mutations in CP-CML and their increasing frequency with progression is that ASXL1 loss-of-function provides a permissive epigenetic lesion that is compatible with CP biology but primes cells for subsequent transformation. Since ASXL1 is a positive regulator of PRC2, its disruption is expected to lower PRC2 activity, loosen epigenetic control of self-renewal and differentiation programs, and thereby to reduce the number of additional steps required to achieve a BP transcriptional state (34, 85, 86). This would explain why ASXL1-mutant clones are already detectable in CP-CML but become enriched as disease advances to BP. Similarly, mutations in *ASXL1* are among the most common genetic abnormalities found in clonal hematopoiesis, often arising years before overt myeloid neoplasia and conferring a competitive advantage to mutant stem cells (87, 88). In contrast to *ASXL1*, *TET2, DTMT3A, EZH2* and the splicing regulators are rarely mutated and not enriched in BP, suggesting these variants do not significantly impact disease progression. These data suggest fundamental differences between CML and other myeloid neoplasms. As precise data on the clonal architecture of BP-CML are unavailable, we do not know whether clonal evolution is predominantly linear or parallel. However, BCR::ABL1 independence, epigenetic dysregulation and TKI resistance parallel each other, suggesting that the BCR::ABL1 independence of advanced CML reflects progressive re-programming to a different cellular state that is less dependent on BCR::ABL1 kinase activity. Remarkably however, although multi-aberrant genotypes are common in BP-CML, loss of Ph has not been reported in patients progressing on TKIs (this is not to be confused with the emergence of clonally unrelated Ph^{-} MDS or AML (89)). Thus, BCR::ABL1 may continue to provide a net gain, even if kinase activity is suppressed, and even in BP-CML, BCR::ABL1 kinase independence does not equate BCR::ABL independence (Figure 2).

PERSPECTIVE

In resource-rich countries with access to state-of-the-art medical care for most patients, CML has morphed into a chronic condition of steadily increasing prevalence. Although we lack precise data, it is certain that CML outcomes are far worse in economically less fortunate parts of the world, and much could be improved without any new drugs, by enabling better access to care. However, even with optimal care, overt TKI resistance and progression to advanced phase would still occur in some patients, and the majority would still have to remain on TKI therapy. A few considerations may inform strategies to improve on this. First, overt clinical TKI resistance often combines BCR::ABL1 dependent and -independent mechanisms that cannot be mapped by mutational screening alone. Functional assays may provide additional guidance for therapy selection. Second, TKI exposure of LSCs does not completely revert BCR::ABL1-induced epigenetic and metabolic reprogramming, suggesting certain vulnerabilities persist. Alternatively, preventing LSCs from reprogramming may retain them in a more vulnerable state. Third, BCR::ABL1 scaffold functions may play a bigger role than often appreciated, supporting the development of clinical degraders. Fourth, for many patients CML is only one of several chronic conditions. In the US, 30% of patients aged 60 - 70 and 50% of those aged 70 - 80 are prescribed five of more daily medications. As adherence is known to drop sharply once the list of daily medication exceeds three, we can only guess how much non-adherence contributes to TKI resistance. The solution? Making TFR a reality for most patients. Clearly, long-term TKI therapy cannot be the final answer to CML.

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Table 1. Mutations identified in ABL1 and their association with clinical and *in vitro* resistance

Domain	Subregion	Mutation	Imatinib		Nilotinib		Dasatinib		Ponatinib		Bosutinib		Asciminib		Resistance Status	Ref
			Clinical	In vitro	Clinical	In vitro	Clinical	In vitro	Clinical	In vitro	Clinical	In vitro	Clinical	In vitro		
CAP	CAP	R47C		Low		Low		Low								(90)
SH3	SH3 domain	K84N		Low		Low		Low								(90)
SH3-SH2 connector	SH3-SH2 connector	E123Q		Low		Low		Low								(90)
SH2	SH2 domain	Y139D		NR		NR		NR								(90)
	SH2 domain	G144E		Low		Low		Low								(90)
	SH2 domain	S154N		Low		Low		Low								(90)
	SH2 domain	A196V		Low		Low		Low								(90)
	SH2 domain C-terminal region	T212R	R	Int		Low		Low								(90)
SH2-KD linker	SH2-kinase domain linker	P223S		Low		Low		Low		Low		Low		V		(91, 92)
SH2-KD linker	SH2-kinase domain linker	N231D/I		Low		Low		Low								(90)
SH2-KD linker	SH2-kinase domain linker	L237M	R													(93)
SH2-KD linker	SH2-kinase domain linker	M237V			R		R		R		R					(94, 95)
SH2-KD linker	SH2-kinase domain linker	M237I				Low										(96)
SH2-KD linker	SH2-kinase domain linker	T240M						NR								(97)
TKD	N-lobe, β1 strand	I242T	R													(98)
TKD	N-lobe β1 strand	M244V	R	Low		Low		Low		Low		Low	R	High		(20, 37, 94, 95, 99-103)
TKD	P-loop	K247R													Resistance not demonstrated	(104, 105)
TKD	P-loop	L248R		High		High		High		Int		High		Low		(20, 100)

TKD	P-loop	L248V	R	Low	R	Low	R	Int		Low	R	Low		High		(20, 96, 100-102, 106-108)
TKD	P-loop	G250A				Low										(96)
TKD	P-loop	G250E	R	Int	R	Int		V		Int		Int		V		(20, 24, 94, 95, 99-102, 106, 107, 109)
TKD	P-loop	G250R	R													(110)
TKD	P-loop	G250H		Low		Low		Low		Low		Low		Low		(91, 92)
TKD	P-loop	G250V				Low										(96)
TKD	P-loop	Q252H	R	Low	R	Low		Low		Int		Low		Variable		(91, 92, 94, 99-102, 106, 107)
TKD	P-loop	Q252R	R											Int		(110, 111)
TKD	P-loop	Y253C				Int										(112)
TKD	P-loop	Y253F	R	V		V		Low		Low		Low		Int		(20, 96, 100, 101, 107, 108)
TKD	P-loop	Y253H	R	Int	R	Variable		Low		Low		Low		Low		(24, 91, 94, 95, 99-102, 106, 109, 113)
TKD	P-loop	E255D				Low										(96)
TKD	P-loop	E255R				Int										(96)

TKD	P-loop	E255K/V	R	V	V	V	V	V	V	V	V	V		Low		(37, 91, 94-97, 99-101, 106, 107, 114, 115)
TKD	P-loop	V256G	R													(109)
TKD	P-loop	E258D													Resistance not demonstrated	(116)
TKD	N-lobe, β2 strand	W261L													Resistance not demonstrated	(116)
TKD	N-lobe, β3-αC loop	L273M	R													(93, 108)
TKD	N-lobe, β3-αC loop	E275K/Q													Resistance not demonstrated	(97, 117)
TKD	N-lobe, β3-αC loop	D276G	R	V		Low		Low		Low		Low		High		(96, 99-102, 107, 118, 119)
TKD	N-lobe, β3-αC loop	D276V													Resistance not demonstrated	(95)
TKD	N-lobe, β3-αC loop	T277A													Resistance not demonstrated	(97)
TKD	N-lobe, β3-αC loop	E279K		Int		Low		Low		Low		Low				(94, 99, 100, 107)
TKD	N-lobe, β3-αC loop	F279L	R													(102)
TKD	N-lobe, αC helix	E281K				Low										(96)
TKD	N-lobe, αC helix	E281*	R													(109)
TKD	N-lobe, αC helix	E285N				V										(96)
TKD	N-lobe, αC helix	E292Q	R													(120)
TKD	N-lobe, αC helix	E292L		Low		Low		Low		Low		Low				(100)
TKD	N-lobe, αC helix	E292V	R													(102)
TKD	N-lobe, αC-β4 loop	K294E		Low		Low		Low		Low		Low		V		(91, 119)

TKD	N-lobe, C-β4 loop	D295G	R													(108)
TKD	N-lobe, C-β4 loop	L298V	R													(102)
TKD	N-lobe, C-β4 loop	V299L	R	Low		Low	R	V	R	Low	R	V		V		(91, 92, 94, 99-102, 106, 107, 113, 121)
TKD	N-lobe, β4 strand	L301I	R													(109)
TKD	N-lobe, β4- β 5 loop	F311V				V										(96)
TKD	N-lobe, β4- β 5 loop	F311I	R			Low										(96, 102, 108)
TKD	Gatekeeper, β5 strand	T315V		High		High		High		High		High				(100)
TKD	Gatekeeper, β5 strand	T315I	R	High	R	High	R	High	S	Low	R	V	S	V		(20, 91, 94, 99-101, 106, 107, 113, 114, 122, 123)
TKD	Gatekeeper, β5 strand	T315A		Low		Low		High		Low		Int				(100, 101)
TKD	Gatekeeper, β5 strand	T315M		High		High		High		High				V		(24, 124)
TKD	Contact site, hinge region	F317I													Probable dasatinib and ponatinib resistance	(97, 125)
TKD	Contact site, hinge region	F317L	R	Low	R	Low	R	Int		Low		Low		High		(20, 94, 95, 100,

																101, 106, 107, 109, 113, 122, 126)
TKD	Contact site, hinge region	F317R		Low		Low		High		Int		High				(100)
TKD	Contact site, hinge region	F317C			V	V	R	High								(125, 127)
TKD	Contact site, hinge region	F317V		Low		Low		High		Low		Int				(95, 100, 101, 106, 113, 122)
TKD	hinge region	Y320H	R													(109)
TKD	hinge region	L324Q	R													(101, 120) Next-
TKD	C-lobe, E helix	A337T/V		Low		Low		Low		Low		Low	R	High		(20, 24, 91, 92, 94, 119, 123)
TKD	C-lobe, E helix	Y342H	R													(98, 128)
TKD	C-lobe E helix	M343T		Low		Low		Low		Low		Low				(100)
TKD	C-lobe E helix	A344V													Probable imatinib resistance	(95)
TKD	C-lobe E helix	A344P												High		(24)
TKD	C-lobe E helix	S348L				Low										(96)
TKD	C-lobe E helix	S349L				Low										(96)
TKD	C-lobe E helix	M351V			R											(106, 122)
TKD	C-lobe E helix	M351T	R	Low	R	Low		Low		Low		Low	R			(37, 94, 100, 102, 106, 107, 109, 123)

TKD	C-lobe □E helix	E355G	R	Low	R	Low		Low		Low		Low		V		(91, 92, 95, 96, 99, 101, 106, 108)
TKD	C-lobe □E helix	E355A	R			Low										(95, 96, 102)
TKD	C-lobe □E helix	K357T													Probable imatinib resistance	(95)
TKD	C-lobe □E helix	K357E													Resistance not demonstrated	(106)
TKD	C-lobe	V359F													Resistance not demonstrated	(114)
TKD	N-lobe-C-lobe interface	F359I	R	Int	R	V		Low		Low		Low	R	V		(24, 94, 95, 100, 101, 106, 123)
TKD	N-lobe-C-lobe interface	F359C	R		R	Int								High		(24, 95, 96, 101, 102, 113, 122)
TKD	N-lobe-C-lobe interface	F359V	R	V		V		Low		Low		Low		V		(20, 24, 91, 95, 96, 107)
TKD	N-lobe-C-lobe interface	L364I	R													(102)
TKD	N-lobe-C-lobe interface	L370P													Predicted high resistance to imatinib and nilotinib, moderate to bosutinib and	(129)

															axitinib	
TKD	N-lobe-C-lobe interface	V371A	R													(130)
TKD	N-lobe-C-lobe interface	E373D	R													(109)
TKD	N-lobe-C-lobe interface	E373K													Probable imatinib resistance	(120, 131)
TKD	N-lobe-C-lobe interface	V379I		Low												(132)
TKD	N-lobe-C-lobe interface	A380S				Low										(96)
TKD	Activation loop	D381N	R													(109)
TKD	Activation loop	F382L													Resistance not demonstrated	(120, 133)
TKD	Activation loop	L384M	R	V		Low		V		Low		Low				(99, 100, 107, 108)
TKD	Activation loop	L387F				V										(96)
TKD	Activation loop	L387W	R													(134)
TKD	Activation loop	L387M	R				R									(95, 99, 101, 102, 106)
TKD	Activation loop	M388L				Low										(96)
TKD	Activation loop	Y393C													Resistance not demonstrated	(98)
TKD	Activation loop	Y393*	R													(109)
TKD	Activation loop	H396R	R	Int		Low		Low		Int		Low		Low		(24, 94, 99-102, 106-108)
TKD	Activation loop	H396P		V		Low		Low		Low		Low				(100, 107)
TKD	Activation loop	H396A	R													(102)
TKD	Activation loop	A397P	R													(94, 135, 136)
TKD	Activation loop	G398R		Low		Low		Low				Low				(107)
TKD	Activation loop	A407P													Resistance not demonstrated	(99)

TKD	C-lobe, αF helix	N414I	R													(94)
TKD	C-lobe, αF helix	S417Y	R													(98)
TKD	C-lobe, αF helix	S417F	R													(137)
TKD	C-lobe, αF helix	I418S/V	R													(98, 135)
TKD	C-lobe, αF helix	A433T	R													(120, 138)
TKD	C-lobe, αF-αG loop	S438C	R													(139, 140)
TKD	C-lobe, αG helix	E450A/V	R													(98, 135, 141)
TKD	C-lobe, αG helix	E450G	R													(98, 122, 142, 143)
TKD	C-lobe, αG helix	E450K					R									(94)
TKD	C-lobe, αG helix	E453K													Ponatinib resistance (with additional mutations); probable imatinib resistance.	(23, 144)
TKD	C-lobe, αG helix	E453G													Resistance not demonstrated	(145)
TKD	C-lobe, αG helix	E453A	R													(93, 95)
TKD	C-lobe, αG helix	E453V	R													(39, 146)
TKD	C-lobe, αG-αH loop	E459G	R													(39, 99)
TKD	C-lobe, αG-αH loop	E459Q	R													(102)
TKD	C-lobe, αG-αH loop	E459K		Low	R	Low		Low		Low	R	Low		Low		(91, 94, 95, 99, 122)
TKD	C-lobe, αG-αH loop	G463S		Low		Low		Low		Low		Low		High		(91)
TKD	C-lobe αG-αH loop	C464Y												High		(91)

TKD	C-lobe, □G-□H loop	P465S		Low		Low		Low		Low		Low		High		(24, 91)
TKD	C-lobe, □H helix	V468F		Low		Low		Low		Low		Low	R	High		(24, 91, 92)
TKD	C-lobe, □H helix	M472I	R													(98, 147)
TKD	C-lobe, □I helix	P480L	R													(98, 148)
TKD	C-lobe, □I helix	S481P			R											(94)
TKD	C-lobe, □I helix	F486S	R	Int		Low		V		Low		Low				(100-102, 107, 122)
TKD	C-lobe, □I-□I' loop	F497L												High		(91)
TKD	C-lobe, □I' helix	I502L		Low		Low		Low		Low		Low	R	V		(91, 92)
TKD	C-lobe, □I' helix	L510P												High		(119)
TKD	C-lobe, □I' helix	E507G													Resistance not demonstrated	(149)
TKD	C-lobe, □I' helix	E543A													Resistance not demonstrated	(122)

R = documented clinical resistance; V = variable resistance (conflicting or context-dependent clinical/in vitro data); NR = no significant resistance compared to WT ABL1; TKD = ABL1 tyrosine kinase domain; Low/Int/High = relative in vitro IC□□ categories (definitions based on thresholds used in the cited studies); Mutations annotated with an asterisk (*) indicate a nonsense mutation resulting in a premature stop codon; “Resistance not demonstrated” = mutation reported in the literature but without reproducible evidence of conferring TKI resistance.

Table 2. Gene detection rate in chronic and advanced stages of chronic myeloid leukemia and its clinical relevance.

Category	Role	Genes	Biological Function	Chronic phase	Blast phase	Clinical relevance	Ref
Epigenetic Regulator	Chromatin Remodeling	*ASXL1*	Epigenetic regulator involved in chromatin remodeling and regulation of HOX genes	13.0%	24.4%*	•Lower rates of molecular/cytogenetic responses •Lower rates of progression-free survival/event-free survival •TKI resistance	(150-153)
	Transcriptional Corepressor	*BCOR*	Transcriptional corepressor interacting with epigenetic complexes for gene silencing	3.4%	4.5%	•Lower rates of molecular/cytogenetic responses •Lower rates of progression-free survival/event-free survival	(33, 35, 128, 150, 151, 153-155)
	Transcriptional Corepressor	*BCOLR1*	Transcriptional corepressor like BCOR, involved in gene repression	NR	9.3%	There is no evidence of clinical significance	(33, 35, 150, 155)
	DNA Methylation	*DNMT3A*	DNA methyltransferase responsible for de novo methylation, regulating gene expression	2.8%	2.7%*	•Lower rates of molecular/cytogenetic responses •Lower rates of progression-free survival/event-free survival •Markers of disease progression to BP/AP •Risk of relapse after discontinuation of TKI	(35, 81, 128, 150, 153, 154, 156, 157)
	Histone Methylation	*EZH2*	Histone methyltransferase, part of PRC2 complex, mediates H3K27 trimethylation for gene repression	1.7%	1.6%	•Lower rates of molecular/cytogenetic responses •Lower rates of progression-free survival/event-free survival	(33, 35, 81, 128, 153, 155, 157)
	DNA Demethylation	*TET2*	DNA demethylase converting 5-methylcytosine to 5-hydroxymethylcytosine, involved in hematopoiesis	3.5%	3.3%*	•Markers of disease progression to BP/AP •Risk of relapse after discontinuation of TKI	(35, 128, 150, 153)

	Metabolic Enzyme	*IDH1*	Metabolic enzyme producing 2-hydroxyglutarate, affecting epigenetic regulation via TET2 inhibition	1.1%	3.6%	•Lower rates of molecular/cytogenetic responses •Lower rates of progression-free survival/event-free survival	(33, 35, 128, 150, 153)
	Metabolic Enzyme	*IDH2*	Produces 2-hydroxyglutarate and alters epigenetic regulation	NR	2.4%		
	Histone Acetylation	*EP300*	Histone acetyltransferase and transcriptional coactivator regulating chromatin accessibility.	1.7%	NR	There is no evidence of clinical significance	(81, 154)
	Histone Acetylation	*CREBBP*	Histone acetyltransferase and transcriptional coactivator involved in gene expression regulation.	2.6%	NR	There is no evidence of clinical significance	(81, 154)
	Histone Methylation	*KMT2D*	Histone methyltransferase involved in H3K4 methylation and transcriptional activation	2.0%	3.2%	There is no evidence of clinical significance	(33, 35, 81, 154)
	Histone Methylation	*SETD1B*	Histone methyltransferase involved in H3K4 methylation and gene activation	1.6%	3.4%	There is no evidence of clinical significance	(33, 35, 155)
	Histone Demethylation	*KDM6A*	Histone demethylase removing H3K27me3 marks, counteracting PRC2-mediated repression	NR	2.2%*	There is no evidence of clinical significance	(35, 150)
	Histone Methylation	*SETD2*	Histone methyltransferase mediating H3K36 trimethylation, involved in transcription elongation	NR	5.7%	There is no evidence of clinical significance	(33, 151)
	Chromatin Remodeling	*PHF6*	Transcriptional regulator involved in chromatin remodeling and hematopoietic development	NR	3.4%	There is no evidence of clinical significance	(33, 35, 128, 150, 155)

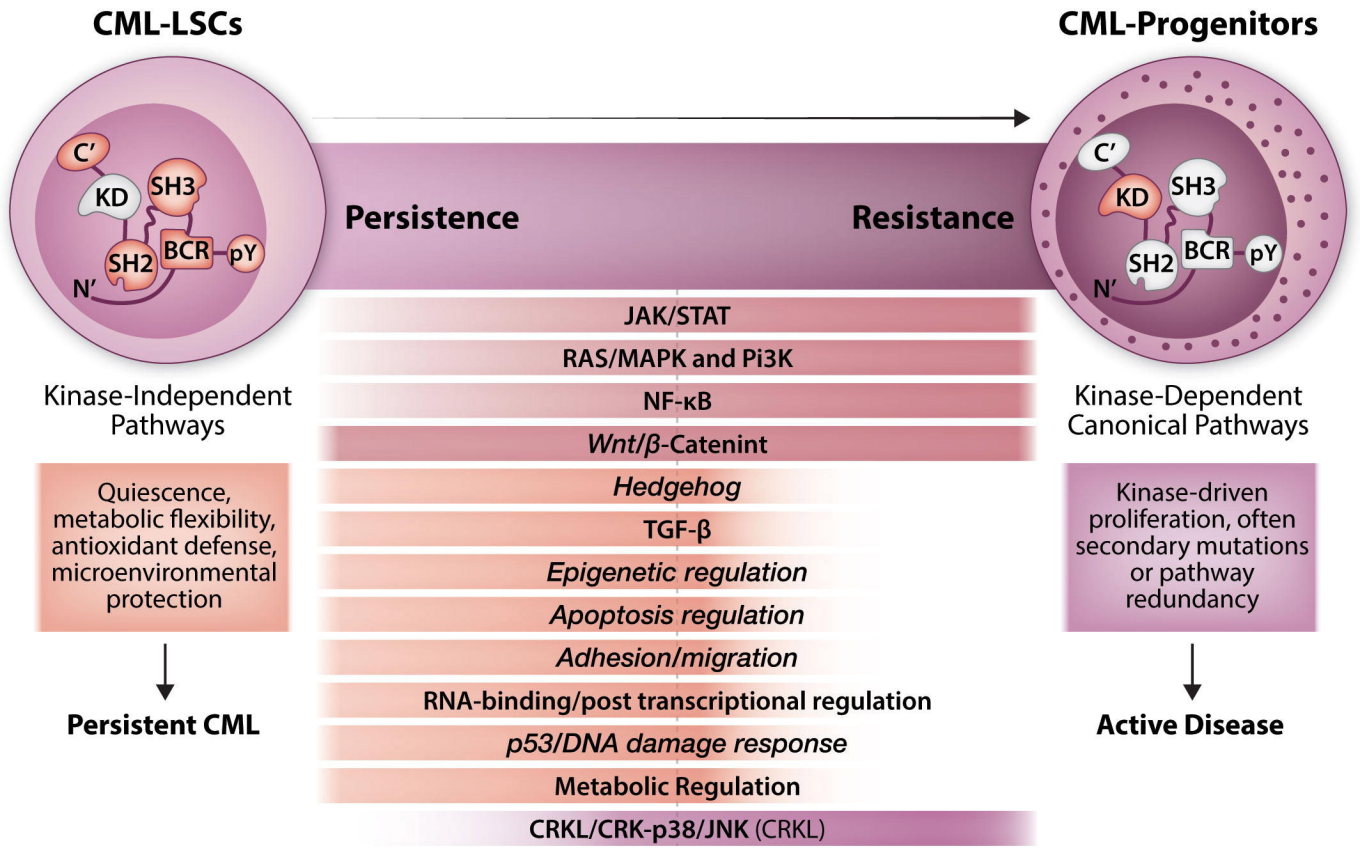
	Transcriptional Regulator	*SETBP1*	Transcriptional regulator associated with cell proliferation and aggressive leukemia phenotypes	NR	1.2%	There is no evidence of clinical significance	(35, 128)
RNA Splicing Factor	Splice Site Recognition	*U2AF1*	RNA splicing factor regulating splice site recognition and pre-mRNA processing	NR	1.2%	There is no evidence of clinical significance	(33, 35)
Cohesin Complex	Chromosome Segregation	*STAG2*	Cohesin complex component regulating chromatin structure and chromosome segregation	0.9%	NR	There is no evidence of clinical significance	(128, 150)
		STAG1	Cohesin complex component involved in sister chromatid cohesion and gene regulation	NR	1.3%*	There is no evidence of clinical significance	(35, 150)
Transcription Factor	Hematopoiesis	*GATA2*	Transcription factor essential for hematopoietic stem cell maintenance and differentiation	0.6%	7.0%	There is no evidence of clinical significance	(33, 35, 128, 155)
	Lineage Commitment	*RUNX1*	Transcription factor regulating hematopoietic lineage commitment and differentiation	2.5%	22.5%*	•Markers of disease progression to BP/AP •Risk of relapse after discontinuation of TKI	(33, 35, 128, 150-153, 155)
	Tumor Suppressor	*WT1*	Transcription factor involved in development, tumor suppression, and apoptosis regulation	NR	5.3%*	There is no evidence of clinical significance	(35, 128, 150)
	Tumor Suppressor	*TP53*	Tumor suppressor regulating cell cycle, DNA repair, and apoptosis in response to stress	1.8%	7.0%	There is no evidence of clinical significance	(33, 35, 150, 154, 157)

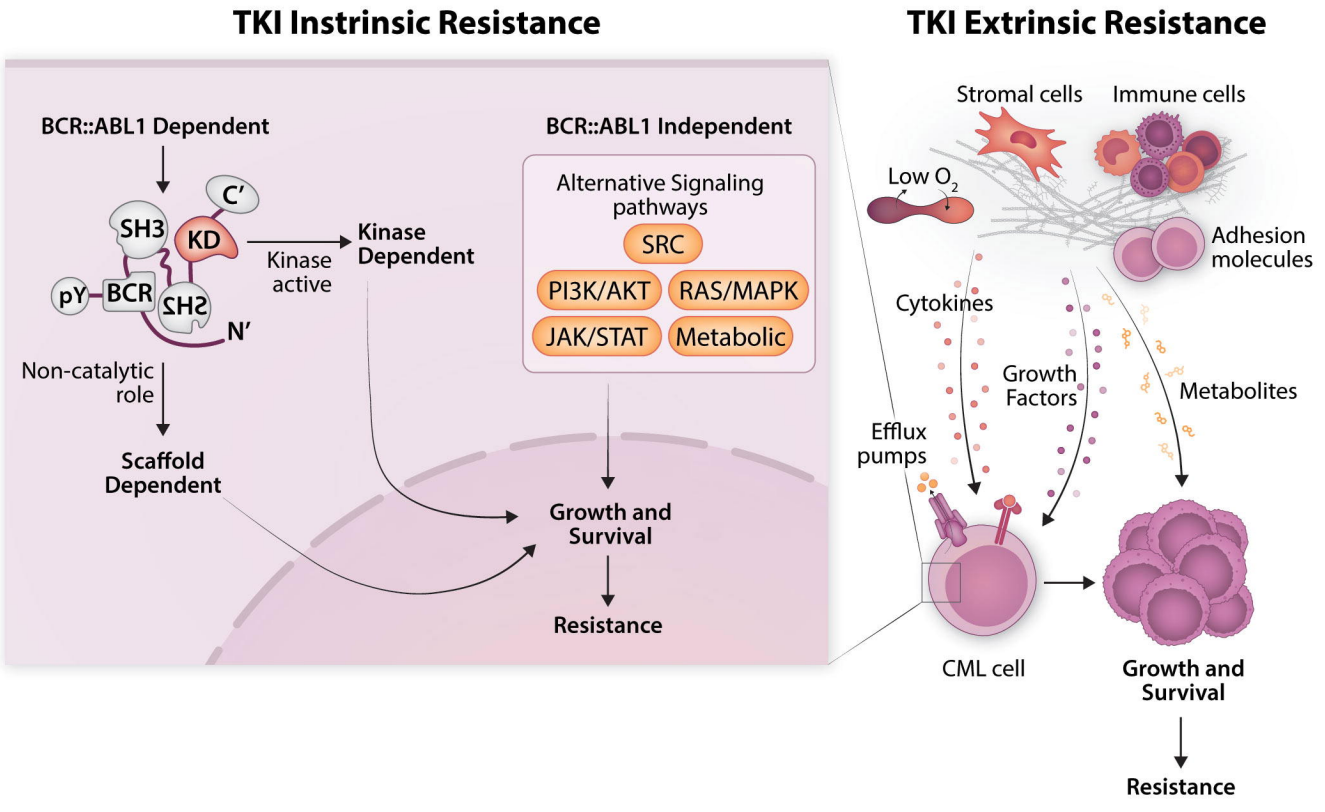
	Lymphoid Differentiation	*IKZF1*	Transcription factor critical for lymphoid lineage development, especially B-cell differentiation	3.4%	16%*	•Lower rates of molecular/cytogenetic responses •Lower rates of progression-free survival/event-free survival •Markers of disease progression to BP/AP •Risk of relapse after discontinuation of TKI	(33-35, 81, 150, 152-155)
Signal Transduction	RAS Pathway	*NRAS*	Signal transduction molecule regulating cell proliferation and survival via MAPK pathway	NR	3.2%	There is no evidence of clinical significance	(35, 128, 151, 152)
		RAS	Family of GTPases involved in cell signaling, proliferation, and differentiation	NR	9%*	There is no evidence of clinical significance	(150)
		PTPN11	Protein tyrosine phosphatase involved in RAS/MAPK signaling and hematopoietic development	NR	2.2%	There is no evidence of clinical significance	(35, 150, 152)
	MAPK Activation	*KIAA1549*	Fusion partner in gliomas, activates MAPK pathway when fused with BRAF	2.7%	NR	There is no evidence of clinical significance	(151, 154)
	Tyrosine Kinase Signaling	*BCR*	Fusion partner in BCR-ABL, involved in tyrosine kinase signaling and leukemogenesis	NR	8.0%	There is no evidence of clinical significance	(151, 152)
Others	Ubiquitination	*UBE2A*	Ubiquitin-conjugating enzyme involved in DNA repair and protein degradation	NR	4.8%	There is no evidence of clinical significance	(33, 35, 152, 155)
	Nuclear Export	*XPO1*	Nuclear export protein regulating transport of tumor suppressors and growth regulators	NR	6.1%	There is no evidence of clinical significance	(33, 152, 155)

*Also associated with the accelerated phase. Not Reported (NR) = no association found between the gene and this specific disease phase in the literature reviewed (across all studies included in the table). Tyrosine kinase inhibitor (TKI). The percentages shown in each column were calculated using the total number of patients across all studies cited in that row, summing the patient counts reported in each study.

Figure 1. Hierarchical basis of TKI resistance in CML. Residual CML cells use different survival strategies depending on their position in the hematopoietic hierarchy. Leukemic stem cells (LSCs) persist through kinase-independent pathways that reflect mechanisms used by hematopoietic stem cells. In contrast, progenitor-mediated resistance relies on kinase-dependent canonical pathways that mimic BCR::ABL1 signaling (e.g., JAK/STAT, RAS/MAPK, PI3K), often reinforced by pathway redundancy.

Figure 2. Intrinsic versus extrinsic pathways of TKI resistance. Resistance to tyrosine kinase inhibitors (TKIs) in BCR::ABL1-positive leukemias can be categorized as intrinsic or extrinsic. Intrinsic mechanisms include BCR::ABL1-dependent resistance, which may be kinase-dependent (e.g., kinase domain mutations) or scaffold-dependent (non-catalytic functions sustaining downstream signaling), and BCR::ABL1-independent resistance, involving activation of alternative pathways, metabolic reprogramming, or epigenetic alterations. Extrinsic mechanisms derive from the bone marrow microenvironment and involve cellular, soluble, and solid components.


CML-LSCs
CML-Progenitors
C′
KD
SH3
SH2
BCR
pY
N′
Persistence
Resistance
JAK/STAT
RAS/MAPK and Pi3K
NF-κB
Wnt/β-Catenint
Hedgehog
TGF-β
Epigenetic regulation
Apoptosis regulation
Adhesion/migration
RNA-binding/post transcriptional regulation
p53/DNA damage response
Metabolic Regulation
CRKL/CRK-p38/JNK (CRKL)
Kinase-Independent Pathways
Quiescence, metabolic flexibility, antioxidant defense, microenvironmental protection
Persistent CML
Kinase-Dependent Canonical Pathways
Kinase-driven proliferation, often secondary mutations or pathway redundancy
Active Disease


TKI Instrinsic Resistance
BCR::ABL1 Dependent
SH3
KD
C′
pY
BCR
SH2
N′
Kinase active
Kinase Dependent
Non-catalytic role
Scaffold Dependent
BCR::ABL1 Independent
Alternative Signaling pathways
SRC
PI3K/AKT
RAS/MAPK
JAK/STAT
Metabolic
Growth and Survival
Resistance
TKI Extrinsic Resistance
Stromal cells
Immune cells
Low O2
Adhesion molecules
Cytokines
Growth Factors
Metabolites
Efflux pumps
CML cell
Growth and Survival
Resistance