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Mechanisms of resistance to ABL kinase inhibition in CML and the development of next generation ABL kinase inhibitors

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Keywords

Chronic myeloid leukemia (CML); tyrosine kinase inhibitor (TKI); BCR-ABL1; treatment-free remission (TFR); drug resistance; mutation

INTRODUCTION

Every year, more than 8000 new cases of chronic myeloid leukemia (CML) are diagnosed in the United States[1]. BCR-ABL1, a fusion protein kinase derived from a reciprocal translocation between chromosomes 9 and 22, is necessary and sufficient for CML pathogenesis[2]. Tyrosine kinase inhibitors (TKIs) of BCR-ABL1 have revolutionized CML therapy, with life expectancy now close to that of the general population[3]. As a result, the prevalence of CML is growing, as patients on TKIs live with what is more and more viewed as a chronic ailment rather than a potentially lethal disease. It is estimated that over 25% of CML patients will switch TKIs at least once during their lifetime due to TKI intolerance or resistance[4]. Mutations in the kinase domain (KD) of BCR-ABL1 are the most extensively studied mechanism of TKI resistance in CML, but fail to explain anywhere from 20–40% of resistant cases. Activation of alternative, BCR-ABL1-independent survival pathways has been mechanistically implicated in these cases, and may also explain the phenomenon of persistence in responding patients who fail to clear minimal residual disease (MRD) or experience recurrence upon discontinuation of therapy despite achieving deep molecular response (DMR, BCR-ABL1 ≤ 0.01% on the international scale, IS).

DEFINITIONS

The National Comprehensive Cancer Network and the 2013 European LeukemiaNet (ELN) guidelines recommend cytogenetic and/or molecular monitoring at 3, 6 and 12 months into

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frontline TKI therapy[5,6]. ELN recommendations categorize the molecular and cytogenetic responses at each time interval as “optimal”, “warning” or “failure”. Optimal responses are associated with a life expectancy similar to that of the general population, whereas failure is associated with TKI resistance and increased risk of disease progression/death, necessitating a change in TKI therapy. Failure to achieve complete hematologic response (CHR; normalization of peripheral blood counts; resolution of splenomegaly and CML-related symptoms) or complete cytogenetic response (CCyR; 0% Ph^+ metaphases based on analysis of 20 bone marrow cells) within an allocated duration of time constitutes TKI failure, as does loss of these milestones or progression to accelerated phase (AP-CML) or blastic phase (BP-CML) at any time point. Whether failure to achieve major molecular response (MMR; BCR-ABL1 ≤ 0.1% on the IS) in patients with CCyR defines failure is subject to ongoing debate. Similarly, confirmed loss of MMR while CCyR is maintained does not technically constitute failure, although most of these patients will go on to lose CCyR[7].

Overt resistance such as loss of CHR or even progression to AP/BC-CML is associated with unfavorable clinical outcomes and represents a situation very different from persistent low-level disease associated with MRD which is clinically relevant only in the context of TKI discontinuation. Primary resistance implies failure to achieve time-dependent endpoints of CHR, CCyR and MMR upon initiation of TKI therapy, while secondary (acquired) resistance is defined as the loss of response[8]. At the mechanistic level, we classify TKI resistance as either BCR-ABL1-dependent or BCR-ABL1 independent (Figure 1). Although this distinction seems formalistic, it does have a great degree of clinical relevance, as it informs the strategy required to combat resistance: BCR-ABL1-dependent resistance is reliant upon mechanisms that subvert effective BCR-ABL1 kinase inhibition, such as point mutations in the kinase domain that impair drug binding or cellular/biological processes that interfere with TKI availability and result in suboptimal drug concentrations at the target. In contrast, BCR-ABL1-independent resistance is mediated through alternative survival pathways operating in the context of effective TKI inhibition of BCR-ABL1. Overt clinical resistance is observed via both mechanisms, although acquired resistance is more likely to be BCR-ABL1 dependent, while primary resistance tends to be BCR-ABL1-independent. In BCR-ABL1-dependent resistance, achieving or restoring BCR-ABL1 inhibition is expected to induce or recapture responses, and the most effective approach is the use of alternate TKIs. For obvious reasons this strategy in isolation will not be effective in BCR-ABL1-independent resistance. In this review, we will discuss the mechanisms underlying BCR-ABL1-dependent and independent resistance and therapeutic strategies designed to circumvent them.

BCR-ABL1 DEPENDENT RESISTANCE

BCR-ABL1 KD Mutations

General Considerations: The active sites of tyrosine kinases exist in two principal conformations that are distinct by the position of key structural motifs, including the activation loop (A-loop) that controls access of substrate to the catalytic site, the ATP-binding loop (P-loop) and the highly conserved aspartate-phenylalanine-glycine (DFG) motif that coordinates an adenosine triphosphate (ATP)-bound magnesium ion. In the inactive conformation, the activation loop is in a closed position, and the DFG in an outward

("DFG out") orientation. In contrast, in active kinases the A-loop is in an open conformation, and the DFG motif is oriented toward the catalytic site ("DFG-in") (Figure 2)[9]. Depending on whether they recognize an active or inactive kinase conformation, TKIs are referred to as type I or type II inhibitors, respectively[10]. Although all active site inhibitors are essentially ATP-competitive, type II inhibitors could be considered as stabilizers of an inactive enzyme conformation, while type I inhibitors compete more directly with ATP for binding. Of the approved BCR-ABL1 TKIs, imatinib, nilotinib and ponatinib are type II inhibitors, dasatinib is a type I inhibitor, and bosutinib exhibits features of both[11–15]. These general structural distinctions have practical consequences as they inform the number and types of mutations that confer resistance to a given TKI. Generally, type II inhibitors exhibit more stringent binding requirements, exposing more mutational vulnerabilities, but have the advantage of increased selectivity[16]. Type I inhibitors tend to be more promiscuous, but less prone to mutational escape.

Clinically observed BCR-ABL1 KD mutations and structure-function relationships: Anywhere from 50–90% of CML patients who experience hematologic relapse on imatinib have been reported to harbor KD mutations[17–20]. Point substitutions at just twelve residues (M244, G250, Q252, Y253, E255, V299, F311, T315, F317, M351, F359 and H396) account for most resistance-associated KD mutations (Figure 3A)[21]. KD mutations develop with greater frequency in AP/BP-CML than in CP-CML[18]. For instance a study of 297 patients with primary or acquired resistance to imatinib reported KD mutations in 27% of CP patients, 52% AP patients, 75% myeloid BC patients and 83% lymphoid BC patients[22]. This suggests that reactivation of BCR-ABL1 signaling is critical to conferring an aggressive clinical phenotype. KD mutations can also be detected at low levels in patients at diagnosis, and may in some cases become clinically relevant upon selection of clones by TKI therapy[23,24]. However, as this is not a predictable development, testing for KD mutations at diagnosis is not generally recommended[5,24]. Interestingly, the duration of disease prior to initiation of TKI therapy correlates with the frequency of KD mutations, which supports a role for BCR-ABL1 induced self-mutagenesis[18]. Moreover, advanced phase CML, clonal cytogenetic evolution and KD mutation rate are correlated, suggesting a temporal relationship between uninhibited exposure to BCR-ABL1 kinase activity and degree of genomic instability[25].

Of the approved TKIs, imatinib exhibits the broadest spectrum of vulnerabilities and more than 50 different imatinib-resistance KD mutations have been described[26,27]. Solving the crystal structure of ABL1 in complex with an imatinib analogue was critical for understanding KD mutation-based imatinib resistance. In contrast to expectations imatinib was found to recognize an inactive kinase conformation, with the A-loop in a closed position. Additionally, there was extensive 'downward' displacement of the P-loop[11]. Lastly, imatinib was found to form a hydrogen bond with threonine 315. This binding mode is reflected in the types of KD mutations associated with imatinib resistance[28]. P-loop mutations are thought to prevent the structural adjustments required for optimal drug binding, the T315I mutant causes a steric clash and A-loop mutations stabilize the kinase in an active conformation from which imatinib is excluded. The degree of resistance conferred by the various KD mutations varies greatly, and some (such as M351T or F311L) remain

amenable to dose escalation. In contrast, second-generation TKIs such as dasatinib and nilotinib retain inhibitory activity against the majority of mutants conferring imatinib resistance, with the notable exception of the T315I 'gatekeeper' mutation[29]. Nilotinib was developed from the imatinib scaffold, but has a much improved topological fit, greatly increasing binding affinity. As a result, nilotinib captures many imatinib resistant mutants, although their relative sensitivities to imatinib and nilotinib are similar[13,30]. Thus nilotinib overcomes resistance through tighter binding to a very similar (inactive) ABL1 conformation. Dasatinib was initially reported to bind to ABL1 with less stringent conformational requirements compared to imatinib, but sophisticated nuclear magnetic resonance studies suggest it is a type I inhibitor[12]. The dasatinib resistance mutation spectrum is distinct and includes V299 and F317 as hotspots[31]. However, both nilotinib and dasatinib make a hydrogen bond with T315 and consequently have no activity against T315I. Bosutinib's resistance mutation spectrum is similar to that of dasatinib, suggesting that type I binding is dominant[32]. Ponatinib in contrast is a type II inhibitor that binds ABL1 in a conformation that is quite similar to that observed with imatinib, except that no hydrogen bond is formed with T315 (Figure 3B)[33]. Owing to this and its high target affinity ponatinib exhibits activity against all single BCR-ABL1 mutants at achievable plasma concentrations. In vitro mutagenesis assays developed by us and others fairly accurately predict clinical mutations, validating the fascinating link between structural analysis and clinical observations[33]. Clinically, the type of BCR-ABL1 mutation informs the selection of salvage therapy and represents a prime example of individualized cancer therapy. It is important to note though that the convenient heat maps displaying the differential activity of the approved TKIs toward the various KD mutants are a guide, but not a dogma (Figure 4). For example achievable plasma concentrations and plasma protein binding are additional variables not captured by in vitro assays of BCR-ABL1 expressing cell lines. Further, correlations are tight only toward the negative side. Thus, the presence of a T315I mutation predicts resistance, but there is no guarantee that a patient with a 'sensitive' mutant will respond to a given TKI. Failure to respond to TKI therapy in this setting could be due to alternative BCR-ABL1-dependent mechanisms of resistance (e.g. efflux pumps, see below), or to BCR-ABL1 independent mechanisms.

No single BCR-ABL1 KD mutation has been demonstrated to confer resistance to ponatinib. However, T315I-inclusive compound mutations, defined as a BCR-ABL1 allele with two or more mutations including T315I, have been associated with ponatinib failure[21] in advanced phase CML and Philadelphia chromosome-positive (PH^{+}) acute lymphoblastic leukemia (ALL). A recent analysis of CP CML patients in the PACE trial failed to demonstrate that baseline compound mutation status, regardless of T315I inclusion, affects cytogenetic or molecular responses to ponatinib in this cohort[34].

Increased BCR-ABL1 expression—Increased BCR-ABL1 expression via BCR-ABL1 gene amplification, Ph duplication and differential regulation of oncogene transcription has been demonstrated in patients, but its relationship to acquired clinical resistance is less certain than in cases of KD mutations. High levels of the BCR-ABL1 oncoprotein are associated with more advanced phase disease, often preceding the development of overt resistance via KD mutations[35]. Thus, higher levels of BCR-ABL1 may allow for sufficient

kinase activity to persist despite the presence of TKIs, enabling leukemia cell survival until a KD mutation is acquired and confers overt resistance. One indication that these relationships are complex is the seemingly paradoxical observation that primary $CD34^+$ CML cells engineered to express high levels of BCR-ABL1 have been reported to exhibit increased sensitivity to imatinib *in vitro*[19,28,36,37].

Drug influx/efflux pumps

OCT-1: Organic-cation transporter-1 (OCT-1) is a cellular influx pump for imatinib that has been demonstrated to influence intracellular drug availability. Low OCT-1 activity imparts BCR-ABL1 dependent imatinib resistance. High OCT-1 activity is predictive of improved MMR rates, event free survival (EFS) and overall survival (OS) in patients treated with imatinib[38,39]. Patients with low OCT-1 activity and imatinib trough plasma levels <1200ng/mL have inferior outcomes and benefit from imatinib dose intensification[40]. Imatinib trough levels <1200ng/mL do not necessarily predict inferior outcomes in patients with high OCT-1 activity and these patients are likely to meet molecular milestones on standard-dose imatinib. OCT-1 does not regulate cellular uptake of dasatinib, nilotinib or ponatinib[41–43]. In the future baseline OCT-1 testing may identify candidates for trough imatinib monitoring and imatinib dose intensification, thereby avoiding unnecessary TKI switching due to perceived imatinib failure, but it is not part of current routine clinical practice. Similarly, although several members of the ATP-binding cassette (ABC) transporter family, including ABCB1 and ABCG2, have been implicated in TKI resistance, testing for polymorphisms and increased expression of ABC transporters is not clinically routine[27,37,43–48 49,50].

TKI bioavailability: All of the TKIs used in CML undergo extensive hepatic first-pass metabolism by CYP3A4 and strong inducers of CYP3A4 can contribute to TKI resistance. Patients on TKIs should undergo thorough medication reconciliation to avoid potential drug-drug interactions that can negatively impact TKI efficacy. Common CYP3A4-inducing medications and supplements include dexamethasone, rifampicin, phenobarbital, phenytoin, carbamazepine and St. John's wort[51]. Gastric pH-modifying medications such as H2 antagonists and proton pump inhibitors can affect the bioavailability of dasatinib due to the drug's poor solubility in solutions with a pH >4.0. These patients must be counseled to take antacids 2 hours prior or 2 hours after dasatinib administration to avoid decreases in dasatinib exposure that can occur with their concomitant administration[52,53].

BCR-ABL1 INDEPENDENT RESISTANCE

General considerations—Point mutations in BCR-ABL1 are an important mechanism of TKI resistance in CML, but nearly 40% of cases of clinical TKI failure occur in the setting of sustained BCR-ABL1 inhibition[54]. In this scenario, activation of alternative survival pathways must be responsible for primary or secondary resistance. Conceptually CML cell survival can be mediated through cell-autonomous (leukemia cell intrinsic) mechanisms or through cell-extrinsic microenvironmental factors provided by the bone marrow niche[55]. It is worth noting that while BCR-ABL1 independent resistance can confer overt resistance in active disease, it is also an important contributor to MRD, likely accounting for leukemia stem cell persistence despite DMR to TKI therapy. Multiple (and

counting) signaling pathways have been implicated in BCR-ABL1-independent resistance (Table 1). We have proposed that various upstream pathways may converge on common downstream mediators, offering therapeutic opportunities despite the diversity of upstream signaling[56]. Moreover it seems that the pathways activated by extrinsic and intrinsic resistance mechanisms overlap. In this frame of thinking, extrinsic resistance may enable survival of leukemogenic cells despite TKI inhibition of BCR-ABL1, until the surviving cells manage to activate the very same pathway through cell-intrinsic mechanisms, leading to overt resistance.

STAT3—STAT3 activation has been demonstrated to impart survival cues to leukemic cells via cell-intrinsic and extrinsic mechanisms. Co-culture of TKI-sensitive CML primary cells with HS-5 human bone marrow stromal cells was shown to promote STAT3^{Y705} phosphorylation and leukemia cell survival through soluble BM-derived factors despite BCR-ABL1 inhibition[57,58]. Moreover, in the absence of BM-derived factors, BCR-ABL1 independent activation of STAT3 was demonstrated to be a recurring feature of TKI-resistant cell lines and primary CML cells from patients with clinical resistance to multiple TKIs, suggesting that cell-autonomous activation of STAT3 can mediate CML cell survival[56]. Thus, consistent with the concepts described above, pro-survival cues appear to converge on STAT3 as a crucial distal signal integrator and arbiter of drug resistance. As a result, synthetic lethality approaches designed to inhibit both BCR-ABL1 and pSTAT3^{Y705} hold therapeutic potential, both in active disease and as a tactic to eliminate MRD.

PI3K/AKT—PI3K signaling is required for the proliferation and growth of CML cells[59]. Activation of the PI3K/AKT/mTOR pathway has been shown to facilitate primary CML cell survival during imatinib treatment until overt resistance through secondary mutations emerges[60]. Co-treatment of CML primary cells with nilotinib and the PI3K inhibitor NVP-BEZ235 was shown to inhibit cell growth and increase apoptosis[61]. Increased cytoplasmic retention of FOXO1, a transcription factor downstream of the PI3K signaling axis, has been reported to contribute to BCR-ABL1 independent resistance in TKI-resistant CML cell lines[54]. Elevation in FOXO1 levels has also been demonstrated in primary cells from relapsed CML patients lacking BCR-ABL1 KD mutations. TKI-resistant cells appear to be sensitive to combination drug strategies involving BCR-ABL1 TKIs and PI3K inhibitors that facilitate nuclear translocation of FOXO1.

RAF/MEK/ERK—Enhanced MAP kinase signaling has previously been observed in imatinib-treated CD34^{+} CML progenitor cells[62]. More recently Ma and colleagues performed a large-scale RNA interference screen that revealed increased RAF/MEK/ERK pathway activity mediated through PRKCH in BCR-ABL1-independent imatinib-resistant CML cell lines and patient samples[63]. They found that dual treatment with imatinib and the MEK inhibitor trametinib preferentially killed human CML CD34^{+} cells while sparing normal hematopoietic cells and prolonged survival in their murine models of BCR-ABL1-independent imatinib-resistant CML. In line with this, another study described paradoxical RAS-dependent activation of the RAF/MEK/ERK pathway in nilotinib-treated primary CML cells containing T315I and found that nilotinib synergizes with MEK inhibition to induce synthetic lethality in these cells[64]. In TKI-sensitive CML cells, MEK activity appears

to facilitate BCR-ABL1-mediated oncogene addiction, suggesting that activation of this pathway is critical for leukemia cell survival and a potential target for combination drug inhibition strategies[65].

Nucleocytoplasmic transport—More recently, *XPO1* and *RAN*, components of the nucleocytoplasmic transport complex, were identified as genes whose shRNA-mediated knockdown decreased cell proliferation in a BCR-ABL1-independent imatinib-resistant cell line[66]. Both shRNA-mediated inhibition of RAN and treatment with the XPO1 inhibitor KPT-330 (selinexor) increased the sensitivity of resistant cells to imatinib. KPT-330 has also demonstrated preclinical anti-leukemic activity in mouse models of CML and was observed to decrease leukocytosis and palliate symptoms in a TKI-resistant patient with AP-CML who was provided the drug on a compassionate use basis[67].

EZH2—EZH2, a histone methyltransferase that provides the catalytic subunit of polycomb repressive complex 2 (PRC2), has been shown to be overexpressed in CML leukemia stem cells (LSCs). Two recent publications have highlighted the importance of EZH2 misregulation and its association with reprogramming of H3K27me3 targets in LSCs, resulting in LSC protection from apoptosis and TKI resistance[68,69]. EZH2 inactivation was shown to delay the development of leukemia and prolong survival in mouse models of CML independent of BCR-ABL1 mutational status. In mice with pre-existing gene inactivation of *EZH2* through CRISPR/Cas9-mediated gene editing slowed disease progression and extended survival. Combination treatment with nilotinib and EZH2 inhibitors in CML primary cells engrafted into NOD/SCID mice led to a greater reduction of the LSC population compared to nilotinib treatment alone. Normal hematopoietic stem and progenitor cells appear to be spared from EZH2 inhibition, perhaps due to compensation from EZH1, which is expressed at higher levels in normal HSCs compared to LSCs. The selective vulnerability of LSCs to EZH2 inhibition may provide a therapeutic window to eradicate TKI-persistent LSCs with minimal effects on normal hematopoiesis.

Numerous other BCR-ABL1 independent factors have been proposed to contribute to CML LSC persistence and TKI resistance, including activation of SRC family kinases, Wnt-β-catenin, hypoxia-inducible factor 1α, arachidonate 15-lipoxygenase, miR-126, p53, MYC, ADAR1, SIRT1, RAD21 heat shock proteins, PP2A, Fap1, apoptotic regulators, the Hedgehog pathway and the IL-2/CD25 signaling circuit[55,70–95]. The number of theoretical synthetic lethality approaches involving TKIs and other inhibitors is destined to grow as new resistance mechanisms are unearthed, yet it remains unclear which combinations harbor clinical potential above and beyond TKI monotherapy.

NEW THERAPIES

Tyrosine kinase inhibitors

ABL001: One of the most anticipated new therapies for CML is ABL001, a novel allosteric inhibitor of BCR-ABL1 targeting the myristoyl pocket of the ABL1 kinase. In physiological conditions, the myristoylated N-terminus of ABL1 serves to negatively regulate kinase activity, but is lost upon fusion with BCR in CML. ABL001 was designed to restore this autoregulatory function to the BCR-ABL1 fusion protein, thereby inhibiting oncogenic

signaling. Single-agent ABL001 led to tumor regression in mice xenografted with the KCL22 CML cell line, though all tumors eventually recurred. *In vivo* combination treatment with nilotinib and ABL001 induced complete and sustained regression of disease in mice, with no relapses observed as long as 5 months out from active drug treament[96]. These encouraging results led to a dose-finding phase I trial of ABL001 monotherapy in CP and AP CML patients with failure of ≥ 2 TKIs due to resistance/intolerance[97]. Over 50% of patients enrolled had failed ≥ 3 TKIs. Initial results from the trial are promising – 82% of TKI resistant patients in cytogenetic relapse achieved MCyR by 3 months, including 55% who achieved CCyR. Nearly 30% of TKI-resistant patients achieved MMR by 5 months, and clinical activity was pronounced across a range of mutations. A single relapse was attributed to a mutation in the myristoyl pocket[97]. Overall the drug was well-tolerated, with common grade 3 toxicities including lipase elevation and cytopenias. At the time of last reporting, the maximum tolerated dose had not been reached. Other arms of the Phase I study are assessing the safety and tolerability of ABL001 in combination with imatinib, nilotinib and dasatinib, respectively.

Several other TKIs were previously in development for CML, including bafetinib (BCR-ABL1/Lyn inhibitor), and rebastinib (ABL1/TIE2 inhibitor), but have been sidelined due to poor efficacy in early phase clinical trials[98,99]. A phase I trial of the intravenous ABL1/Aurora kinase inhibitor danusertib produced modest responses in T315I-positive, TKI-resistant AP/BC CML and Ph^+ ALL[100]. The VEGFR inhibitor axitinib has been found to inhibit BCR-ABL1 mutants with substitutions at positions 315 and 299, but its clinical use is limited by this mutational selectivity[101,102]. Radotinib, a second-generation oral BCR-ABL1 inhibitor with an almost identical chemical structure as nilotinib, is approved for second-line treatment of CML in South Korea. An ongoing Phase 3 study investigating radotinib versus imatinib in newly diagnosed CML demonstrated superior 12-month CCyR and MMR rates with radotinib 300mg BID (CCyR: 91% vs 76%; MMR: 52% vs 30%)[103]. Not surprisingly, the in vitro efficacy of radotinib against single BCR-ABL1 mutants appears to be similar to that of nilotinib[104].

Drug combinations to eradicate LSCs and eliminate MRD—Patients who have maintained long-term (one to two years minimum) DMR on TKI therapy may be candidates for TKI discontinuation. When treated with single-agent TKI therapy, at best half of newly diagnosed CML patients will eventually be eligible for TKI discontinuation trials, and of these, at most 50–60% will successfully maintain treatment-free remission (TFR) one year following TKI discontinuation[105]. The finding that a portion of patients are “operationally cured” following TKI treatment is surprising given the wealth of data suggesting CML LSCs are not eradicated by BCR-ABL1 inhibition. It also remains unclear why patients with seemingly identical deep responses segregate in their responses to TKI discontinuation. Recent data has emerged to support the role of immune surveillance by NK and T cells in maintaining successful TFR, implying that alternative biological factors contribute to optimal disease control[106]. Various TKI discontinuation trials are ongoing, and attempts to clarify the clinical and biologic characteristics predictive of successful TFR are reflected in a trend toward more liberalized patient eligibility criteria and an emphasis on correlative studies (Table 2).

TKI discontinuation is an evolving goal of CML therapy and has been embraced by patients motivated to come off these chronic medications due to undesirable side effects, which, in some cases, can be quite serious (i.e. pulmonary hypertension on dasatinib or arterial occlusive events on nilotinib). The reality that the majority of CML patients will never attain TFR with current therapies has led to efforts to combine TKIs with other drugs in hopes of eliminating TKI-persistent LSCs and the reservoir of cells responsible for MRD.

TKIs plus immune therapies: Prior to imatinib, interferon-α-(IFN) based therapy was standard of care for CML. Anecdotal evidence suggests that IFN preferentially targets leukemic stem cells in CML, as demonstrated by the fact a small minority of CML patients treated with IFN alone were functionally cured of their disease[107]. Randomized trials of imatinib and pegylated IFN report improved molecular response rates with combination therapy compared to imatinib alone[108,109]. With the advent of TKI discontinuation and documentation of successful TFRs, there has been renewed interest in pegylated IFN as an adjunct to TKI therapy in promoting DMR. This had led to early phase trials investigating pegylated IFN in combination with second-generation TKIs. Non-randomized trials of nilotinib or dasatinib in combination with pegylated IFN in newly diagnosed CML patients have reported 12-month $MR^{4.5}$ rates of 17% and 27–30%, respectively, which compare favorably to the 12-month $MR^{4.5}$ rates observed in the registration trials of frontline nilotinib (ENESTnd) and dasatinib (DASISION)[110–114]. A phase 3 randomized trial of IFN in combination with nilotinib is underway in Germany. There remains considerable interest in developing novel immune therapies against a variety of tumor antigens and while early-phase trials investigating peptide vaccines have had mixed results, antibody-based treatments may hold promise[115–122].

TKIs plus inhibitors of additional pathways: Despite mounting evidence implicating diverse pathways in BCR-ABL1-independent resistance and LSC persistence, there are a limited number of clinical trials investigating inhibitors of these pathways in combination with TKIs.

Leukemic stem and progenitor cells may be protected in the bone marrow niche via JAK2/STAT5 activation by exogenous growth factors in the setting of BCR-ABL1 inhibition[57,123,124]. CML $CD34^+$ cells display reduced engraftment when treated ex vivo with the combination of TKI and ruxolitinib (a clinically available JAK2 inhibitor) and transplanted into NSG mice[124]. The impact of the addition of ruxolitinib to baseline TKI therapy in CML is being studied in a phase 1/2 trial (NCT01751425) and the specific combination of ruxolitinib and nilotinib in CML and Ph^+ ALL is being investigated in a separate phase 1/2 study (NCT02253277).

Pioglitazone, an agonist of peroxisome proliferator-activated receptor-γ (PPARγ) belonging to the glitazone family of anti-diabetic drugs, has been found to induce apoptosis in LSCs when used in combination with imatinib, presumably by downregulating STAT5 transcriptional targets, including HIF2α and CITED2[125]. The addition of pioglitazone to TKI therapy in three CML patients unable to reach CMR after several years of continuous imatinib treatment was associated with sustained $MR^{4.5}$ in all three patients at 6 months to 1 year following initial pioglitazone exposure. These findings led to phase II trial combining

imatinib and pioglitazone in patients with persistent MRD on imatinib. The incidence of PCR-negativity was reported at 57% for the combination group and 27% for a historical cohort receiving imatinib alone. Currently there are several trials investigating pioglitazone in combination with TKIs for CML, including one study (PIO2STOP) attempting to define its use in a second trial of TKI discontinuation for patients who experienced loss of MMR after initial TKI discontinuation.

CONCLUSIONS

Due to improved survival, the prevalence of CML is estimated to exceed 180,000 cases by 2050, thereby establishing CML as the most common form of leukemia in the United States[126]. While excellent progress has been made through the introduction of targeted molecular therapy over the last two decades, new strategies to eliminate MRD and increase the pool of candidates eligible for trials of TFR are needed. Eliminating TKI resistance and LSC persistence by dual targeting of BCR-ABL1 and alternative pathways appears to be the most promising therapeutic avenue to decrease leukemic disease burden and potentiate “operational cures.” The number of alternative pathways posited to establish synthetic lethality with TKIs is overwhelming, and it will take time and effort to sift through the multiple permutations with rigorous clinical testing. Ultimately though, responses to cancer therapy depend not just on the efficacy of target inhibition, but also on factors such as patient compliance and tolerability of side effects that need to be addressed with a completely different set of tools. It is for these reasons that mechanisms of resistance will always keep pace with therapeutic developments, and we will be contending with them for as long as we continue our fight against cancer.

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Synopsis

Chronic myeloid leukemia (CML) is increasingly viewed as a chronic illness, with most patients expected to have a life expectancy close to that of the general population. Despite the great progress that has been made using BCR-ABL1 tyrosine kinase inhibitors (TKIs), drug resistance via BCR-ABL1-dependent and BCR-ABL1-independent mechanisms continues to be an issue for many patients. BCR-ABL1-dependent resistance is primarily mediated through oncoprotein kinase domain mutations and usually results in overt clinical resistance to TKIs. However, BCR-ABL1-independent resistance, which occurs in the setting of effective BCR-ABL1 inhibition, has become increasingly recognized a major contributor to minimal residual disease (MRD) and efforts to eradicate persistent leukemic stem cells (LSCs) have largely focused on combination therapy with TKIs and drugs targeting these pathways.

Key Points

1. Over 25% of CML patients will switch TKIs during their lifetime due to resistance or intolerance. While most cases of clinical resistance are due to kinase domain mutations (BCR-ABL1-dependent resistance), 20–40% of patients exhibit resistance despite effective BCR-ABL1 inhibition (BCR-ABL1-independent resistance).
2. Ponatinib is the only TKI effective against the T315I BCR-ABL1 mutation. Ponatinib's activity against this mutant isoform derives from its lack of dependence on forming a critical hydrogen bond with residue T315 for high-affinity binding to BCR-ABL1.
3. Diverse pathways involving growth factors, epigenetic regulators and apoptotic machinery have been implicated in BCR-ABL1-independent resistance. BCR-ABL1-independent resistance can be classified as cell-extrinsic or cell-intrinsic depending on the relative influence of the microenvironment.
4. CML leukemic stem cells (LSCs) are resistant to TKI therapy and contribute to minimal residual disease (MRD). Combination strategies to eradicate MRD using TKIs and other drugs are an intense focus of investigation in CML.
5. A minority of CML patients who achieve sustained deep molecular responses on TKI therapy are able to discontinue treatment without molecular recurrence, entering a state called "treatment-free remission (TFR)." Multiple TKI discontinuation trials are ongoing worldwide and will help determine which patients are most likely to have successful TFR and what biological factors govern maintenance of response.

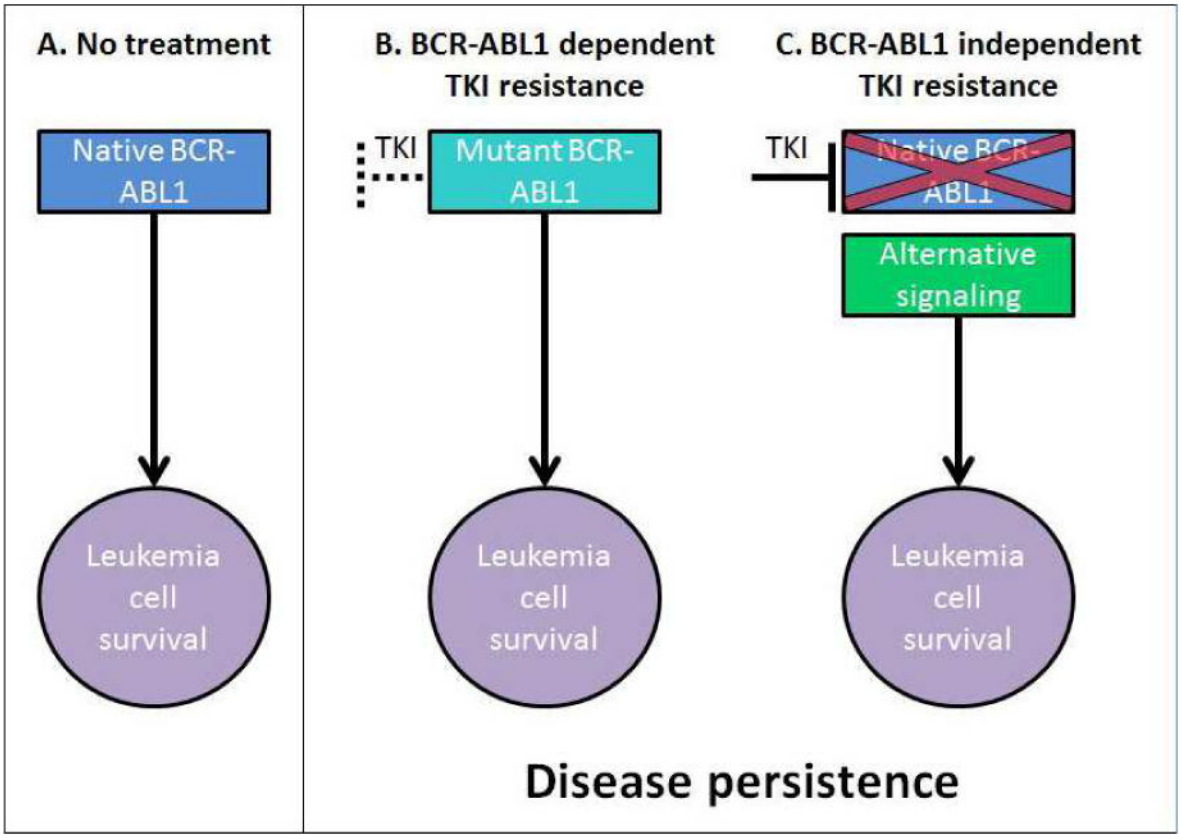

Figure 1.

BCR-ABL1-dependent vs. independent resistance. (A) Native BCR-ABL1 signaling in the absence of TKI inhibition is necessary and sufficient for leukemogenesis in CML. (B) Kinase domain mutations in BCR-ABL1 can alter the binding of TKIs and lead to reconstitution of BCR-ABL1 signaling. (C) In the setting of effective BCR-ABL1 inhibition with TKIs, leukemia cells persist due to activation of alternative survival pathways.

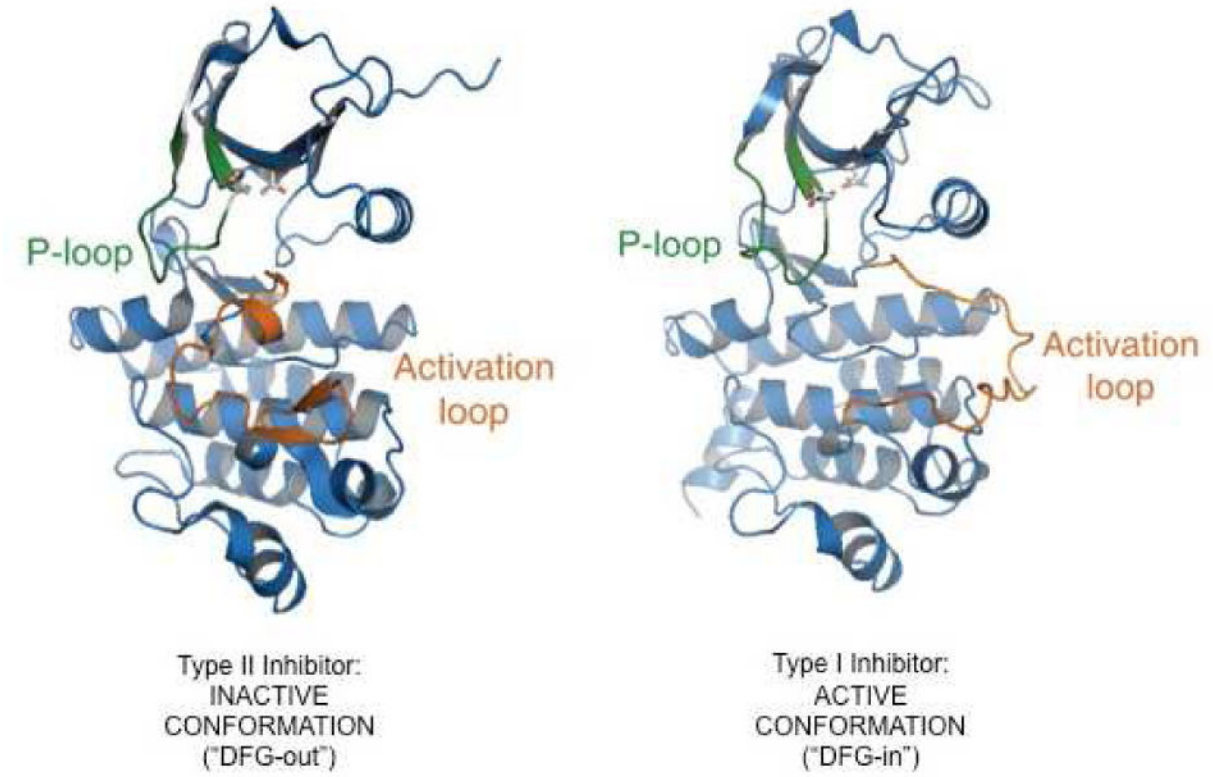

Figure 2.

Type I and Type II inhibitors. (A) Type II inhibitors stabilize the inactive conformation of BCR-ABL1 in which the activation loop is closed and the DFG is in an outward ("DFG out") orientation. (B) Type I inhibitors are ATP-competitive, binding to BCR-ABL1 when the activation loop is in an open position conformation and the DFG motif is oriented toward the catalytic site ("DFG-in").

Courtesy of T. Clackson, PhD, Cambridge, MA.

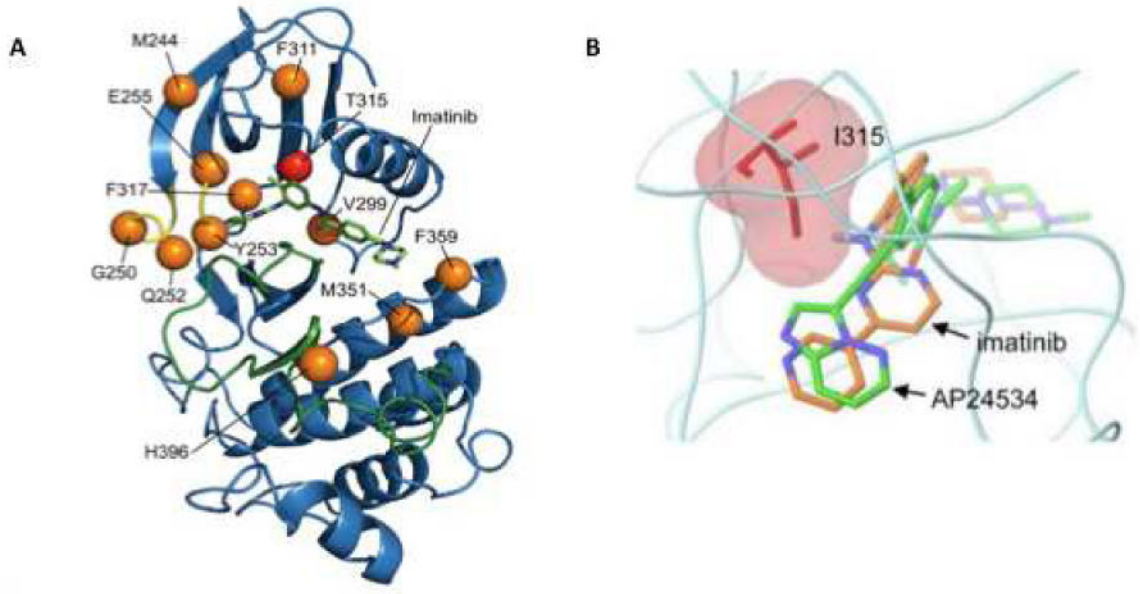

Figure 3.

Key residues influence BCR-ABL1-dependent resistance to TKIs. (A) Crystal structure of the ABL1 kinase domain in complex with imatinib. Twelve positions (in orange, T315 in red) account for most clinical BCR-ABL1 TKI resistance. The phosphate-binding (yellow) and activation loops (green) are indicated. (B) Superposition of imatinib and AP24534 (ponatinib) highlighting the effect of the Thr to Ile mutation. High-affinity binding of imatinib and other 2G TKIs to BCR-ABL1 requires a critical hydrogen bond with residue T315, which is eliminated upon the conversion of threonine to isoleucine. Unlike other clinically available TKIs, ponatinib does not form a hydrogen bond with T315 and has activity against the T315I mutant form of BCR-ABL1.

Figure 3A: *From* Zabriskie MS, Eide CA, Tantravahi SK, et al. BCR-ABL1 compound mutations combining key kinase domain positions confer clinical resistance to ponatinib in Ph chromosome-positive leukemia. Cancer Cell 2014; 26(3); 430; with permission.

Figure 3B: *From* O'Hare T, Shakespeare WC, Zhu X, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell 2009; 16(5): 403; with permission.

		IC50-fold increase (WT = 1)				
		Imatinib	Bosutinib	Dasatinib	Nilotinib	Ponatinib
	Parental	10.8	38.3	568.3	38.4	570.0
	WT	1	1	1	1	1
P-loop	M244V	0.9	0.9	2.0	1.2	3.2
	L248R	14.6	22.9	12.5	30.2	6.2
	L248V	3.5	3.5	5.1	2.8	3.4
	G250E	6.9	4.3	4.4	4.6	6.0
	Q252H	1.4	0.8	3.1	2.6	6.1
	Y253F	3.6	1.0	1.6	3.2	3.7
	Y253H	8.7	0.6	2.6	36.8	2.6
	E255K	6.0	9.5	5.6	6.7	8.4
	E255V	17.0	5.5	3.4	10.3	12.9
C-helix	D276G	2.2	0.6	1.4	2.0	2.1
	E279K	3.6	1.0	1.6	2.0	3.0
	E292L	0.7	1.1	1.3	1.8	2.0
ATP binding region	V299L	1.5	26.1	8.7	1.3	0.6
	T315A	1.7	6.0	58.9	2.7	0.4
	T315I	17.5	45.4	75.0	39.4	3.0
	T315V	12.2	29.3	738.8	57.0	2.1
	F317L	2.6	2.4	4.5	2.2	0.7
	F317R	2.3	33.5	114.8	2.3	4.9
	F317V	0.4	11.5	21.3	0.5	2.3
SH2-contact	M343T	1.2	1.1	0.9	0.8	0.9
	M351T	1.8	0.7	0.9	0.4	1.2
Substrate binding region	F359I	6.0	2.9	3.0	16.3	2.9
	F359V	2.9	0.9	1.5	5.2	4.4
A-loop	L384M	1.3	0.5	2.2	2.3	2.2
	H396P	2.4	0.4	1.1	2.4	1.4
	H396R	3.9	0.8	1.6	3.1	5.9
C-terminal lobe	F486S	8.1	2.3	3.0	1.9	2.1
	L248R + F359I	11.7	39.3	13.7	96.2	17.7

Sensitive	<2
Moderately resistant	2.1–4
Resistant	4.1–10
Highly resistant	>10

Figure 4.

Activity of TKIs against mutant isoforms of BCR-ABL1 in Ba/F3 cells. The relative increase in IC50 value over wild-type BCR-ABL1 is depicted for each TKI against single BCR-ABL1 mutants. Green indicates sensitive mutants, yellow indicates moderate resistance and yellow indicates marked resistance. In patients, TKI efficacy is dependent on other factors, such as oral and cellular bioavailability.

From Eiring AM, Deininger MW. Individualizing kinase-targeted cancer therapy: the paradigm of chronic myeloid leukemia. Genome Biol 2014;15(9):461; with permission.

Table 1

Targets for eradication of LSCs in CML

Target	References	Trial	Drug(s) tested	Comments
Wnt/β-catenin MNK	Zhao et al. Cancer Cell 2007[72] Lim et al. PNAS 2013[73]	+	PRI-724	Difficult target
Hedgehog	Dierks et al. Cancer Cell 2008[84] Zhao et al. Nature 2009	+	BMS-833923 LDE225	Failed (toxicity)
5-Lipoxygenase	Chen et al. Nat Genet 2009[85]	+	Zileuton	Currently recruiting
BCL6	Hurtz et al. JExMed 2011	−	RI-BPI	Small molecule inhibitor in development
MYC	Reavie et al. Cancer Cell 2013[94] Abraham et al. Nature 2016[95]	–	–	Difficult target
PP2A	Neviani et al. J Clin Invest 2007; 2013[86]	−	–	Fingolimod approved for MS
PPAR-γ	Prost et al. Nature 2015[125]	+	Pioglitazone	Ongoing
SIRT1	Bhatia et al. Cancer Cell 2012	+	Panobinostat Vorinostat	Tested in refractory CML
Rad52	Cramer-Morales et al. Blood 2013[87]	−	–	–
MEK	Ma et al. Sci Transl Med 2014[63] Packer et al. Cancer Cell 2011[64]	+	MEK-162	Ongoing
BCL2 family	Goff et al. Cancer Stem Cell 2013	+	Obatoclax	Tested in advanced hematologic malignancies, including CML-BC
Autophagy	Bellodi et al. J Clin Invest 2009[90]	+	Hydroxychloroquine	Ongoing (CHOICES)
PML	Ito et al. Nature 2008[91]	+	As2O3	Ongoing
JAK2	Traer et al. Leukemia 2012[57] Neviani et al. J Clin Invest 2013[86]	+	Ruxolitinib	Ongoing
ADAR1	Jiang et al. PNAS 2013[93]	−	–	–
STAT3	Eiring et al. Leukemia 2014[56]	−	–	Difficult target
EZH2	Xie et al.[69] Scott et al.[68] Cancer Discovery 2016	–	–	–
Heat shock proteins	Peng et al. Blood 2007[88]	+	STA-9090	Ongoing
Fap1	Huang et al. Leukemia 2016[92]	−	–	–
BCR-ABL1	Pinilla-Ibarz et al. Blood 2000[115] Bocchia et al. Lancet 2005[116]	+	Breakpoint peptide vaccines	Suggestion of activity
PR1	Molldrem et al. Nat Med 2000[117] Rezvani et al. Haematologica 2011[118]	+	Peptide vaccines	Negative studies
WT1	Gao et al. Blood 2000[119] Dubrovsky et al. Blood 2014[120]	+	Peptide vaccines Peptide-specific antibody WT transduced autologous T cells	Ongoing (some)
IL1RAP	Järås M et al. PNAS 2010[121]	−	Antibody	–
IL3R (CD123)	Frolova et al. Br J Haematol 2014[122]	−	DT-conjugated antibody (SL-401; SL-501)	–

Table 2

Summary of TKI discontinuation studies

Adapted from Saußele S, Richter J, Hochhaus A, et al. The concept of treatment-free remission in chronic myeloid leukemia. Leukemia 2016; 30(8): 1641; with permission.

Trial	Patients reported	Treatment prior to discontinuation	Eligibility for TKI discontinuation by MR	Threshold for restarting TKI	TFR% (median follow-up time)
Imatinib discontinuation trials					
STIM1	100	Imatinib +/− prior IFN	MR^5(≥2y)	≥2 consecutive samples with detectable PCR and a 1-log increase	39% (55m)
STIM2	124	Imatinib	$MR^{4.5}$(≥2y)	≥2 consecutive samples with detectable PCR and a 1-log increase	46% (2y)
TWISTER	40	Imatinib +/− prior IFN	$MR^{4.5}$(≥2y)	Detectable PCR	45% (42m)
A-STIM	80	Imatinib +/− prior IFN	Undetectable PCR (≥2y) with low level positives occasionally allowed	Loss of MMR	64% (23m)
ISAV	112	Imatinib	Undetectable PCR (18 months)	Loss of MMR	51.9% at 36 months (21m)
KID	90	Imatinib +/− prior IFN	$MR^{4.5}$(≥2y)	Loss of MMR	50% (26.6m)
HOVON	18	Imatinib	$MR^{4.5}$(≥2y)	Detectable PCR	33% (36m)
Imatinib and/or 2G-TKI discontinuation trials					
STOP-2G TKI	52	Nilotinib or dasatinib	$MR^{4.5}$(≥2y)	Loss of MMR	61% (6m); ongoing
ENEST Freedom	190	Nilotinib	$MR^{4.5}$(≥1y)	Loss of MMR	51.6% (week 48);ongoing
ENESTop	126	2^{nd}-line Nilotinib	$MR^{4.5}$(≥1y)	Loss of MMR or confirmed loss of MR^4	57.9% (week 48);ongoing
ENEST Path	1058 (estimated)	Imatinib followed by Nilotinib	MR^4 (≥1–2y)	Loss of MMR or confirmed loss of MR^4	Ongoing
ENEST Goal	300 (estimated)	Imatinib without MMR followed by Nilotinib	$MR^{4.5}$(≥1–2y)	Confirmed loss of MR^4	Ongoing
DADI	63	Second-line dasatinib	MR^4(≥1y)	Loss of MR^4	49% (6m)
DASFREE	79(estimated)	Dasatinib	$MR^{4.5}$(≥1y)	Loss of MMR	Ongoing
CML V (TIGER)	652(estimated)	Nilotinib vs. Nilotinib + IFN	MR^4((≥1y)	Loss of MMK	Ongoing
LAST	173(estimated)	Imatinib, nilotinib, dasatinib or bosutinib	MR^4(≥2y)	Detectable PCR	Ongoing
DESTINY	168 (estimated)	Imatinib, nilotinib or dasatinib	Patients in MMR or MR^4 (≥1y) who can maintain MMR response on half-dose TKI for 12 months	Loss of MMR	Ongoing

Trial	Patients reported	Treatment prior to discontinuation	Eligibility for TKI discontinuation by MR	Threshold for restarting TKI	TFR% (median follow-up time)
EURO-SKI	200	Imatinib, nilotinib or dasatinib	MR^4(≥1y)	Loss of MMR	61% (6m); ongoing