*Teaser Chronic myeloid leukemia cells are armed with several resistance mechanisms that can make current drugs ineffective. A better understanding of resistance mechanisms is yielding new approaches to management of the disease.Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm the hallmark of which, the breakpoint cluster region-Abelson (BCR-ABL) oncogene, has been the target of tyrosine kinase inhibitors (TKIs), which have significantly improved the survival of patients with CML. However, because of an increase in TKI resistance, it is becoming imperative to identify resistance mechanisms so that drug therapies can be better prescribed and new agents developed. In this review, we discuss the various BCR-ABL-dependent and -independent mechanisms of resistance observed in CML, and the range of therapeutic solutions available to overcome such resistance and to ultimately improve the survival of patients with CML.*



# Current outlook on drug resistance in chronic myeloid leukemia (CML) and potential therapeutic options

**Daniel Nisakar Meenakshi Sundaram[1], Xiaoyan Jiang[2], Joseph M. Brandwein[3], Juliana Valencia-Serna[4], K.C. Remant[4] and Hasan Uludağ[1,4,5]**

[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada
[2] Terry Fox Laboratory, British Columbia Cancer Agency and Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada
[3] Department of Medicine, University of Alberta, Edmonton, AB, Canada
[4] Department of Chemical and Materials Engineering, University of Alberta, Edmonton, AB, Canada
[5] Department of Biomedical Engineering, University of Alberta, Edmonton, AB, Canada

**Daniel Nisakar Meenakshi Sundaram** is a PhD student in the Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta. He completed his MSc degree with a specialization in biotechnology at the University of Madras (India). He is conducting research on advanced drug delivery formulations and RNAi therapies for cancer.

**Xiaoyan Jiang** is a professor of medical genetics at the University of British Columbia (Canada) and an adjunct professor at the Shanghai Institute of Medical Genetics, Shanghai Jiaotong University (China). She is also a health research scholar at the Michael Smith Foundation with expertise on the cellular and molecular biology of leukemia; her research interests include leukemic stem cell biology, gene regulation, drug resistance, and proteomics.

**Hasan Uludag** is a professor of chemical and materials engineering at the University of Alberta (Canada), with appointments at the Department of Biomedical Engineering and Faculty of Pharmacy and Pharmaceutical Sciences. He is an expert on biomaterials, tissue engineering, and drug delivery, with recent research interests in molecular therapies derived from nucleic acids with a specialization on bone diseases and cancer.

## Introduction

CML is a multilineage myeloproliferative neoplasm that originates from hematopoietic stem cells (HSCs). It affects 1–2 per 100 000 adults worldwide and is characterized by the uncontrolled proliferation of HSCs, in particular an excessive number of granulocytes produced in the bone marrow. The cytogenetic hallmark of CML is a reciprocal chromosomal translocation that occurs between the long arms of chromosome 9 and 22, t(9;22)(q34;q11) forming a shortened chromosome 22, known as the Philadelphia chromosome (Ph) found in 95% of patients with CML. The resulting *BCR-ABL* fusion gene encodes an oncoprotein ($p210^{BCR\text{-}ABL}$) with increased tyrosine kinase activity, contributing to uncontrolled proliferation, growth factor-independent survival, modified cell adhesion, and apoptosis inhibition [1–3]. CML progresses in three phases: a chronic phase (CP) representing 85–90% of patients at diagnosis, progressing to an accelerated phase (AP), and then to either a myeloid or lymphoid blast crisis. With the introduction of BCR-ABL TKIs, the overall survival of patients with CML in CP was drastically improved, with imatinib mesylate (IM) becoming the gold standard for first-line treatment, and is now available as a generic drug in many countries [4,5]. However, emerging IM resistance and therapeutic failure have led to the development of second- and third-generation TKIs with increased potency in the treatment of CML; however, they also elicit inadequate responses and fail to prevent disease progression in some patients [6–9]. Further failure modes of TKI monotherapies have provoked comprehensive studies exploring various BCR-ABL-dependent and -independent mechanisms of drug resistance. Here, we discuss these mechanisms of drug resistance in CML, and the range of therapeutic solutions available to overcome such resistance and ultimately help to improve CML treatment. *In vitro* and *in vivo* findings on drug resistance mechanisms and their clinical relevance are presented, highlighting the importance of targeting multiple mediators to overcome CML drug resistance. Readers are referred to previous reviews summarizing drug resistance in CML [10,11], as well as emerging information on important mediators and key pathways for a more in-depth analysis of the molecular and cellular mechanisms of resistance that promote the survival of

*Corresponding author:*


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drug-resistant leukemic cells, including biological functions of miRNAs and their target genes, and properties of cancer stem cells (CSCs) with their protective niches and microenvironment. Promising preclinical approaches as well as challenges to effectively eradicate drug-resistant cells, particularly leukemic stem cells (LSCs), are also detailed in this review.

## Drug resistance in CML

The growth dependence of CML on constitutively activated BCR-ABL tyrosine kinase allows its targeting by TKIs, of which the first-generation IM has become the frontline therapy. An 8-year follow-up study on CP-CML indicated an estimated event-free survival rate of 81% and overall survival rate of 93% [12]. However, drug resistance was described through *in vitro* and *in vivo* studies (Fig. 1), shortly followed by clinical reports of resistance to IM and the development of the second-generation TKIs dasatinib (DA), nilotinib (NI), and bosutinib (BO), and the third-generation ponatinib (PO). Drug resistance in patients can be classified into two major groups: (i) BCR-ABL dependent and (ii) BCR-ABL independent.

### *BCR-ABL-dependent resistance*

BCR-ABL-dependent resistance can result from mutations of the BCR-ABL kinase domain, mutations outside BCR-ABL kinase domains, compound mutations, defective DNA repair mechanisms, and amplification and/or overexpression of BCR-ABL, which all ultimately impair the effectiveness of TKI treatment in patients.

#### ***Mutations within the BCR-ABL kinase domain***

Mutations in the ABL kinase domain and other domains that control the conformation of the kinase domain hinder the binding of drugs by altering the BCR-ABL conformation or by hampering the binding altogether, leading to resistance. Mutations are the most common resistance mechanism, with >100 different mutations identified to date. They can be observed at various structural subunits of the kinase domain and are classified into four categories affecting: (i) the ATP-binding P-loop, between amino acids (aa) 244 and 255; (ii) the IM direct binding site between aa 315 and 317; (iii) the C-loop (catalytic domain) between aa 350 and 363; and (iv) the A-loop (activation loop) between aa 381 and 402 [13] (Fig. 2).

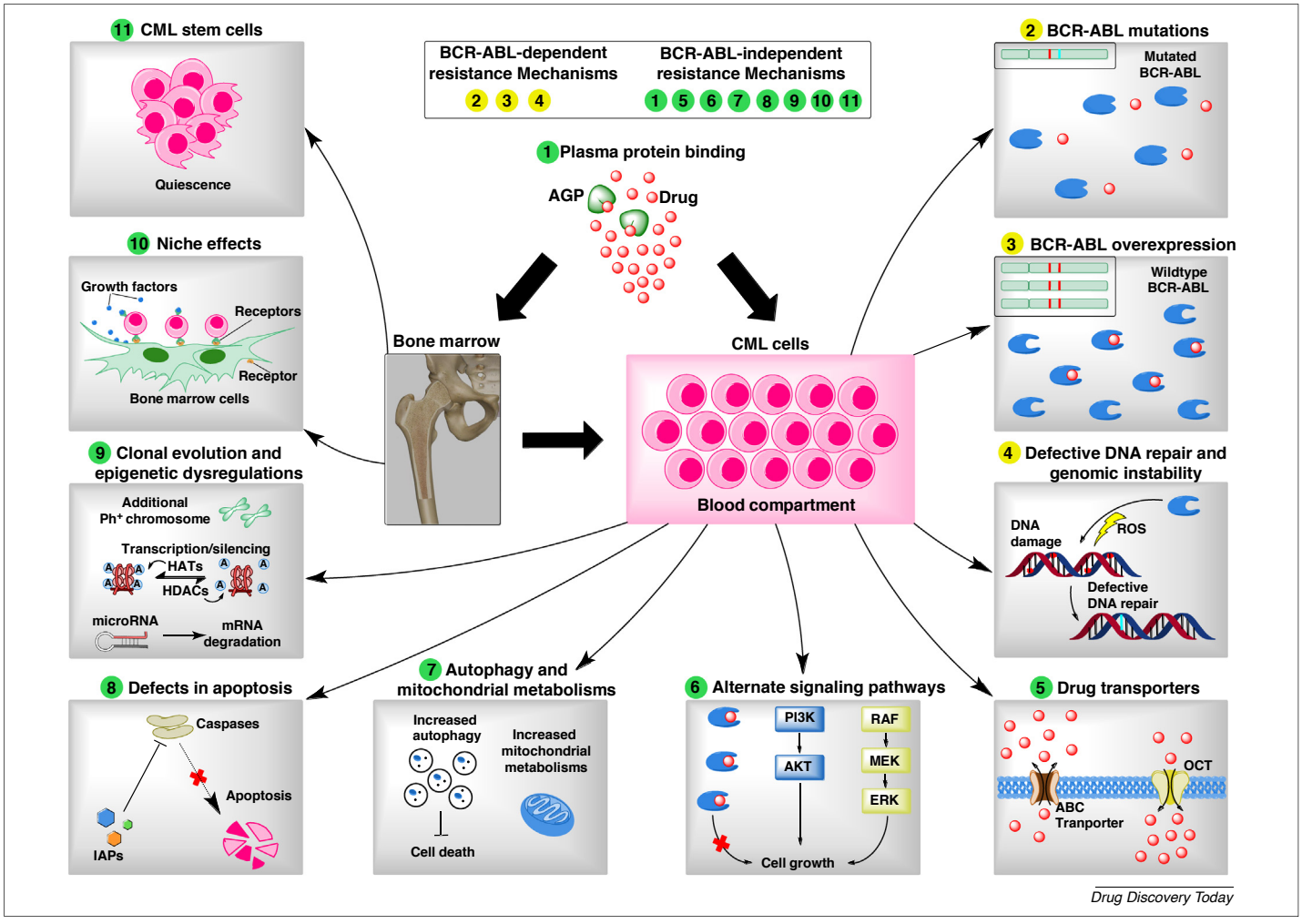


**FIGURE 1**

Resistance mechanisms in chronic myeloid leukemia (CML). Breakpoint cluster region-Abelson (BCR-ABL)-dependent mechanisms are indicated by yellow circles that include: (2) BCR-ABL mutations, (3) BCR-ABL overexpression, and (4) defective DNA repair and genomic instability. BCR-ABL-independent mechanisms are indicated by green circles that include: (1) plasma protein binding (AGP, α1-acid glycoprotein), (5) drug transporters, (6) alternate signaling pathways, (7) autophagy and mitochondrial metabolism, (8) defects in apoptosis (inhibitors of apoptosis proteins, IAPs), (9) clonal evolution and epigenetic dysregulations, (10) niche effects and (11) CML stem cells.

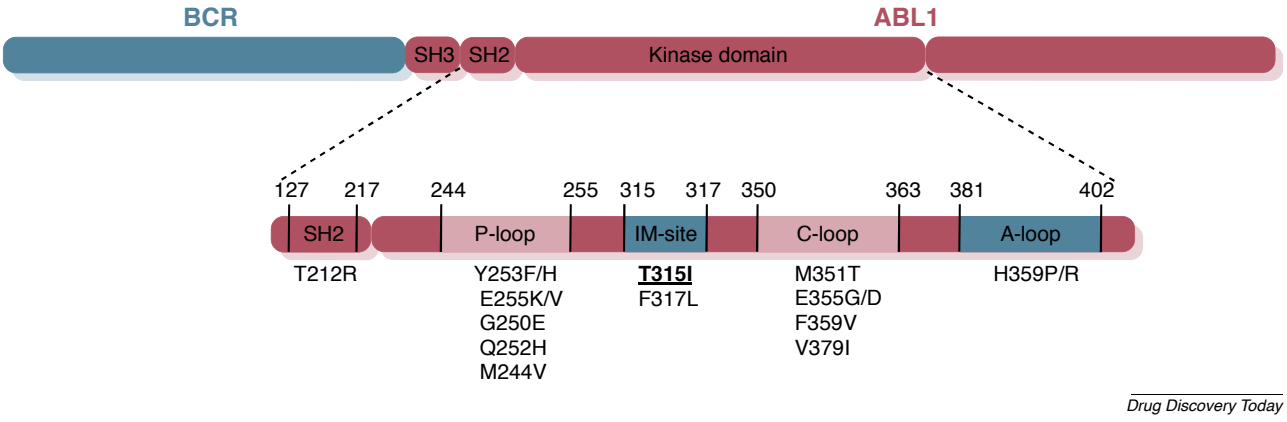


**FIGURE 2**

Most frequent mutations in breakpoint cluster region-Abelson 1 (BCR-ABL1). The figure displays the specific domains, their location (amino acid), with the most common mutations recorded in each region and the highly resistant T315I mutation (bold and underlined). SH3, SH2 (Src homology domains), P-loop (ATP-binding loop), imatinib mesylate (IM) site (IM direct-binding site), C-loop (catalytic loop), and A-loop (activation loop) are shown.

Following IM binding, the P-loop changes its confirmation (so-called 'induced-fit' site), which helps in H-bond formation with Y253. However, Y253H/F mutations impair the induced-fit interaction of IM, thereby exhibiting decreased sensitivity not only to IM, but also to NI treatment [14,15]. Various other P-loop mutations also exhibit less sensitivity to complete resistance, accounting for 36–48% of all mutations [16].

The widely observed T315I mutation in the IM-binding site is a result of a single nucleotide change from C to T at position 944 in ABL kinase, which leads to replacement of threonine at position 315 with isoleucine. T315, the gatekeeper residue for ABL that aids in the formation of H-bonding with the TKIs, is changed for a bulky isoleucine that hinders the interaction of the inhibitors [17]. T315I mutation is the most common mutation, detected in 4–15% of patients with IM-resistant CML, and is highly resistant to IM [10]. Other mutations have been reported in this region, and treatment of the 'Gatekeeper' T315I mutation is more challenging because it confers resistance to all second-generation TKIs [15].

The closed (inactive) conformation of the activation loop is vital for drug binding. Phosphorylation of Y393 has a pivotal role in stabilizing the open (active) conformation, and proximity of Y393 to H396 influences this conformation. Thus, H396P/R mutation in the activation loop could stabilize the open conformation or destabilize the closed conformation, thereby inhibiting the binding of drug because ABL remains active for an extended period [18,19]. The C-loop aa M351, E355, F359, and V379 provide a strong foundation for the activation loop, because they are positioned close to the region of drug binding. The M351T, E355G/D, and F359 V mutations can alter ABL conformation, whereas V379I is also assumed to induce a conformational change [14,20].

### *Mutations outside the BCR-ABL kinase domain*

The SH2, SH3, and Cap domains are involved in the autoinhibition of ABL kinase [21]. The T212R mutation in the SH2 domain stabilizes the active conformation of BCR-ABL kinase, thereby inhibiting IM binding with increased levels of *BCR-ABL* transcripts *in vitro* and in patients without the presence of any kinase domain mutations [22] (Fig. 2). Although mutations in these domains are uncommon, other mutations might also be involved in the destabilization of the inactive BCR-ABL conformation [23]. *BCR-ABL* mutations can also arise before TKI treatment and not all mutations lead to drug resistance [24].

### *Compound mutations*

Polyclonal mutations, resulting from two or more point mutations in the kinase domain of separate BCR-ABL proteins, and compound mutations, resulting from two mutations within the same BCR-ABL protein, also contribute to drug resistance [14]. Not all mutations contribute to compound mutations, because only 12 positions have been reported to be involved in drug resistance [25]. Sequential TKI treatment could be one of the driving factors for the steady increase in the incidence of compound mutations [26]. As shown in Table 1, we can observe the dominance of T315I-related compound mutations exhibiting resistance to the recently developed TKI PO. Although contrasting evidence reports the efficiency of PO irrespective of any compound mutation, the percentage of T315I-compound mutations was lower in this particular study, which could have contributed to the positive effects of PO treatment [27]. A recent study reported the L248R/F359I compound mutation to be highly resistant to all TKIs, from IM to PO [19]. In most of these reports, mutational studies were performed after encountering TKI resistance in patients (following several years of treatment) with subsequent changes in the drug [within the same generation (e.g., DA to NI/BO) or from one generation to the other (e.g., IM to DA/PO)]. There can also be an increase in the number of new mutations along with the change of TKI. This could be minimized by: (i) screening patients for the presence of possible pre-existing mutations before treatment; and (ii) the use of non-TKIs in combination with a low TKI dosage, along with frequent monitoring of mutations and drug response in patients.

### *Defective DNA repair mechanisms and genomic instability*

DNA repair is an important component of the DNA damage response by which cells can recover, although its efficacy and accuracy vary, affecting the cell cycle, induction of malignancy, cell death, and transfer of genetic information. The production of reactive oxygen species (ROS) by BCR-ABL can cause DNA damage and faulty DNA repair, leading to mutations. The base excision

**TABLE 1**

**Representative individual and compound mutations inside and outside the ABL kinase domain[a,b,c,d]**

| | Imatinib | Nilotinib | Dasatinib | Ponatinib | Mutation location in ABL kinase |
|---|---|---|---|---|---|
| Sensitive | | M244V [S1,S2] L248V [S2]; Q252H [S2] | M244V [S1,S2]; Y253F/H [S3] | M244V [S4] | P-loop |
| | | D276G [S5]; V299L [S2]; F486S [S5] | | | Other |
| | | F317L/V/C [S6] | | T315I [S7]; F317L [S4,S7] | DBS |
| | | | F359C/V [S3,S8] | M351T [S8] | C-loop |
| | | L387F [S2] | H396P/R [S4,S8] | H396P [S9] | A-loop |
| | | E123Q [S10]; T212R [S10] | | | SH2 (outside ABL KD) |
| Less sensitive | M244V [S11]; Q252H [S11] | Y253H/F [S5,S12,S13]; E255K/V [S5,S13] | Q252H [S13]; E255K/V [S5,S13] | E255K/V [S4] | P-loop |
| | E275K [S10]; D276G [S5]; F486S [S5] | | V299L [S5,S13]; | | Other |
| | F317L/VI/C [S12] | | F317L/VI/C [S5,S13,S14] | | DBS |
| | M351T [S15,S16]; E355G/D/A [S12]; F359V/C/I [S12] | M351T [S8,S9]; F359V/C/I [S5,S13,S15] | | | C-loop |
| | L387F [S2]; H396P/R [S5,S9] | H396P/R [S9] | | | A-loop |
| | | | Y253H/E255V [S17]; Y253H/T315I [S8] | Q252H/T315I [S18,S19]; F317L/E255V [S4]; T315I/M351T [S18, S19]; T315I/F359V [S18,S19]; T315I/H396R [S8,S18,S19]; F317L/E459K [S4] | Compound mutation |
| | E123Q [S10] | | T212R [S10] | | SH2 (outside ABL KD) |
| Resistant | L248V [S5]; G250E [S11]; Y253H/F [S5, S11]; E255K/V [S5, S11] | | | | P-loop |
| | V299L [S13]; F311L [S20] | | | | Other |
| | T315I [S7,S11] | T315I [S7,S11] | T315I [S7,S11] | | DBS |
| | | | | H396R [S9] | A-loop |
| | L248R/F359I [S9] | | L248R/F359I [S9]; M351T/E255K [S18] | L248R/F359I [S9]; Y253H/T315I [S8]; T315I/G250E [S18]; E255V/T315I [S18]; T315I/M351T [S19]; T315I/L384M [S421]; T315I/E453K [S8]; F317L/F359V/C [S8, S19]; T315I/E459K [S4]; F317L/L384M [S4] | Compound mutation |
| | T212R [S10] | | | | SH2 (outside ABL KD) |

[a] Mutation sensitivity to specific tyrosine kinase inhibitors is indicated.
[b] Locations of the mutations are categorized based on whether they are located on the P-loop (aa 244–255 of ABL), DBS (aa 315–317 of ABL), C-loop (aa 350–363 of ABL), A-loop (aa 381-402), and KD (ABL kinase domain).
[c] The references are provided in the supplemental information online.
[d] Also see Fig. 2 in the main text for additional information.

repair (BER) pathway by which DNA repair occurs is crucial for the proper prevention of accumulation of point mutations and, in CML-CP cells, BCR-ABL kinase was shown to inhibit uracil DNA glycosylase (UNG2) activity, which is one of the glycosylases in the BER pathway, leading to the accumulation of point mutations and facilitating drug resistance [28]. Early studies provided strong evidence that the *BCR-ABL* fusion gene markedly enhances genomic instability in BCR-ABL$^+$ murine HSCs, mediated by elevated levels of ROS and affecting the genome broadly [29]. Interestingly, the rapid generation of BCR-ABL mutants was then demonstrated in primary CML cells or in BCR-ABL-transduced adult human bone marrow *in vitro* [30,31]. Notably, the frequency of *BCR-ABL* mutants observed in primary CML cells, including pre-existing *BCR-ABL* mutations, appears to be higher in LSCs than in more mature cells. Recent studies further demonstrated that Rac2 GTPase can alter mitochondrial membrane potential through the mitochondrial respiratory chain complex III (MRC-cIII), which can generate high levels of ROS in CML-CP stem and progenitor cells. MRC-cIII-generated ROS can promote oxidative DNA damage to trigger genomic instability, resulting in the accumulation of

chromosomal aberrations and TKI-resistant BCR-ABL mutants [32,33]. These studies suggest that BCR-ABL-induced genomic instability originates in the most primitive TKI-refractory LSCs that contribute to drug resistance and disease progression in CML.

### *Overexpression of BCR-ABL*

Genomic amplification of *BCR-ABL* gene as well as overexpression of BCR-ABL transcripts have been detected in patients with drug-resistant CML [34]. Patients treated with IM showed an increase in *BCR-ABL* copy number and experienced resistance [17]. *BCR-ABL* could be reversed after IM discontinuation, indicating the dynamic nature of this mechanism [17]. The overexpression of *BCR-ABL* in 50% of TKI-resistant cell lines was the initial mechanism of resistance identified, because no mutations emerged in the absence of *BCR-ABL* overexpression. However, other modes of resistance have also been also described [35]. The level of *BCR-ABL* expression correlated with mutation emergence in patients, but might not translate to increased leukemic burden [36]. However, contrasting evidence on *BCR-ABL* amplification has been reported whereby *BCR-ABL* amplification was absent in IM resistance: a follow-up study in a 68-year-old patient who developed secondary resistance to IM displayed not only genomic *BCR-ABL* amplification, but also an increase in *BCR-ABL* transcripts, which were also demonstrated in other studies [37,38]. Nevertheless, the low frequency of *BCR-ABL* gene amplification compared with resistance-causing mutations should not overshadow the importance of this mechanism [39].

## *BCR-ABL independent resistance*

Although BCR-ABL-dependent resistance mechanisms are most common, other mechanisms of resistance include activation of alternate prosurvival signaling pathways, drug influx–efflux activity, clonal evolution, epigenetic modifications, inherently resistant stem cells, the bone marrow stromal microenvironment, and elevated levels of inhibitors of apoptosis proteins.

### *Alternate prosurvival pathways*

One of the earliest findings from studies of BCR-ABL-transduced HSCs was the activation of an autocrine mechanism that could confer partial or complete growth factor autonomy [2,40]. This finding was then demonstrated in most $CD34^+$ cells from patients with CML that display a constitutively activated production of IL-3 and granulocyte-colony stimulating factor (G-CSF), which accounts for increased STAT5 phosphorylation [41]. In primitive CML cells, this is silenced when they become quiescent and is then reversed when they begin to proliferate. Increased levels of GM-CSF protected CML cells against IM and NI through activation of the JAK2/STAT5 pathway, independent of *BCR-ABL*. GM-CSF levels were elevated in patients with CML exhibiting IM resistance independent of any *BCR-ABL* mutation, suggesting a contribution to IM and NI resistance [42,43]. IM treatment can induce the PI3K/Akt/mTor signaling pathway, which is essential in mediating early IM resistance [44]. The overexpression of: (i) FOXO1, which is downstream of the PI3K pathway; (ii) PRKCH, a Protein Kinase C (PKC) and activator of c-RAF (RAF/MEK/ERK) signaling; and (iii) SRC family kinase (SFK)-LYN also contribute towards BCR-ABL-independent resistance [45–47].

Overexpression of various proteins has been documented among TKI-resistant CML cells, including: (i) PFKFB3 [48]; (ii) nuclear β-catenin, NFκB-p65, and Akirin-2 protein [49]; (iii) tumor progression locus Tpl2 (COT1 kinase, MAP3K8) along with SFKs NFκB and MEK/ERK [50]. Other findings indicated the involvement of exosomes (30–120-nm vesicles) with the transfer of miR-365 from resistant to sensitive cells [51]. Some of the signaling pathways involved in BCR-ABL-dependent survival overlap with alternate signaling pathways independent of BCR-ABL, such as JAK/STAT and RAF/MEK/ERK, which could be 'overlapping' therapeutic targets.

### *Changes in drug transporters and plasma protein binding*

The activity of transporters is associated with TKI resistance, because the availability of intracellular drug is crucial to achieve a clinical outcome. Membrane influx pumps, such as human organic cation transporter 1 (hOCT1), a key transporter required for IM uptake, and ATP-binding cassette (ABC) members that encode key efflux pumps, have important roles [52]. A high OCT1 content appears to be predictive of major molecular response and low OCT1 is associated with a suboptimal response, with a dose increase needed to achieve optimal response [53]. By contrast, other studies have shown no correlation between OCT1 SNPs and clinical IM response [54]. Similarly, the ABC efflux transporter P-glycoprotein (P-gp or MDR1 protein) can reduce intracellular IM levels when overexpressed, adversely affecting the therapeutic efficacy [55]. Other ABC transporters (ABCA3, ABCC2, and ABCG2) can also contribute to TKI resistance [56]. $CD34^+$ cells from patients with CML expressed extremely low OCT1 and high ABC transporters with slightly elevated levels on LSCs compared with their more mature progeny, which are also predictive of poor long-term outcomes to IM therapy clinically [57–62]. Laboratory tests can be carried out within the first month of treatment, offering the potential for the early identification of patients who are most likely to respond suboptimally to IM therapy and, thus, who might benefit from receiving a more potent TKI and/or an allosteric ABL1 inhibitor as frontline treatment [63].

### *Clonal evolution and epigenetic dysregulations*

Additional chromosomal abnormalities in $Ph^+$ cells are often detected in 30% of patients in the AP and 80% of patients in the blast crisis. Frequently reported clonal abnormalities include an additional Ph+ chromosome, trisomy 8, and chromosome 17 abnormalities, all of which contribute to diminishing the effect of IM [23,64]. Detection of these in Ph− cells in patients with CML during IM treatment has also been reported, although this is not limited to patients with IM resistance, because it could also be detected in patients undergoing interferon-α or DA treatments [65]. Interestingly, changes in gene expression regulated by epigenetic modifications, such as DNA methylation, histone modification, and noncoding (nc)RNAs, can also contribute to drug resistance in CML. Hyper- and hypomethylation of numerous genes, including tumor suppressors affecting proliferation, differentiation, cell-cycle regulation, DNA repair and apoptosis induction, have been well documented [66]. Numerous studies have identified large number of genes altered by methylation, with more recently reported *HOXA4* and *HOXA5* gene methylation, affecting differentiation and contributing towards IM resistance in patients with CML [67]. In general, the number of methylated genes was higher for patients with drug-resistant and intolerant CML, and the CpG island in the promoter region was the most

recognized mechanism of DNA methylation leading to gene suppression [68,69]. Histone modifications, such as acetylation by histone acetyl transferases (HATs) and hypoacetylation by histone deacetylase (HDAC), leading to gene transcription and silencing, respectively, can also contribute to drug resistance [70,71]. ncRNAs were recently recognized as epigenetic regulators that can manage mRNA and protein levels via several mechanisms. mRNA-binding miRNAs can silence or activate gene expression; examples of dysregulated miRNAs identified in CML are summarized in Table 2. The identification of numerous miRNAs that are directly involved in CML resistance by altering the expression of various genes, including drug efflux pump, antiapoptotic proteins, and signaling pathways, highlights the significance of considering miRNA-based therapy along with conventional methods.

### *Leukemic stem cells*

CML stem cells are a rare population that have been established as being inherently resistant to TKIs and a key population for driving relapse and disease progression. Strong evidence indicates that LSCs have multiple features expected to promote TKI resistance, including deregulated expression of *BCR-ABL*, a high degree of genetic instability, and *BCR-ABL*-independent survival [30,59,72]. Independent groups have reported that *BCR-ABL* expression is elevated in the most primitive subset of lin–CD34$^+$CD38– CML stem cells and is then rapidly and progressively reduced as these cells differentiate [59,72,73]. Interestingly, the levels of *BCR-ABL* transcripts present in CML LSCs with highly proliferative activities are higher than those present in the G0 fraction of quiescent cells, which appears not to be dependent on BCR-ABL kinase activity for survival and explains why all US Food and Drug Administration (FDA)-approved TKIs are ineffective against primitive quiescent CML cells [74–76]. Developing new treatments to eradicate this population is a longstanding challenge. The resistance of LSCs could also arise because of the increased expression of PRKCH, thereby activating the RAF/MEK/ERK signaling pathway in murine CML stem cells, TKI-resistant cells, and in IM-resistant human CML stem cells [47]. High levels of BCR-ABL and phosphorylated-CrKL leading to increased BCR-ABL activity could be another reason for observed resistance in CD34$^+$CD38- populations. Increasing IM dose resulted in nonspecific cell death without altering phosphorylated-CrKL levels, because IM is known to inhibit other targets, such as c-kit (a human stem cell factor receptor) and PDGF-R [72,77]. The presence of external factors, such as autocrine production of cytokines and a stromal-support microenvironment, enhance the survival of CML LSCs irrespective of BCR-ABL inhibition by IM. It has been reported that Abelson helper integration site-1 (AHI-1), a scaffold oncoprotein, is deregulated in LSCs and interacts with multiple kinases and other proteins (BCR-ABL, JAK2, β-catenin, DNM2, and PP2A) to enhance leukemia-initiating activity and resistance to TKIs [78–80]. Combinations of TKIs with a JAK2 inhibitor or a PP2A inhibitor to disrupt the AHI-1-mediated protein complex sensitized drug-insensitive LSCs to TKIs both *in vitro* and in preclinical xenotransplant models [80,81]. In addition, the high expression of human estrogen receptor alpha 36 (ERα36), an alternative splicing variant of ERα66, has been demonstrated in CD34$^+$ CML cells and T315I mutant cells and is abnormally localized in plasma membrane and cytoplasm, which could be another factor in the observed TKI resistance [82].

**TABLE 2**

**Recently identified miRNAs that are dysregulated (down- or upregulated) in CML leading to drug resistance[a,b,c]**

| miRNA | Regulation | Cells | Outcome | Target | Refs |
|---|---|---|---|---|---|
| miR-101 | Downregulation | K562 cells | Overexpression inhibited proliferation and induced apoptosis | Decreased antiapoptotic (*Bcl2*, *BclxL*, *Mcl1*, *XIAP*, *survivin*) and proliferative genes (*c-Myc*, *CCND1*) | [S21] |
| miR-124-3p | Downregulation | K562, KU812, patient BMNCs | Inhibition of SOCS3, cell proliferation, and drug resistance | Decreased expression of *B4GALT* | [S22] |
| miR-146a | Downregulation | K562/ADM | Overexpression resensitized cells to adriamycin (ADM) | Increased expression of *CXCR4* | [S23] |
| miR-181c | Downregulation | K562/ADR, CML/MDR | Overexpression resensitized cells to adriamycin | Decreased expression of ST8SIA4 | [S24] |
| miR-199b | Downregulation | Patients with CML | IM resistance | Caused by deletion in 9q34.1 region (*ABL*) | [S25] |
| miR-212 | Downregulation | K562 cells | Inhibition improved cell viability, reduced apoptosis, and decreased cytotoxicity caused by IM treatment | Upregulation of *ABCG2* efflux pump | [S26] |
| miR-217 | Downregulation | K562 cells | IM resistance | Increased expression of *DNMT3A* | [S27] |
| miR-3142 | Overexpression | K562/ADR, CML/MDR | Increased colony formation; enhanced resistance to ADR | Decreased expression of *PTEN* and activation of PI3K/Akt pathway | [S28] |
| miR-451 | Downregulation | Patients with CML | Inhibition led to IM resistance | Increased levels of *MYC* | [S29] |
| miR-574-3p | Downregulation | K562 cells | Overexpression inhibited proliferation and induced apoptosis | Suppression of IL-6/JAK/ STAT3 pathway | [S30] |
| miR-9 | Downregulation | K562/ADR, CML/MDR | Overexpression resensitized cells to multiple drugs also *in vivo* | Decreased expression of *ABCB1* efflux pump and P-gp proteins | [S31] |

[a] Summary of the ability of miRNAs to alter other targets along with specific cell types used in the studies.
[b] Abbreviations: ABC, ATP-binding cassette transporter; ADR/ADM, adriamycin resistant; B4GALT1, beta-1;4-Galactosyltransferase 1; DNMT3A, DNA methyltransferases 3 alpha; PTEN, phosphatase and tensin homolog; SOCS3, suppressor of cytokine signaling; ST8SIA4, sialyltransferase 8 (alpha-2; 8-polysialyltransferase).
[c] The references are provided in the supplemental material online.

Activation of Wnt/β-catenin signaling in which TKI treatment enhances CD70 ligand-induced CD27 signaling, leading to nuclear translocation of β-catenin and activating Wnt target genes, supports cell survival independent of BCR-ABL. Involvement of Hedgehog signaling is another mechanism of resistance in CML stem cells [83]. The activation of STAT3 in the JAK/STAT pathway and expression of BCL-6, a zinc finger transcription factor, can be upregulated following TKI treatment in CML stem cells [84]. In addition, a rare population of CD34- cells with stem cell properties was shown to be relatively resistant to IM, suggesting a potential contribution of this cell population to disease persistence [85]. Thus, the innate as well as acquired mechanisms that make CML stem cells resistant to TKIs has prompted considerable interest in developing strategies to target these cells more effectively.

### *Bone marrow microenvironment*

Cell–cell contact mediated by various receptors in bone marrow stroma along with cytokines, chemokines, and growth factors secreted by stromal cells can contribute towards drug resistance [86]. Stromal derived factor-1 (SDF-1 or CXCL12), which acts through the CXCR4 receptor, can modulate CML cell survival; CXCR4 expression is altered with BCR-ABL activity, leading to the defective adhesion of CML cells to bone marrow stroma. IM treatment can induce CXCR4 and BCL-XL expression, leading to migration and/or homing to bone marrow and protection from drug-induced cell death. Bone marrow samples collected from patients with IM-resistant CML displayed an increase in protective FGF2 levels without any BCR-ABL mutation [87]. High levels of IL-7 in the bone marrow microenvironment, secreted by mesenchymal stem cells (MSC), was shown to protect against IM-induced apoptosis [88]. High levels of IL-1β can help the migration of cells towards the stroma [89]. The production of placental growth factor (PGF) by stromal cells stimulates the proliferation of *BCR-ABL* cells via Flt1 (VEGFR1) and helps to overcome the effects of IM [90]. Studies with stromal conditioned media were shown to cause and/or enhance resistance towards IM *in vitro,* associated with an increase in the levels of the STAT3 target genes *BCLxL*, *MCL1* and *Survivin* [91]. Co-culture of CML cells with bone marrow stromal cells (BMSCs) can induce the expression of heme oxygenase-1 (HO-1, heat shock protein 32) in the latter and galectin-3 (GAL-3) in the former, providing antiapoptotic protection, multidrug resistance, proliferation, and bone marrow homing [92,93].

Integrins have a major role in cell adhesion-mediated drug resistance. Various intracellular signaling pathways can be regulated via 24 different receptors that are formed by dimerization of 18α and 8β integrin subunits, the expression of which varies significantly from cell to cell [94]. The well-studied $\beta_1$ integrins VLA4 ($\alpha_4\beta_1$) and VLA-5 ($\alpha_5\beta_1$) expressed on CML cells can bind to VCAM-1 and fibronectin expressed on BMSC and extracellular matrix and could activate or downregulate multiple genes to confer drug resistance and act as a sanctuary for minimal residual disease (MRD) [86,91,95,96]. Activation of integrin-linked kinase (ILK) can lead to direct interaction with β-integrins, promoting survival via myriad signaling pathways, such as AKT/PI3K, ERK1/2, STAT3, and Notch1/HES [97]. A subpopulation of CML cells exhibiting IM resistance with high adhesion ability and invasiveness were observed following continuous IM treatment; this was attributed to enhanced expression of $\alpha_V\beta_3$ integrin along with the activation of the focal adhesion kinase (FAK)/AKT and ERK1/2 pathways [98].

The cytoplasmic domain of the $Ca^{2+}$-dependent adhesion molecule Cadherin can bind to intracellular β-catenin, which is linked to the actin cytoskeleton, stabilizing cell–cell adhesion. Co-culture of $CD34^+$ CML cells with MSC can activate WNT-β-catenin signaling and, because of its association with N-cadherin, protects CML cells from TKIs [99]. The direct interaction between MSC and CML cells via the CXCR4 receptor effectively protects leukemic cells from IM-induced cell death and homes CML cells to the bone marrow [100]. The overexpression of hyaluronan-receptor CD44, selectins, and osteopontin also promotes the homing and engraftment of CML cells in bone marrow that bears N-selectins on the endothelium [101].

The inherently hypoxic bone marrow environment can prolong the survival of CML cells during TKI treatment. The hypoxic environment leads to activation of hypoxia-inducible factor-1α (HIF-1α, a transcriptional factor), aiding the survival of CML cells despite BCR-ABL reduction by IM treatment, and evading IM-induced apoptosis. Gene-profiling studies indicated the upregulation of various prosurvival genes, which were partially attenuated by IM treatment; however, several genes still promoted the survival of CML cells in the stromal environment [102]. These observations support the role of the bone marrow niche as a sanctuary for cells responsible for MRD as well as drug resistance (Fig. 3).

### *Defects in apoptosis (inhibitors of apoptosis proteins, IAPs)*

IAPs can inhibit drug-induced apoptosis. The upregulation of IAPs in TKI-resistant CML cell lines as well as primitive patient cells has been documented [11]. The expression of BIRC6 was increased in IM-resistant cells and was dependent on the SRC family kinase LYN. Similarly, XIAP, which can directly inhibit caspases-3, 7, and 9, was upregulated in drug-resistant CML cells. Other IAPs, such as survivin and MCL-1, were reported to contribute to drug resistance, but to a lesser extent in CML [103].

### *Autophagy and mitochondrial metabolisms*

Recent evidence on enhanced autophagy as a survival mechanism in IM-treated CML cell lines and patient cells has identified key autophagy proteins, such as autophagy-related 5 (ATG5), ATG7, and ATG4B, and their role in CML drug resistance [104,105]. Reports of increased dependence on mitochondrial oxidative phosphorylation (OXPHOS), as evident by high ROS levels and DNA damage in CML LSCs compared with normal HSCs, could provide an additional specific treatment strategy to treat CML drug resistance [33,106].

## Therapeutic approaches against resistance

Patients with CML who do not respond to IM treatment with 400 mg/day dose in CP and 600 mg/day for AP are considered as treatment failures. Increasing the IM dose from 400 mg/day to 800 mg/day could be tolerated and therapeutically beneficial. Multiple studies have reported the therapeutic outcome of dose escalation among patients with CML-CP experiencing suboptimal response or cytogenetic relapse (appearance of $Ph^+$ metaphases), patients with mutations that exhibit low-level resistance, and patients who experience resistance because of inadequate levels of IM in the plasma and *BCR-ABL* amplification. However, this approach appears to be ineffective for all other cases of IM resistance [23,107]. Inhibition of the drug efflux transporter P-gp helped to increase the intracellular concentration of IM,

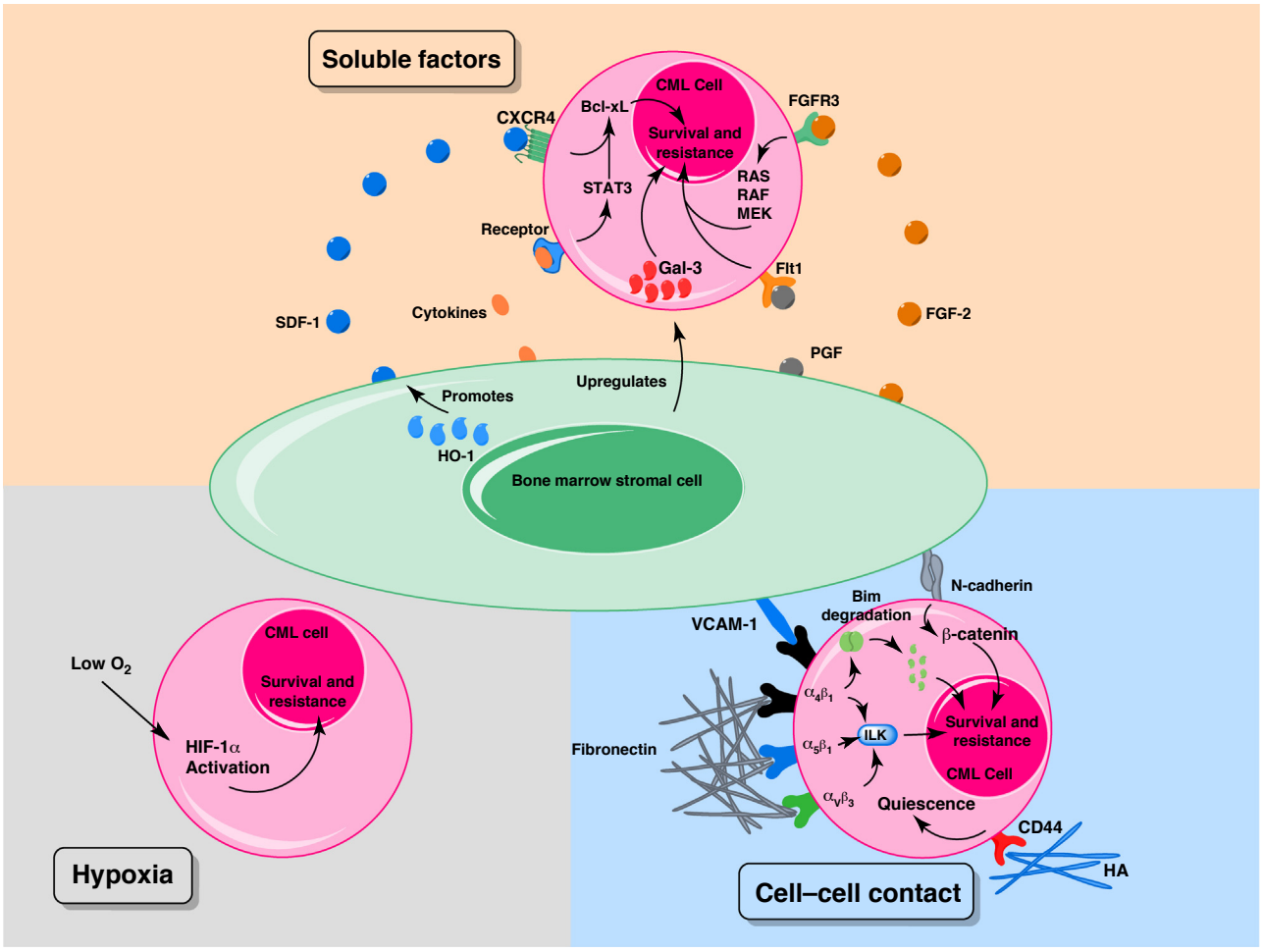


**FIGURE 3**

Overview of select soluble factors, cellular receptors, and their mediators altering different pathways along with hypoxia in the bone marrow microenvironment (BMM) that act as a protective niche and contribute to drug resistance. Abbreviations: FGF-2, fibroblast growth factor-2; FGFR3, fibroblast growth factor receptor-3; GAL-3, galectin-3; HA, hyaluronan; HIF-1α, integrins $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_3$ and hypoxia-inducible factor-1α; HO-1, heme oxygenase-1; ILK, integrin-linked kinase; PGF, placental growth factor; SDF-1, stromal derived factor-1; STAT3, signal transducer and activator of transcription-3; VCAM-1, vascular cell adhesion molecule-1.

which in turn addressed IM resistance. Reversin 205, a P-gp inhibitor, was able to decrease the $IC_{50}$ of IM significantly in IM-resistant cell lines, thus resensitizing the cells to IM [108]. This combinational approach can also be implemented for treating LSC because they express high levels of P-gp, and to increase the intracellular concentration of other drugs (non-TKIs) that are affected or dependent on P-gp concentration. However, this approach might fail in T315I mutant cells, where IM cannot bind to BCR-ABL irrespective of its intracellular concentration. Here, we summarize the treatment options that have been explored in clinical trials, *in vivo* and *in vitro,* which have the potential to address different BCR-ABL-dependent and -independent resistance mechanisms (Fig. 4).

### *Targeting BCR-ABL-dependent resistance by directly targeting BCR-ABL*

#### *Targeting the BCR-ABL kinase domain*

First-generation IM specifically binds to the ATP-binding site of the inactive BCR-ABL oncoprotein and inhibits its phosphorylation and downstream proteins in signal transduction. Resistance or intolerance to IM treatment necessitated the development of second-generation DA, which can bind to both active (open) and inactive (closed) BCR-ABL conformations, thus exhibiting improved therapeutic efficiency [109]. Preclinical testing revealed that DA was 100–300-fold more potent, which could be because of its potential to additionally target SRC family kinase. The Dasasion study demonstrated that, compared with IM, DA use as a frontline therapy produced faster and deeper remissions, and was associated with lower rates of transformation to AP or blast phase [110]. Several clinical trials have shown DA efficacy in patients with IM-resistant CML despite the presence of mutations in *BCR-ABL*. However, poor response was observed in patients with T315I mutations. Various new mutations were also detected in patients treated with DA, which cautions proper monitoring of patients. In addition, patients treated with DA may experience severe nonhematological toxicities, such as pleural effusions and pulmonary hypertension, and reports of DA resistance independent of any mutation warrants the need to explore other modes of resistance mechanisms [23,111].

NI is another second-generation TKI and is 10–30-fold more potent in BCR-ABL inhibition because of higher affinity as well as

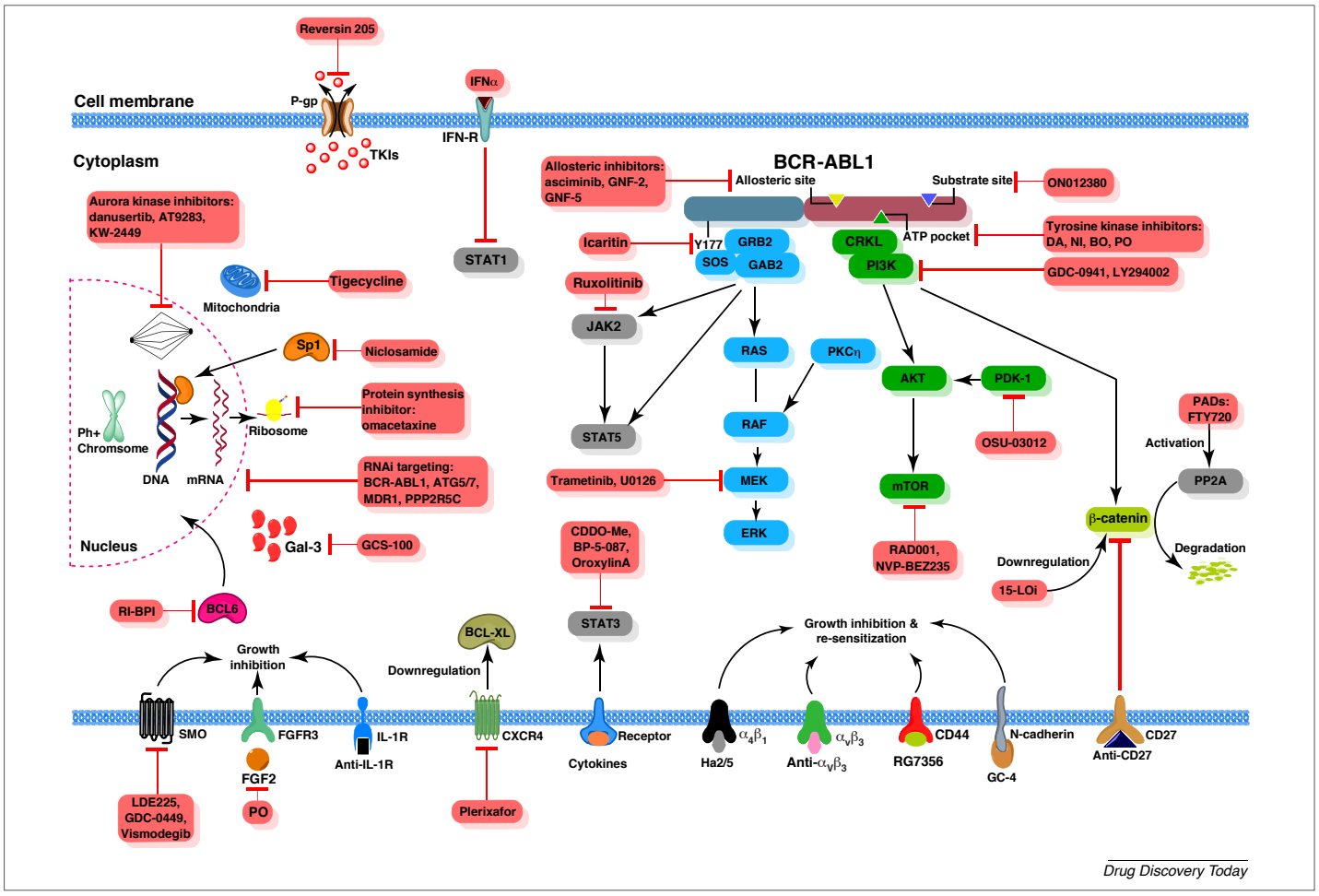


**FIGURE 4**

Therapeutic options available for imatinib mesylate (IM) resistance and chronic myeloid leukemia (CML) stem cells. Various treatment options (indicated in red boxes) have been explored to tackle breakpoint cluster region-Abelson (BCR-ABL)-dependent and -independent mechanisms, with specific inhibitors targeting intracellular signaling pathways and cell surface receptors, as a monotherapy and/or as combinational therapy with tyrosine kinase inhibitors (TKIs). For additional definitions, please see the main text.

higher activity against IM-resistant mutations [112,113]. In an *in vitro* study involving 33 *BCR-ABL* mutations resistant to IM, NI was effective against 32 mutations by reducing autophosphorylation and proliferation at concentrations appropriate for clinical use. Unlike IM, both DA and NI operate independent of OCT, which could explain their higher activity. However, NI was also ineffective in patients with T315I mutation, similar to DA and IM. As with DA, NI used as a frontline therapy has been demonstrated to produce deeper responses compared with IM, with a lower incidence of transformation. The more recently approved second-generation TKI BO exhibits 10–20-fold higher BCR-ABL inhibition compared with IM, with the ability to bind both inactive and active conformations [114,115]. It can target SRC family kinases, c-kit, and PDGF, in addition to BCR-ABL. BO was also effective in patients with CML-CP who were previously treated with IM followed by DA and/or NI; adverse effects include transient diarrhea, hematological abnormalities, and abnormal liver function. However, all second-generation TKIs have an inability to target T315I mutants [23,114].

The third-generation TKI PO can act effectively on T315I mutations because of the C≡C linker that helps to bind T315I mutations without any steric interference [116,117]. It can bind to the closed BCR-ABL conformation and is believed to be effective against almost all *BCR-ABL* mutations. It is 500-fold more potent than IM and effective against mutant as well as wild-type BCR-ABL protein [15,118]. The PACE study demonstrated the efficacy of PO in patients with CML with T315I mutations [119]. Common adverse effects, including abdominal pain and thrombocytopenia, are attributed to its ability to target other kinases, such as SRC family kinases, c-KIT and growth factor receptors. It has also been associated with an excess risk of severe arterial thrombotic complications [119]. In particular, cardiac failure and congestive heart failure in some patients have been a concern. Further clinical trials have investigated optimized dosing schemes to reduce adverse effects for treatment of patients with TKI-resistant CML [120]. However, reports of BCR-ABL-independent resistance as well as detection of new compound mutations in PO treatment groups have also appeared [121,122].

### *Targeting the BCR-ABL allosteric site*

Allosteric inhibitors bind to sites that regulate BCR-ABL kinase activity, rather than to the ATP-binding site. Asciminib *(ABL001)* is an allosteric inhibitor that binds to the myristoylation site, changing the conformation of the kinase domain to maintain it inactive.

Asciminib has become valuable to treat TKI resistance mediated by *BCR-ABL* mutations and promising results led to clinical trials in combination with NI, which was effective in patients carrying T315I mutation, with acceptable toxicity. Asciminib monotherapy was also effective in patients who failed two or more TKIs, although the threat of mutations at the myristate-binding site needs to be monitored for long-term use, because previous allosteric BCR-ABL inhibitors, GNF-2 and GNF-5, were ineffective in these mutations [115]. However, overexpression of ABC efflux proteins was shown to result in asciminib resistance *in vitro* [123].

#### *Targeting the BCR-ABL substrate site*

ON012380 is an inhibitor of the substrate-binding domain of BCR-ABL and was found to be tenfold more potent than IM without any hematotoxicity. Given its ATP-independent inhibition of cell growth, it was effective in T315I-mutant cells, resulting in growth inhibition. It also showed synergistic outcome with IM and inhibited other kinases, such as PDGF receptor kinases and SRC family kinases [124,125].

### *Targeting BCR-ABL-dependent resistance by indirectly targeting BCR-ABL*

#### *Targeting Aurora kinases*

Aurora kinases have key roles in centrosome duplication, chromosome alignment, and mitotic checkpoint arrest. Their overexpression has made them a therapeutic target and their inhibition can be highly beneficial for the treatment of TKI resistance. The Aurora kinase inhibitor, danusertib, was shown to inhibit all known aurora kinases along with BCR-ABL tyrosine kinase. It was effective in both wild-type as well as T315I-mutant *BCR-ABL* cells and its clinical trials are at various stages. A Phase II study with MK-0457, another Aurora kinase inhibitor, among patients with T315I-mutated CML in CP and AP stages showed minimal response, with neutropenia as the common adverse effect [126]. Other Aurora kinase inhibitors, such as AT9283 and KW-2449, were also effective against T315I mutation *in vitro*, but only the latter helped to eradicate this mutation in a patient with CML [23,127,128].

#### *Targeting protein synthesis*

Omacetaxine mepesuccinate is a subcutaneous version of homoharringtonine, which inhibits protein synthesis by binding to the 80S ribosome in a BCR-ABL-independent manner. This leads to a decrease in the intracellular levels of various antiapoptotic regulatory proteins, such as MCL-1 and BCL-2, which was demonstrated in T315I mutant *BCR-ABL* cell lines and mouse models [129]. Given its unique mechanism of action, patients with T315I mutation and TKI failure showed beneficial outcome in their overall survival [130,131].

#### *Targeting transcription factors*

Niclosamide is an anthelmintic for the treatment of tapeworm infection, but it can also inhibit various intracellular signaling pathways, such as Wnt/β-catenin, STAT3, and Notch. Results of a recent study to evaluate the therapeutic potential of niclosamide for IM resistance with T315I mutation (in cell lines and in a mouse model) were promising; niclosamide inhibited the transcription of both wild-type *BCR-ABL* as well as T315I *BCR-ABL* by suppressing the transcription factor SP1. This reduced phosphorylated STAT5 and AKT, with MCL-1 and XIAP helping to induce apoptosis and growth inhibition, in addition to acting in synergy with IM [132].

### *Targeting BCR-ABL-independent resistance*

#### *Interferon-α (IFNα)*

IFNα is a cytokine that was the gold standard for treatment of CML before TKIs and was able to improve the median survival time to 5 years. Several groups have reported the benefit of using IFNα combined with TKIs in patients with drug-resistant T315I CML. IFNα exhibits antiviral, antiproliferative, and immunomodulatory activity along with its ability to induce cell differentiation. PEGylated IFNα, when administered with IM/DA, was effective in T315I mutants and was also able to successfully eradicate this mutation in patients with CML [133,134].

#### *Targeting signaling pathways*

Targeting the JAK pathway with ruxolitinib effectively inactivated STAT5 and, with NI, provided beneficial outcome in growth inhibition of CML stem and progenitor cells that were resistant to TKIs [135]. Blocking the JAK-2 pathway led to decreased levels of GM-CSF-induced STAT5 activation, thereby inhibiting the proliferation of drug-resistant cells [42]. A combinational treatment targeting STAT3, STAT5, and HO-1 was shown to overcome a range of resistances, including CML stem cells, highly resistant *BCR-ABL* subclones, and T315I-inclusive mutations. In this study, the STAT3 inhibitor CDDO-Me (bardoxolone methyl) in combination with TKIs showed synergistic effects on growth inhibition and apoptosis induction in drug-resistant CML cells. The outcome was more effective when combined with zinc protoporphyrin IX (ZnPP), which inhibits HO-1, leading to increased apoptosis [136]. Another study targeting STAT3 with BP-5-087, a salicylic acid-based inhibitor, in combination with IM, reduced the survival of BCR-ABL-independent resistant CML cells [137].

Inhibition of the mechanistic target of rapamycin (mTOR) with RAD001 (everolimus) was shown to prevent IM-induced AKT activation and resistance development [44]. More recently, mTOR inhibition with NVP-BEZ235 in a PO-resistant cell line, which exhibit BCR-ABL-independent activation of mTOR, was shown to induce cell death. Moreover, inhibition of autophagy by hydroxychloroquine helped to sensitize these cells to mTOR inhibitor treatment *in vitro* and *in vivo*, resulting in higher cell death [138].

A class-I PI3K inhibitor, GDC-0941 (pictilisib) was able to inhibit growth and induce apoptosis in a dual TKI (IM and DA)-resistant cell line by downregulating the transcription factor FOXO1. The BCR-ABL-independent resistant cells were also sensitive to a combinational treatment of DA and GDC-0941, along with a significant inhibition of AKT substrates [45]. Another inhibitor, LY294002 in combination with IM or alone, was able to induce apoptosis and autophagy in *BCR-ABL*-positive cells by causing endoplasmic reticulum stress, which could be further explored for IM-resistant cells [139]. Inhibition of phosphoinositide-dependent kinase-1 (PDK-1), which is key in AKT signaling, using OSU-03012 was able to resensitize resistant cells to IM because of lower AKT phosphorylation [140].

The simultaneous inhibition of the RAF/MEK/ERK pathway by the MEK inhibitor trametinib (GSK1120212) and IM exhibited synergistic outcome in cells from patients with BCR-ABL-independent, IM-resistant CML [47]. Another study using the MEK inhibitor U0126 in combination with DA (SFK and BCR-ABL inhibition) significantly decreased the survival of IM-resistant cells, whereas the U0126/IM combination did not have any beneficial effect, highlighting the importance of targeting both MEK-ERK and SFK [50].

### Targeting bone marrow microenvironment

The rise of drug resistance has made HSC transplantation (HSCT) an important option again for patients with CML-CP with T315I mutation who fail to respond to two TKIs and for patients with CML-AP [141]. A recent study comparing the efficacy of PO versus HSCT provided strong evidence in favor of HSCT for patients with T315I-positive CML-AP, whereas PO was the most effective in patients with T315I-positive CML-CP [142].

The growth of CML stem cells independent of BCR-ABL and their inherent resistance to TKIs has led to the targeting of numerous intracellular pathways that are crucial in bone marrow milieu. The MEK inhibitor, trametinib, targeting the RAF/MEK/ERK pathway, was successful in inducing apoptosis in both murine and human stem cells with minimal effect on normal HSCs [47]. Targeting the upregulated ERα36 in TKI-resistant $CD34^+$ CML stem cells and the T315I mutant cell line by the flavonoid Icaritin (SNG162) impeded cellular growth and induced apoptosis. The outcomes were synergistically improved with TKIs, and a detailed mechanistic study revealed the disruption of BCR-ABL/Tyr177/GRB2 complex interactions along with RAS/MAPK pathway inhibition, because this was activated by the binding of GRB2 at the SH2 domain, initiated by the phosphorylation of Tyr177 by ABL [82]. The β-catenin signaling in CML stem cells can be counteracted by: (i) antibodies blocking the interaction of CD70 and CD27; (ii) protein phosphatase 2A-activating drugs (PADs), such as FTY720, leading to activation of PP2A and degradation of β-catenin; and (iii) 15-lipoxygenase inhibitors (15-LOi), which can downregulate β-catenin. In addition, a combinational treatment of NI and 15-LOi was synergistic in TKI-resistant CML stem cells. The smoothened (SMOs) inhibitors LDE225 (sonidegib) and GDC-0449 were able to inhibit Hedgehog signaling in $CD34^+$ CML-CP and T315I mutant BCR-ABL cells, respectively, in combination with TKIs, without affecting normal HSCs [83]. The STAT3 SH2 domain-binding inhibitor BP-5-087 was also able to restore TKI sensitivity without any toxicity on normal HSCs [137]. The BCL6 inactivator peptide RI-BPI effectively targeted the primary $CD34^+$ as well as the more primitive $CD34^+$CD38- CML population. It was unable to cause any significant change in the viability of cells on its own, but its combination with IM improved its activity [84].

The CXCR4 antagonist plerixafor (AMD3100) in combination with NI provided substantial improvement in the survival of mice. More importantly, plerixafor was effective in reducing NI resistance in CML cells induced by BMSCs, which could be a promising approach to eliminate multidrug resistance [143]. The interruption of the CXCR4/SDF-1 interaction by plerixafor can also downregulate the expression of the antiapoptotic protein BCL-XL in resistant cells [100]. The protection exerted by FGF-2 from the bone marrow microenvironment was successfully neutralized by PO, which was also able to decrease the high FGF-2 concentration in resistant patients [87]. Increased expression and signaling mediated by IL-1R in CML stem cells was successfully treated using an IL-1R antagonist in combination with NI, resulting in decreased cell growth, cell division, and colony formation. The same approach can be extended to other 'protective' interleukins (e.g., IL-1β and IL-7) in the bone marrow microenvironment [89,144].

The anti-PIGF monoclonal antibody 5D11D4 helped to extend the survival of CML mice that were resistant to IM treatment [90]. A monoflavonoid oroxylin A (isolated from the root of *Scutellaria baicalensis* Georgi) effectively resensitized the IM resistance exerted by the presence of stromal derived factors. Oroxylin A was able to inhibit the STAT3 pathway, thereby improving IM sensitivity *in vitro* and *in vivo* [91]. The antiapoptotic protection against IM treatment by HO-1 expression can be counteracted by HO-1 inhibitors, and metalloporphyrins, such as zinc (ZnPPIX), tin (SnPPIX), and chromium protoporphyrin (CrPPIX) [145]. Similarly, the expression of GAL-3 can be encountered by GCS-100, a GAL-3 antagonist and can be used in combination with other TKIs [93].

The binding of CML cells to the bone marrow niche via specific surface receptors can be abrogated using blocking antibodies, such as Ha2/5 targeting β1-integrin, RG7356 targeting CD44, various antibodies targeting $\alpha_V\beta_3$, GC-4 targeting N-cadherin, bicyclams, such as plerixafor, targeting CXCR4, RGD peptides blocking integrins, and RNAi targeting specific receptors, which has been successfully used in acute myeloid leukemia, such as a small interfering (si)RNA/polymer targeting CXCR4 [86,95,146].

### Targeting autophagy and mitochondrial metabolism

The antibiotic tigecycline effectively downregulated the levels of mitochondrial proteins in IM-sensitive and IM-resistant cell lines as well as in patient cells, accompanied by inhibition of mitochondrial respiration and glycolysis, and induction of apoptosis by the caspase-3 pathway. A combination of tigecycline and autophagy inhibitors chloroquine and 3-methyladenine displayed more prominent anticancer activity in these cells [147]. Tigecycline in combination with IM was effective against CML progenitors by strongly inhibiting their colony-forming ability, whereas normal HSCs were unaffected [106]. Similarly, combinational inhibition of autophagy and mTOR, as well as autophagy and the Hedgehog pathway, have been shown to be promising approaches to successfully induce apoptosis in PO-resistant and IM-resistant CML cells, respectively [138].

### Targeting mRNA transcripts

RNAi has been successfully used to target various genes as the basis of leukemia treatment [148], including CML. The knockdown of the *MDR1* gene using the Sleeping Beauty transposon system helped to increase the intracellular concentration of IM, improving its apoptotic efficiency [149]. Similarly, the knockdown of *BCR-ABL* using lipid nanoparticles, which showed high uptake in the bone marrow of leukemic mice, provided evidence of a decrease in leukemic burden and its potential to eradicate multidrug resistance [150]. The use of lipopolymers to deliver BCR-ABL siRNA in CML cells showed a reduction in BCR-ABL mRNA levels within 24 h of treatment and induction of apoptosis [151]. Such an siRNA approach was able to reduce BCR-ABL mRNA levels in IM-resistant K562 cells with similar potency to that of wild-type cells (Fig. 5). Similarly, a recent study using a siRNA/lipopolymer nanoparticle effectively decreased the size of local CML tumors with BCR-ABL siRNA treatment [152]. Other studies silencing BCR-ABL along with growth factor independent-1B (GFI1B), a transcription factor located downstream of BCR-ABL, induced apoptosis in AP-CML [153]. Knockdown of PPP2R5C (a regulatory B subunit of protein phosphatase 2A) in IM-resistant CML cells by nucleofection, significantly reduced their proliferation and induced apoptosis. Successful silencing of the autophagy genes *ATG5* and *ATG7* using siRNA in combination with IM helped to sensitize the cells to IM-

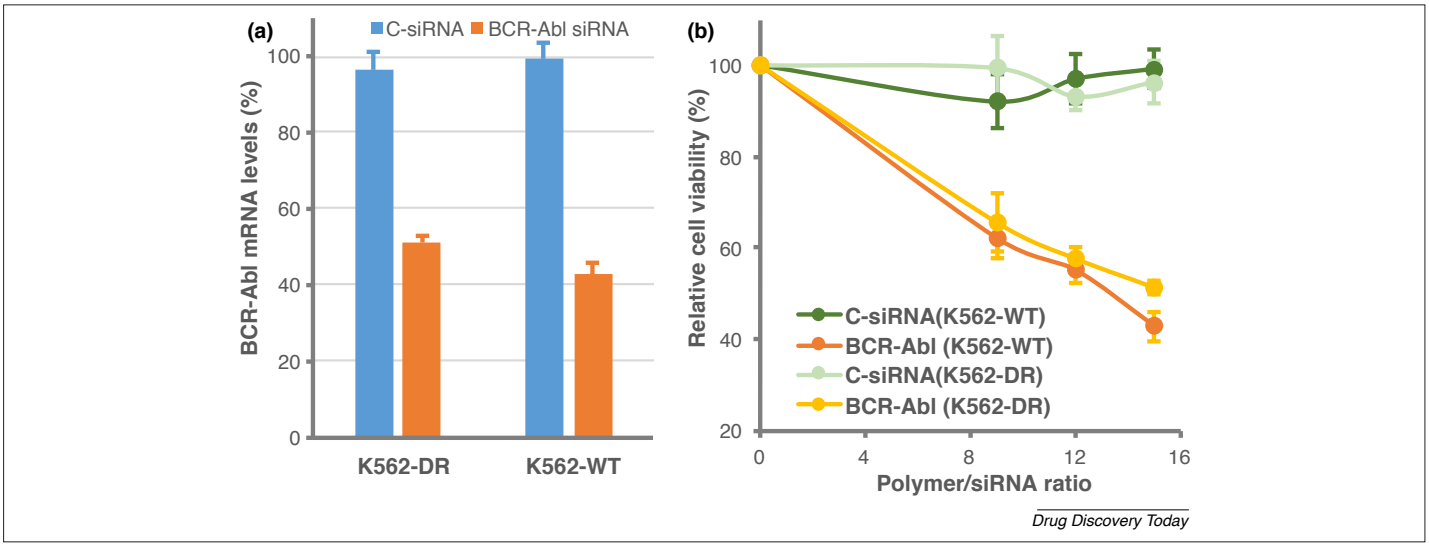


**FIGURE 5**

Silencing of breakpoint cluster region-Abelson (BCR-ABL) expression using RNAi. BCR-ABL-specific small interfering (si)RNAs were delivered nonvirally and BCR-ABL mRNA levels were detected by PCR (a), whereas cell viability was determined by the MTT assay (b). Note the equivalent response to siRNA treatment irrespective of the cell phenotype. Reproduced from [156].

induced cell death and a similar combination with a Hedgehog pathway inhibitor (vismodegib) induced apoptosis in IM-resistant cells [105,154]. All of these highlight the potential of RNAi to overcome CML drug resistance [155], which could find utility in the clinical management of CML.

## Concluding remarks

The emergence of TKI therapies has successfully improved the overall survival of patients with CML and has transformed CML disease from a fatal to a manageable disease; however, because of the inherent plasticity of primitive malignant cells and the array of resistance mechanisms, therapies directed towards a single target might not be a 'cure' for all patients. Proper monitoring of BCR-ABL transcripts, BCR-ABL mutations, and TKI dose, combined with additional therapeutic approaches targeting alternate signaling pathways and cell survival mechanisms in LSCs and BCR-ABL mutant cells will be called for upon TKI resistance. Inhibiting adverse interactions with bone marrow and other protective niches, thereby reducing the impact of soluble and adhesive factors from the local milieu as well as targeting autophagy and mitochondrial oxidative phosphorylation networks, will be an additional strategy to overcome TKI resistance. Understanding dynamic changes in molecular and signaling events regulated by these crucial drivers and/or networks essential for LSC functionality and drug resistance will lead to the development of more effective molecularly targeted therapies, which will be crucial especially for patients at high risk of drug resistance and disease progression. In the long term, endogenous ncRNAs and synthetic interventions based on RNAi (i.e., siRNAs and short hairpin RNAs) could be feasible approaches in case of the failure of more traditional therapies to eradicate minimal residual disease. Personalized approaches for management of drug resistance will require expeditious assessment of individual disease features and are likely to be key for success in overcoming resistance to front-line therapies.

## Conflict of interest

J.V.S., R.K.C., and H.U. declare conflict of interests as founders and shareholders in RJH Biosciences Inc., which holds rights for the commercial development of nonviral RNAi delivery technologies.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drudis.2019.05.007.

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