Chapter XVIII

Can We Cure CML without Transplantation?

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Abstract

A model disease in many respects, chronic myelogenous leukemia (CML) has been called the pristine paradigm for molecularly targeted therapy. Bcr-Abl tyrosine kinase inhibitors (TKIs) have dramatically altered the therapeutic landscape of CML, so much so that many patients diagnosed in chronic phase and treated appropriately now enjoy a life expectancy similar to that of the general population. However, despite the enormous strides made in advancing therapy for this disease, it remains, for the most part, incurable except by allogeneic hematopoietic stem cell transplantation (HSCT). For patients, this means a lifelong commitment to daily TKI therapy. These agents are expensive, and not without side effects, making cure a highly desirable and worthwhile goal. Multiple studies have shown that in contrast to their impressive efficacy against proliferating CML cells, TKIs are unable to eradicate the tiny fraction of quiescent stem cells that are responsible for disease persistence and recurrence upon TKI discontinuation. This has fueled efforts to better understand CML stem cell biology and elucidate survival pathways unique to these cells, the targeted interruption of which could potentially synergize with Bcr-Abl TKIs to confer synthetic lethality while sparing normal hematopoietic stem cells (HSCs). The molecular pathogenesis of CML and mechanisms of resistance to TKIs are reviewed in detail elsewhere in this volume. In this chapter, we provide a brief overview of these topics, summarize key concepts relating to CML stem cell biology, and discuss strategies to effectively target this difficult-to-kill population that might one day result in a cure for CML.

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Introduction

The theory of oncogene addiction [1] posits that some cancers that contain multiple genetic, epigenetic, and chromosomal abnormalities are dependent on or "addicted" to one or a few genes for both maintenance of the malignant phenotype and cell survival. Thus, reversal of only one or a few of these abnormalities can inhibit cancer cell growth and in some cases translate to improved survival rates [2]. This phenomenon is perhaps best exemplified by chronic phase CML (CML-CP). The hallmark of CML, the Philadelphia chromosome (Ph), was first identified in 1960 as a shortened chromosome 22 and named after the city in which it was discovered [3]. Seminal work by Janet Rowley led to the realization in 1973 that the Ph was a consequence of a reciprocal translocation between the long arms of chromosomes 9 and 22 (t(9;22)(q34;q11)) [4]. It was subsequently recognized in the 1980s that this translocation resulted in the formation of a fusion gene, *BCR-ABL1* [5-7], which encodes a constitutively active 210 kilodalton (kd) non-receptor tyrosine kinase (TK), Bcr-Abl [8], that is able to transform hematopoietic cell lines *in vitro* [9-11] and is both necessary and sufficient to cause CML-CP *in vivo* [12,13]. The Bcr-Abl fusion oncoprotein is present in 95% of cases of CML, and nearly all patients with typical CML-CP express the 210kd (major breakpoint) protein [14]. Bcr-Abl signals downstream to and interacts with a myriad of proteins that are responsible for inducing and sustaining the malignant phenotype; this is the subject of several excellent reviews [15,16], and is discussed extensively in other chapters of this book. Untreated, CML-CP progresses inexorably with or without an intervening accelerated phase (AP) in about five years to an acute leukemia-like blast crisis (BC) [17,18] that is characterized by "clonal evolution" with loss of addiction to Bcr-Abl, differentiation arrest, telomere shortening, acquisition of a "mutator phenotype", deficiencies in deoxyribonucleic acid (DNA) repair, failure of genome surveillance, loss of tumor-suppressor gene function [19] and, even today, a very poor prognosis [20].

The discovery of imatinib (Gleevec®, Novartis), a specific inhibitor of the Bcr-Abl TK, marked the beginning of a new era in cancer therapeutics, that of molecularly targeted therapy [21]. The drug moved swiftly through phase I and II trials, demonstrating remarkable efficacy and safety in patients with CML-CP who had failed therapy with interferon alfa (IFN-α) [22,23], as well as substantial activity in patients with CML-BC or Ph[+] acute lymphoblastic leukemia (ALL) [24]. The dramatic results of the pivotal International Randomized Study of Interferon and STI571 (IRIS) firmly established imatinib as the standard of care for newly diagnosed patients with CML-CP [25-27]. Amidst this impressive demonstration of efficacy, however, came evidence of primary and secondary resistance to imatinib; the latter caused most often by mutations in the Bcr-Abl kinase domain [28-31]. Structural biology studies elucidating the crystal structure of imatinib bound to the kinase [32] paved the way for the rapid development of the imatinib congener nilotinib (Tasigna®, Novartis) [33], which provides a better topographical fit, and the dual Abl/Src inhibitor dasatinib (Sprycel®, Bristol-Myers Squibb) [34] which, unlike imatinib, is able to bind to the catalytically active conformation of Bcr-Abl [35]. These more potent second-generation Bcr-Abl TKIs initially received regulatory approval for the treatment of imatinib-resistant or –intolerant patients (reviewed in ref. [36]), but have since moved into the frontline setting based on demonstration of superiority to imatinib in large head-to-head phase III randomized controlled trials (RCTs) in newly diagnosed patients with CML-CP [37,38]. Between them, nilotinib and dasatinib are

able to inhibit all known Bcr-Abl mutants with the notable exception of the T315I "gatekeeper" mutation [39,40]. The search for a clinically relevant Bcr-Abl TKI with activity against the recalcitrant T315I mutant ended with the Food and Drug Administration (FDA) approval of ponatinib (Iclusig™, Ariad), a rationally designed pan-Bcr-Abl inhibitor with robust evidence of efficacy against Bcr-Abl^{T315I} in both preclinical [41] and clinical [42] settings. However, the FDA has since asked Ariad to suspend marketing and sales of ponatinib owing to safety concerns relating to arterial thrombotic events. The development of compound mutations in Bcr-Abl with sequential TKI therapy has also been a significant concern [43,44], and could potentially be minimized by combining inhibitors [40,45,46].

Why Is a Cure Necessary?

The treatment of CML must be ranked as one of the great medical success stories of the past 30 years [47]. Indeed, the life expectancy of patients with CML-CP in the imatinib era might be approaching that of the general population [48]. How, then, is cure a justifiable goal? There are several reasons why. For one, the current pricing of CML drugs in the US is unsustainable [49]. This is readily apparent when one considers that the survival of patients in CML-CP may not be statistically significantly different from that of the general population [50], since each of these drugs is priced at around $100,000 per patient per year [49]. Other important considerations include side effects, compliance issues, concerns relating to fertility and pregnancy, and simply the inconvenience of taking a pill daily for the rest of one's life [51,52]. Eight-year follow-up data from the IRIS were recently presented [53]. At the 8-year data cut-off, 304 (55%) patients originally assigned to imatinib remained on study treatment, and 45% had discontinued imatinib due to adverse events (AEs) (6%), unsatisfactory therapeutic outcome (16%), HSCT (3%), death (3%) or other reasons (17% for withdrawal or lack of renewal of consent and miscellaneous). Of 204 newly diagnosed CML-CP patients treated with imatinib at a single institution in the United Kingdom, 25% had discontinued the drug [54] because of an unsatisfactory response and/or toxicity by 5 years. Indeed, there is data to suggest that the efficacy of imatinib in the "real world" setting is lower than that seen in clinical trials [55]. At 3 years (minimum) of follow-up of the Evaluating Nilotinib Efficacy and Safety Clinical Trial in Newly Diagnosed Patients (ENESTnd), 29.1%, 26.3% and 38.2% of patients in the nilotinib 300 mg twice-daily, nilotinib 400 mg twice-daily and imatinib arms, respectively, discontinued core treatment by the time of data cutoff [56]. Similarly, in the phase III RCT DASISION (Dasatinib versus Imatinib Study In Treatment-Naive CML patients), 23% and 25% of patients in the dasatinib 100 mg daily and imatinib 400 mg daily arms, respectively, had discontinued study treatment by 24 months because of disease progression, treatment failure, AEs, death, withdrawal of consent, non-adherence, pregnancy, loss to follow-up, patient preference or other reasons [57]. Finally, bosutinib (Bosulif®, Pfizer), another second-generation TKI of both Abl and Src was associated with an unexpectedly high discontinuation rate (19%) due to AEs in the phase III Bosutinib Efficacy and Safety in Newly Diagnosed Chronic Myeloid Leukemia (BELA) trial [58]. Long-term therapy with Bcr-Abl TKIs cannot, therefore, be the answer for all patients with CML-CP.

CML Stem Cells: Can TKIS Alone Cure CML?

Stem cells have three distinctive properties: self-renewal, the capability to develop into multiple lineages, and the potential to proliferate extensively, and it is the combination of these three properties that makes stem cells unique [59]. Perhaps the most important property of stem cells is that of self-renewal [60]. Malignant cells that have subverted these properties of normal stem cells are referred to as cancer stem cells, have been described in a range of hematologic and solid malignancies, and particularly well-studied in the former [59,60]. A cancer stem cell may arise by mutation in a normal stem cell, or when a progenitor cell acquires a mutation that causes it to regain the property of self-renewal [59]. Both these phenomena are beautifully illustrated by CML. The earliest evidence of the existence of CML stem cells came in the 1960s and 1970s from observations in recipients of leukocyte transfusions from donors with CML [61,62]. It was later confirmed by genetic marking studies that Ph^+ cells present in autografts from CML patients contributed to relapse after autologous HSCT [63]. Engraftment of nonobese diabetic/severe combined immunodefient (NOD/SCID) mice by primitive normal and leukemic hematopoietic cells from the peripheral blood and bone marrow (BM) of patients with CML-CP was subsequently shown [64,65]. Tessa Holyoake and colleagues first reported the isolation of a deeply but reversibly quiescent subpopulation of leukemic cells in patients with CML with both *in vitro* and *in vivo* stem cell properties [66]. These primitive, quiescent $CD34^+$ CML cells are able to reversibly activate autocrine production and action (through signal transducer and activator of transcription 5 (STAT5) signaling) of interleukin-3 (IL-3) and granulocyte colony stimulating factor (G-CSF) in order to sustain autonomous proliferation *in vitro* [67,68]. Detection of the Ph and of the *BCR-ABL1* gene rearrangement in cells of the myeloid, erythroid, megakaryocytic and B-lymphoid lineages indicates that the initial transformation in CML occurred in a cell with multi-lineage differentiation potential, such as a hematopoietic stem cell (HSC) [69]. Like their normal counterparts, CML stem cells are lineage$^-$$CD34^+$$CD38^-$ cells with long term engraftment potential [70]. The stem cell in CML-CP is believed to be the Ph^+ HSC with intrinsic self-renewal capacity, consistent with the observation that Bcr-Abl fails to confer self-renewal capacity to committed progenitor cells [71]. *JunB* inactivation at the HSC level appears to play a critical role in pathogenesis [72,73]. Upon transformation to CML-BC, however, granulocyte-macrophage progenitor (GMP) cells acquire self-renewal capacity via activation of the β-catenin pathway [74], possibly through glycogen synthase kinase-3-beta (GSK3β) mis-splicing [75] and function as leukemic stem cells (LSCs) in that setting [76].

A wealth of evidence, both preclinical and clinical, supports the notion that Bcr-Abl TKIs do not eliminate CML stem cells, widely believed to be the cause of disease persistence. For example, residual Bcr-Abl$^+$ $CD34^+$ progenitor cells were detected in all subjects in a study of patients with CML-CP on imatinib who had attained a complete cytogenetic response (CCyR) [77]. After a median of 19 months of follow-up, of 333 imatinib-treated patients in the IRIS in a CCyR, only 4% had attained a complete molecular response (CMR) at any point by stringent criteria, although an additional 6% had undetectable levels of *Bcr-Abl* transcripts, but the quality of the ribonucleic acid (RNA) sample was not adequate to ensure that the lower level of sensitivity was more than 4.5 log [78]. In an IRIS sub-study of 53 imatinib-treated patients, undetectable Bcr-Abl was achieved in 18 patients and none of these 18 had lost major molecular response (MMR) after a median follow-up of 33 months [79]. However,

persistence of Bcr-Abl[+] LSCs has been documented in patients with sustained undetectable molecular (minimal) residual disease (UMRD) induced by IFNα or TKI therapy [80], and in those treated with imatinib for at least four years while in CCyR and CMR [81]. In the French Stop Imatinib (STIM) study, imatinib treatment was discontinued in 100 patients with CML in CMR (defined as a >5-log reduction in Bcr-Abl and Abl levels and undetectable transcripts on quantitative reverse transcriptase polymerase chain reaction (RT-PCR, Q-PCR)) of at least 2 years' duration [82]. 42 (61%) of 69 patients with at least 12 months of follow-up relapsed; all of these individuals responded to reintroduction of imatinib. In the very recently reported Australian TWISTER study, 40 patients with CML-CP and UMRD (by conventional Q-PCR) for at least 2 years on imatinib discontinued the drug [83]. 21 of these patients had previously received IFN. At a median follow-up of 42 months, 22 (55%) patients had met the study definition of molecular relapse (loss of MMR at any time point, or two consecutive positive samples of any value). Most relapses occurred within 4 months of stopping imatinib, and no relapses beyond 27 months were seen. Highly sensitive patient-specific Bcr-Abl DNA PCR showed persistence of the original CML clone in all patients with stable UMRD, even several years after imatinib withdrawal. All patients who relapsed remained sensitive to imatinib re-treatment, no patient developed a kinase domain mutation, there were no deaths, and no patient progressed to advanced phase [83].

Preclinical studies have shown that neither imatinib [84] nor any of the second generation TKIs [85-88] is able to eradicate (induce apoptosis in) the quiescent stem cell fraction, notwithstanding their potent anti-proliferative effects on CML cells [89]. Underlying these observations is the key concept that although Bcr-Abl is a relevant target in CML stem cells, the latter are not *addicted* to Bcr-Abl [90,91]; inhibition of Bcr-Abl alone, therefore, is unlikely to eliminate CML stem cells. In fact, Bcr-Abl TKIs may promote survival of quiescent CML cells by restoring CXCR4 expression and causing them to migrate to BM microenvironment niches [92]. Mathematical models of the dynamics of molecular responses to imatinib in CML-CP also suggest that the drug is a potent inhibitor of the production of differentiated leukemic cells, but does not deplete LSCs: successful therapy leads to a biphasic exponential decline of leukemic cells, with an initial rapid downslope (α) representing the turnover rate of proliferating differentiated leukemic cells, and a much slower second downslope (β) representing the turnover rate of quiescent leukemic progenitors [93,94]. Other models, however, propose a triphasic exponential decline, suggesting that the last phase might represent effects of imatinib on LSCs [95].

Targeting Bcr-Abl for an "Operational Cure" of CML

Although the inability of Bcr-Abl TKIs in and of themselves to induce apoptosis in CML stem cells is well-established, whether the latter really represent the true enemy has been questioned [51,96]. The theory of "operational cure" postulates that LSCs, similar to their normal counterparts, may alternate between a cycling mode, where they may express Bcr-Abl oncoprotein and be susceptible to the acquisition of additional mutations, and a quiescent mode, where they may express little or no Bcr-Abl oncoprotein, cannot acquire additional mutations and are unaffected by imatinib [97]. Thus, a patient who starts treatment early in

the natural history of CML, and who responds to imatinib clinically, may not have had the opportunity to acquire additional mutations in LSCs. In this case, the persistence, long-term, of quiescent "non-mutated" LSCs despite imatinib treatment may not really matter [97]. That Bcr-Abl transcript levels decline over time on imatinib therapy and increasing numbers of patients achieve CMR or UMRD is well-known [26,54,79]. Additionally, as discussed above, some patients remain transcript negative even after discontinuation of imatinib [82,83]. Persistence of genomic *BCR-ABL1* DNA can be demonstrated in such individuals by highly sensitive patient-specific nested q-PCR; these levels remain stable in patients who sustain CMR, whereas they increase in those who lose CMR after imatinib cessation [98]. Intriguingly, there is some data to suggest that eventual eradication of LSCs may actually be possible on TKI therapy and that TKI discontinuation may work best in patients whose Bcr-Abl transcript levels show a steep β slope [99]. Furthermore, it is possible that persistent and gradually declining Bcr-Abl transcripts in some patients may, in fact, reflect the presence of long-lasting memory B or T cells originally derived from a Bcr-Abl-containing LSC, and not quiescent LSCs that might serve as a reservoir for future disease relapse [52]. Indeed, achievement of cure may not require eradication of the last Bcr-Abl$^+$ cell [100], since these are detectable in the blood of a proportion of normal individuals [101,102]. In light of these considerations, and the ability of dasatinib and nilotinib to induce faster and deeper responses compared to imatinib in newly diagnosed CML-CP patients as well as a lower risk of transformation to AP or BC [56,103], there is interest in discontinuation trials of these more potent agents (e.g., NCT01744665, NCT01627132, NCT01850004). Ponatinib, the only FDA-approved TKI that retains significant activity against Bcr-Abl^{T315I}, was being compared to imatinib in the frontline setting in patients with CML-CP in the Evaluation of Ponatinib versus Imatinib in CML (EPIC) trial (NCT01650805), but this has recently been terminated.

Conformation-specific binding to Bcr-Abl plays a key role in determining TKI resistance in CML [104]. *"Switch-control inhibitors"* bind in a non-adenosine triphosphate (ATP)-competitive fashion to residues (E282, R386) the Abl protein uses to switch between inactive and active conformations [105]. The lead clinical candidate, DCC-2036 (rebastinib), an orally active TKI, potently inhibits Abl by inducing and stabilizing an inactive, inhibitor-bound conformation, and retains efficacy against most clinically relevant CML-resistance mutants, including T315I, both *in vitro* and *in vivo*, although ''P-loop'' mutants (E255V,E255K) maybe less sensitive. Sustained inhibition of Bcr-Abl and downstream pathways was observed in patients with refractory CML enrolled on a phase I clinical trial of this agent [105].

Aurora kinases are serine/threonine kinases that play critical roles in the regulation of mitosis (reviewed in ref. [106]). Small-molecule *aurora kinase inhibitors* (AKIs) constitute an important new class of anti-cancer agents, with particular promise in the treatment of hematologic malignancies, in part because some of these molecules also inhibit other relevant targets, such as Janus kinases (JAK), Bcr-Abl and fms-like tyrosine kinase 3 (FLT3) (reviewed in refs. [107,108]). Furthermore, Bcr-Abl may activate aurora kinases A and B in CML cells via Akt signaling [109]. In the context of CML, most of the excitement surrounding these drugs, particularly prior to the availability of ponatinib, was to do with their ability to inhibit the T315I mutant, although development of some of these agents (e.g., MK-0457 (VX-680), KW-2449) has since been discontinued. However, others (e.g., danusertib (formerly PHA-739358, reviewed in ref. [110]), AT-9283, reviewed in ref. [111])

are currently in phase I and II clinical trials both in hematologic malignancies and solid tumors.

As activation of the Src-family kinase Lyn can serve as a mechanism of resistance to Bcr-Abl TKIs [112], a dual Bcr-Abl/Lyn kinase inhibitor (bafetinib, CytRx) was developed and phase I and II trials completed both in hematologic malignancies and solid tumors [113]; however, this agent is not active against Bcr-Abl^{T315I}. In contrast, HG-7-85-01 is a novel small-molecule type II inhibitor of wild-type and gatekeeper mutants of Bcr-Abl (T315I), c-Kit, Src and platelet derived growth factor receptor alpha (PDGFRα) with an unprecedented tolerance for a range of gatekeeper side chains, without becoming a promiscuous kinase inhibitor [114].

ON012380 and ON044580 are *substrate-competitive* inhibitors of Bcr-Abl that are unaffected by mutations in the ATP-binding site that confer resistance to imatinib [115,116]. ON012380 synergizes with imatinib against wild-type Bcr-Abl and inhibits all known imatinib-resistant mutants both *in vitro* and in xenograft mouse models [115]. ON044580 inhibits JAK2 (including JAK2^{V617F}), which shares a substrate, STAT5, with Bcr-Abl, in addition to inhibiting both wild-type and mutant Bcr-Abl (including Bcr-Abl^{T315I}), and induces apoptosis of imatinib-resistant patient-derived CML cells [116,117].

GNF-2 and its analog, GNF-5 are selective *allosteric* inhibitors [118] of Bcr-Abl that bind to the myristate-binding pocket of Abl and, when combined with the ATP-competitive inhibitors imatinib and nilotinib, suppress the emergence of resistance mutations and display additive inhibitory activity against the T315I mutant *in vitro* as well as *in vivo*, although neither GNF-2 nor GNF-5 individually inhibit Bcr-Abl^{T315I} [119]. An intra-molecular interaction between the SH2 and kinase domains in Bcr-Abl is both necessary and sufficient for high catalytic activity of the enzyme [120]. Disruption of this interface has been shown to inhibit downstream events critical for Bcr-Abl signaling, completely abolish leukemia formation in mice, and increase sensitivity of imatinib-resistant Bcr-Abl mutants to TKIs [120]. An engineered Abl SH2-binding fibronectin type III monobody inhibited Bcr-Abl kinase activity *in vitro* and induced apoptosis in primary CML cells [120].

A number of pharmacokinetic and host factors (such as lack of adherence [121,122]) have been reported to play significant roles in conferring resistance to TKIs, primarily imatinib. For example, enhanced binding of imatinib to plasma alpha-1-acid glycoprotein [123,124] and reduced metabolism of imatinib to its active form by the cytochrome P450 enzyme CYP3A [125] could negatively impact levels of the biologically active drug, and thus diminish the extent of Bcr-Abl inhibition. Drug influx (via the hOCT-1 transporter [126-128]) and efflux (via the P-glycoprotein, also known as MDR-1, or ABCG2 [129-131]) mechanisms have also been implicated. The use of higher doses of imatinib has been advocated [132] in order to mitigate some of these concerns. Furthermore, the contribution of some of these mechanisms to the development of imatinib resistance is questionable [133,134], and their importance at a primitive CD34^{+} CML cell level even less certain [135]. Importantly, the issue of reduced intracellular drug concentrations owing to decreased expression of hOCT-1 appears not to apply to dasatinib [136] or nilotinib [137]. A more detailed discussion of these issues is beyond the scope of this chapter, and appears elsewhere in this volume.

Targeting CML Stem Cells by Bcr-Abl-Independent and "Synthetic Lethal" Strategies

A lot of research in recent years has focused on identifying survival pathways in the quiescent CML stem cells, which are believed to serve as a reservoir of disease essentially unaffected by TKI therapy [138], which primarily targets proliferating cells [89]. Non-proliferating CD34$^+$ CML progenitor cells have been shown to be remarkably resistant to the induction of apoptosis [139]. It has been estimated that non-proliferating CD34$^+$ CML cells make up approximately 0.5% of the total CD34$^+$ compartment [16] and that the average CML patient harbors 5 x 10^7 Ph$^+$ quiescent CD34$^+$ cells, which most authorities consider to represent the major hurdle to a cure of CML [140]. CML stem cells express high levels of native Bcr-Abl, and kinase mutations are uncommon [16,141]. High levels of expression of Bcr-Abl also correlate with disease progression (to BC) in CML, and with the development of imatinib resistance, in part through the more rapid acquisition of mutations, in that setting [142]. Although Bcr-Abl modulates a variety of intracellular signaling pathways in CML cells, Bcr-Abl-independent resistance [143] is an important concept, particularly in the context of LSCs. Hu *et al.* showed that targeting multiple kinase pathways in leukemic progenitors and LSCs is essential for improved treatment of Ph$^+$ leukemia in mice [144]. The phenomenon of alternate pathway activation in CML stem cells was also elegantly demonstrated recently by the generation of induced pluripotent stem cells (iPSCs) from primary CML patient samples [145]. The CML-iPSCs were resistant to imatinib despite consistently expressing Bcr-Abl. Furthermore, there was unequivocal evidence of kinase inhibition by imatinib in these cells, as evidenced by significantly decreased phosphorylation of STAT5 and CRKL (v-crk sarcoma virus CT10 oncogene homolog (avian)-like), an adaptor molecule downstream of Bcr-Abl. However, the phosphorylation status of other essential mediators of survival, viz., extracellular signal-regulated kinase 1/2 (ERK1/2), Akt and Jun-N-terminal kinase (JNK) was unaffected. Hematopoietic cells derived from these CML-iPSCs remained susceptible to imatinib, while the most primitive CD34$^+$38$^-$90$^+$45$^+$ cells were resistant to imatinib [145]. Because Bcr-Abl signaling remains active in LSCs, a major thrust of investigation in this area has been to develop "synthetic lethal" [146] combinations of Bcr-Abl TKIs with agents that selectively interrupt pathways critical to the survival of LSCs, while sparing normal HSCs; these are discussed in detail below. A variety of agents, representing different drug classes, such as DNA methyltransferase inhibitors (DNMTIs), MEK inhibitors, CDK inhibitors and tumor necrosis factor-related apoptosis inducing ligand (TRAIL) are active in CML and may synergize with TKIs; these are not considered further in this chapter, and have been reviewed [147].

Inhibition of Autophagy

Autophagy ("self-eating") is a homeostatic process that takes place in all eukaryotic cells and involves the sequestration of cytoplasmic components in double-membraned autophagosomes that subsequently fuse with lysosomes, where their contents are degraded (reviewed in ref. [148]). Autophagy acts as a survival mechanism under conditions of stress, maintaining cellular integrity by regenerating metabolic precursors and clearing subcellular

debris, but is also widely implicated in cancer pathophysiology, where it can be cytoprotective. Bellodi *et al.* demonstrated that imatinib induces autophagy in CML cells, and that pharmacologic inhibition of autophagy can enhance imatinib-induced cell death in CML cell lines and primary CML cells [149]. Significantly, the combination of a TKI with inhibitors of autophagy resulted in near complete elimination of phenotypically and functionally defined CML stem cells [149]. The anti-malarial drug chloroquine inhibits autophagy via induction of lysosomal stress, provoking p53-dependent cell death [150]. CHOICES (chloroquine and imatinib combination to eliminate stem cells) is an ongoing phase II clinical trial (NCT01227135) that is investigating the combination of hydroxychloroquine and imatinib in CML-CP patients treated for at least one year with imatinib who have achieved at least a major cytogenetic response (MCyR) and continue to have molecular evidence of residual disease.

Wnt/β-Catenin Pathway Inhibition

The canonical Wnt pathway plays a critical role in regulating both proliferation and self-renewal in normal HSCs, a function that is subverted in LSCs, and β-catenin is a key effector molecule of this pathway, although dispensable in fully developed adult HSCs (reviewed in ref. [151]). Gene expression profiling studies suggest that the Wnt/β-catenin pathway is deregulated as CML-CP progresses to more advanced phases of the disease [152]. Zhao *et al.* showed that β-catenin-deficient mice formed HSCs that were deficient in long-term growth and maintenance, and that these mice had a profound decrease in their ability to develop Bcr-Abl-induced CML [153]. β-catenin has also been shown to be essential for the survival of imatinib-resistant LSCs in mice with Bcr-Abl-induced CML [154]. Using a conditional mouse model, Heidel *et al.* showed that deletion of β-catenin after CML initiation did not lead to a significant increase in survival; however, deletion of β-catenin synergized with imatinib to delay disease recurrence after imatinib discontinuation and to abrogate CML stem cells [155]. These effects could be mimicked by pharmacologic inhibition of the β-catenin pathway through modulation of prostaglandin signaling using the cyclooxygenase inhibitor indomethacin. AV65 is a novel Wnt/β-catenin signaling inhibitor that reduces the expression of β-catenin in CML cells, resulting in the induction of apoptosis, including in cells harboring Bcr-Abl[T315I] [156]. Furthermore, AV65 inhibited the proliferation of hypoxia-adapted primitive CML cells that overexpress β-catenin, and the combination of AV65 with imatinib synergistically inhibited the proliferation of CML cells [156]. Other components within the Wnt signaling cascade that could potentially be targeted within LSCs, while sparing normal HSCs, include CD27 [157] and GSK3β [158]. An RNA interference-based synthetic lethality screen with imatinib identified numerous components of a Wnt/Ca^{2+}/NFAT signaling pathway [159]. Antagonism of this pathway led to impaired NFAT activity, decreased cytokine production, and enhanced sensitivity to Bcr-Abl inhibition [159]. Furthermore, NFAT inhibition with cyclosporin A facilitated leukemia cell elimination by dasatinib and markedly improved survival in a mouse model of Ph[+] ALL [159].

Hedgehog Pathway Inhibition

The Hedgehog (Hh) family of secreted proteins controls cell proliferation, differentiation and tissue patterning in a dose-dependent manner, playing an essential role in numerous processes during embryonic development; however, uncontrolled activation of this pathway (e.g., due to mutations) can result in specific types of cancer (reviewed in ref. [160]). Binding of any of the three mammalian Hh ligands (Sonic, Desert or Indian Hh) to the transmembrane protein receptor Patched (Ptch) releases another transmembrane protein, Smoothened (Smo), from inhibition by Ptch. Once activated, Smo initiates a signaling cascade that leads to the activation of the Gli transcription factors, resulting in the transcription of Hh target genes that are normally repressed in the absence of ligand binding [160]. Dierks *et al.* showed that Hh signaling is activated in Bcr-Abl[+] LSCs through up-regulation of Smo [161]. The development of re-transplantable Bcr-Abl[+] leukemias was abolished in the absence of Smo expression, and pharmacological Smo inhibition reduced LSCs *in vivo* and enhanced time to relapse after the end of treatment [161]. Zhao and co-workers demonstrated that Hh signaling is essential for maintenance of CML stem cells, showing that loss of Smo impaired HSC renewal, depleted CML stem cells and decreased induction of CML by the Bcr-Abl oncoprotein [162]. Pharmacological inhibition of Hh signaling impaired not only the propagation of CML driven by wild-type Bcr-Abl, but also the growth of imatinib-resistant mouse and human CML [162]. The FDA approval of a Smo inhibitor, vismodegib (Erivedge™, Genentech) for patients with advanced basal cell carcinoma [163] makes this a particularly exciting area of clinical investigation, especially since Hh signaling is dispensable for adult HSC function [164]. LDE225 (Novartis) is an orally bioavailable Smo antagonist that is currently being studied in combination with nilotinib in a phase I trial (NCT01456676) in patients with CML-CP or –AP who have failed therapy with other Bcr-Abl TKIs.

ALOX5 Inhibition

Chen and colleagues have demonstrated that arachidonate 5-lipoxygenase (5-LO) (ALOX5) is a critical regulator for CML stem cells [165]. Alox5 deficiency caused impairment of the function of LSCs but not normal HSCs via effects on differentiation, cell division and survival of long-term LSCs (LT-LSCs), resulting in depletion of LSCs and a failure of CML development in mice [165]. Treatment of mouse xenograft models of CML with a 5-LO inhibitor (zileuton) also impaired the function of LSCs similarly by affecting LT-LSCs, and prolonged survival [165]. Zileuton (Zyflo™, Cornerstone) is a 5-LO inhibitor that is approved by the FDA for the prevention and treatment of asthma [166]. Based on preclinical evidence of additive effects *in vivo,* a phase I clinical trial of zileuton in combination with imatinib in patients with CML-CP already on imatinib (NCT01130688) was initiated, but was recently terminated. *Scd1* (stearoyl-Co-A-desaturase) is a gene that plays a tumor-suppressive role in LSCs (through effects on p53, B-cell lymphoma-2 (Bcl-2) and phosphatase and tensin homolog (PTEN)) with no effect on normal HSC function, and is down-regulated in CML stem cells [167]. Deletion of *Scd1* causes acceleration of CML development and conversely overexpression of *Scd1* delays CML development [167]. Interestingly, induction of *Scd1* expression by a peroxisome proliferator-activated receptor gamma (PPARγ) agonist suppresses LSCs and delays CML development in a Bcr-Abl-

induced CML mouse model, suggesting a new anti-LSC strategy through enhancing *Scd1* activity [167].

Histone Deacetylase Inhibitors (HDACIs)

There is evidence suggesting that HDACIs, an important class of anti-cancer agents with diverse mechanisms of action (reviewed in ref. [168]), kill proliferating and non-proliferating tumor cells with equal efficacy [169]. Among the pleiotropic actions of HDACIs is the ability, via inhibition of HDAC6, to disrupt the chaperone function of heat shock protein 90 (Hsp90) [170], of which Bcr-Abl is a client, leading to proteasomal degradation of the latter [171]. Thus, the investigational HDACI dacinostat (Novartis) exhibited significant activity against imatinib-sensitive and –resistant Bcr-Abl[+] myeloid leukemia cell lines and primary CML-BC cells, both *in vitro* and *in vivo*, and synergized with imatinib [172,173]. The mechanism involved acetylation of Hsp90 and inhibition of its chaperone function, thereby promoting the proteasomal degradation of Bcr-Abl [173]. Synergistic interactions between the HDACI vorinostat (Zolinza®, Merck) and imatinib or dasatinib against CML cell lines and primary CML cells, including those expressing the imatinib-resistant Bcr-Abl mutants E255K and T315I [174,175], and those with overexpression of Bcr-Abl [176], have been described. Lowering of Bcr-Abl levels and induction of apoptosis in human CML cell lines and primary CML cells, including those expressing Bcr-Abl[T315I], were observed with the investigational HDACI panobinostat (Novartis) and synergism with nilotinib was demonstrated [177]. Zhang *et al.* have shown that HDACIs combined with imatinib can effectively induce apoptosis in quiescent CML progenitors resistant to imatinib alone, and eliminate CML stem cells capable of engrafting immunodeficient mice [178]. *In vivo* administration of HDACIs with imatinib markedly diminished LSCs in a transgenic mouse model of CML [178]. These findings form the basis of a recently completed phase I trial (NCT00686218) of panobinostat in combination with imatinib in patients with CML-CP treated with imatinib for at least a year and in MCyR (or better) with molecular evidence of disease persistence.

Hsp90 Inhibition

As noted above in the context of HDACIs, p210 Bcr-Abl is complexed with the chaperone protein Hsp90 (reviewed in ref. [179]) in CML cells, and disruption of this association by Hsp90 inhibitors such as geldanamycin leads to proteasomal degradation of p210 Bcr-Abl [171]; similar considerations apply to v-Src. Significantly, these oncogenic kinases are more susceptible to geldanamycin-induced degradation than their normal cellular counterparts, c-Abl and c-Src [171]. Geldanamycin and its analog 17-allyl-amino-17-demethoxygeldanamycin (17-AAG) lower Bcr-Abl levels and induce apoptosis and differentiation of human Bcr-Abl^{+} cells [180], including those bearing the imatinib-resistant mutants T315I and E255K [181]. Additionally, 17-AAG may inhibit the function of P-glycoprotein [182]. Most significantly, this strategy (using IPI-504, an orally administered Hsp90 inhibitor) has been shown to be effective *in vivo* in a murine model of CML against both wild type Bcr-Abl and Bcr-Abl[T315I], to suppress CML stem cells, and to synergize with

imatinib [183]. Combination of the Hsp90 inhibitor NVP-AUY922 (Novartis) with nilotinib was more effective at reducing the outgrowth of resistant cell clones in a random Bcr-Abl mutagenesis screen than either drug individually, and targeted both wild-type and mutant forms of Bcr-Abl (including Bcr-Abl^{T315I}) *in vitro* and *in vivo* [184]. As an example of a non-TKI-based combination strategy, co-treatment with 17-AAG and panobinostat synergistically induced apoptosis of CML cell lines, imatinib-refractory leukemia cells expressing Bcr-AblT^{315I} and imatinib-resistant primary CML-BC cells [185]. Ganestespib (Synta) is an Hsp90 inhibitor in phase I/II testing in patients with various hematologic malignancies including CML (NCT00858572, NCT00964873).

Targeting PML

Normally a tumor suppressor [186], the promyelocytic leukemia (PML) protein has been demonstrated to have a critical role in HSC maintenance, and in particular, to be indispensable for the maintenance of CML stem cells [187]. Arsenic trioxide (Trisenox™, Teva Oncology), widely used in the treatment of acute promyelocytic leukemia (APL) [188], degrades the PML protein, and has been shown to decrease the number of quiescent LSCs *in vitro* to a significantly greater extent than normal HSCs [187]. Ito *et al.* found that administration of arsenic trioxide in combination with cytarabine in mice transplanted with cells transduced with Bcr-Abl resulted in apoptosis in the LSC compartment [187]. Arsenic trioxide induced apoptosis in primary human CML cells, an effect that was potentiated by the addition of cytarabine, resulting in significantly more apoptosis than induced by cytarabine alone [187]. Others suggest that the degradation of Bcr-Abl oncoprotein by arsenic trioxide involves an autophagic mechanism [189]. Synergism between imatinib and arsenic sulfide has been shown in a Bcr-Abl$^+$ mouse model of human CML [190]. A phase II trial of arsenic trioxide plus imatinib in patients with CML-CP who fail therapy with imatinib alone has been completed (NCT00250042), and a phase I trial (NCT01397734) evaluating the combination of arsenic trioxide with either imatinib, dasatinib or nilotinib in patients with CML-CP on a stable dose of their TKI and cytogenetic or molecular evidence of persistent disease is currently recruiting participants.

Targeting the CXCXR4/CXCL12 Axis

The chemokine receptor CXCR4 and its ligand CXCL12 are essential for the control of proliferation in HSCs (resulting in their quiescence) and in retaining HSCs within their proper BM niche [191]. Bcr-Abl signaling down-regulates CXCR4, leading to defective adhesion of CML cells to the BM stroma [92]. Imatinib, by blocking Bcr-Abl kinase activity, up-regulates CXCR4 and has been shown to enhance migration of CML cells into protective BM micoenvironmental niches [192], thus promoting stroma-mediated chemo-resistance of CML progenitor cells [92]. Conversely, decreased CXCL12 expression in CML BM, a consequence of increased G-CSF production by the leukemic cells, also may reduce homing and retention of CML stem cells in the BM [193]. Inhibition of CXCR4 signaling by plerixafor (Mozobil®, Sanofi), used widely in conjunction with G-CSF for hematopoietic progenitor cell mobilization in the autologous stem cell transplantation setting [194], disrupts this interaction

of CML cells with the BM microenvironment and sensitizes them to killing by imatinib [192] and nilotinib [195].

PI3K/Akt/mTOR Pathway Inhibition

The phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is a major cellular survival pathway that is frequently dysregulated in cancer [196]. Interestingly, PI3K and Akt are essential for Hh signaling, both in embryonic development and in Hh-dependent tumors [197]. Activation of this pathway is an important mechanism of Bcr-Abl-induced leukemogenesis [15,16] and represents a compensatory response to imatinib treatment [198]. The mTOR inhibitor rapamycin synergizes with imatinib against Bcr-Abl-transformed myeloid and lymphoid cells and increases survival in a murine CML model, and the combination also inhibits imatinib-resistant mutants of Bcr-Abl [199]. The rapamycin derivative everolimus (Afinitor®, Novartis) has been shown to block the late reactivation of mTOR observed in response to imatinib therapy that results from the induction of an mTOR-PI3K feedback loop that drives the retrograde activation of Akt [200]. The dual mTORC2/mTORC1 inhibitor, OSI-027 (OSI), induces potent suppressive effects on primitive leukemic progenitors from CML patients and generates anti-leukemic responses in cells expressing Bcr-Abl^{T315I} [201]. Finally, the dual PI3K/mTOR inhibitor PI-103 was more effective than rapamycin at suppressing the proliferation of mouse precursor B-cell (pre-B-) ALL and human CD19$^+$CD34$^+$ Ph$^+$ ALL leukemia cells treated with imatinib [202]. A phase I/II clinical trial (NCT00093639) of imatinib and everolimus in patients with CML-CP on a stable dose of imatinib who have achieved and maintained a hematological response, but not a CCyR, to imatinib has recently been completed, and another (NCT01188889) for those who have achieved a CCyR but have molecular evidence of disease persistence is planned.

FOXO and Inhibition of Bcl-6 and TGF-β Signaling

Bcr-Abl suppresses the forkhead O transcription factors (FOXO) via activation of the PI3K/Akt/mTOR pathway [203], supporting the proliferation or inhibiting the apoptosis of CML cells [204,205]. The FOXO are also essential for the maintenance of normal HSCs [206,207]. In a seminal study, Naka and colleagues found that the FOXO play a critical role in the maintenance of leukemia-initiating cells (LICs) in CML [208]. Furthermore, they showed that treatment of human CML LICs with a transforming growth factor beta (TGF-β) inhibitor impaired their colony-forming ability *in vitro* [208]. Subsequently, Hurtz *et al.* identified the *BCL6* proto-oncogene as a critical effector downstream of FOXO in self-renewal signaling of CML-initiating cells, acting via repression of Arf and p53 [209]. Importantly, peptide inhibition of Bcl-6 in human CML cells compromised colony formation and leukemia initiation in transplantation assays and selectively eradicated CD34$^+$CD38$^-$ LICs in patient-derived CML samples [209]. In another seminal paper from the same group, Duy *et al.* reported that Bcl-6 is a central component of a protective feedback signaling pathway in Ph$^+$ ALL cells triggered by TKI treatment, demonstrating that targeted inhibition of Bcl-6 leads to eradication of drug-resistant and leukaemia-initiating subclones [210]. TGF-β is a relevant target in a variety of disease states (reviewed in ref. [211]). Potentiation of imatinib-

induced cell death in non-proliferating CD34$^+$ CML cells by a TGF-β kinase inhibitor, SB-431542 (Glaxo Smithkline), has been reported [212].

JAK/STAT Pathway Inhibition

JAK2 plays fundamental roles in cytokine signaling in normal hematopoiesis [213,214] and is strongly implicated in the pathogenesis of the *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs, reviewed in ref. [215]). Blocking cytokine signaling via JAK inhibition along with Bcr-Abl kinase inhibition induces apoptosis in primary CML progenitors [216], and granulocyte macrophage colony-stimulating factor (GM-CSF) induces TKI resistance in primary GMPs via Bcr-Abl-independent activation of the JAK2/STAT5 pathway [217]. It has also been shown that Bcr-Abl stability and oncogenic signaling in CML cells are under the control of JAK2 [218]. Abelson helper integration site 1 (Ahi-1) is a novel oncoprotein whose expression is highly deregulated in CML stem/progenitor cells, where it forms an interaction complex with Bcr-Abl and JAK2 that mediates TKI responsiveness/resistance of these cells [219]. Samanta *et al.* showed that CML cell lines and patient-derived CML cells sensitive or resistant to imatinib underwent apoptosis on exposure to AG490, a potent tyrphostin JAK2 inhibitor [220]. Further studies from the same group demonstrated that JAK2 inhibition (using TG101209, TargeGen) up-regulates SET and activates protein phosphatase 2A (PP2A) and the Shp1 phosphatase, leading to decreased levels of activated Lyn, while sparing normal hematopoietic cells [221]. It has recently been claimed that Bcr-Abl uncouples canonical JAK2-STAT5 signaling in CML, thereby making JAK2 targeting irrelevant, such that STAT5 should be the real target of pharmacologic approaches in this setting [222]. The authors noted that several JAK2 TKIs induced apoptosis in Bcr-Abl$^+$ cells irrespective of whether JAK2 was expressed or not, suggesting direct "off-target" inhibition of Bcr-Abl [222]. In support of this concept, inhibition of STAT5 signaling by indirubicin derivatives was shown to induce apoptosis of imatinib-sensitive and –resistant human CML cell lines, including those expressing Bcr-Abl^{T315I}, and primary patient-derived CD34$^+$ CML cells [223]. Some preclinical studies (both *in vitro* and *in vivo*) of combination treatment with JAK2 and Bcr-Abl TKIs suggest a very narrow therapeutic window for this strategy, owing to toxic effects on normal hematopoietic cells at the doses of JAK2 inhibitors required to restore sensitivity of CML cells to Bcr-Abl TKIs [224]. The investigators demonstrated that soluble factors, signaling via JAK2/STAT3, were responsible for microenvironment-mediated resistance to Bcr-Abl TKIs, and that JAK2 inhibitors alone were only modestly effective in inducing apoptosis in CML cell lines and primary CML CD34$^+$ progenitors, and prolonging survival in a murine model of CML [224]. Although at least additive efficacy for combined JAK2/Bcr-Abl inhibition was noted, the authors concluded that such combinations insufficiently discriminate between normal and CML cells to be of therapeutic use [224]. However, others have very recently shown that combination treatment with imatinib and TG101209 statistically significantly enhances apoptosis of CD34$^+$ leukemic stem/progenitor cells and eliminates their long-term leukemia-initiating activity in immunodeficient mice [225]. Importantly, this approach was effective against treatment-naive CML stem cells from patients who subsequently proved to be resistant to imatinib therapy [225]. Ruxolitinib (Jakafi®, Incyte), the first FDA-approved JAK inhibitor [226,227], is being evaluated for its ability to eradicate minimal residual disease (MRD) in CML in conjunction with Bcr-Abl TKI

therapy by investigators at the M.D. Anderson Cancer Center (MDACC) in a phase I/II trial (NCT01751425).

PP2A Activation

Neviani *et al.* showed that in Bcr-Abl-transformed cells and CML-BC progenitors, the phosphatase activity of the tumor suppressor PP2A is inhibited by Bcr-Abl-induced expression of the PP2A inhibitor SET, and molecular and/or pharmacologic activation of PP2A promotes dephosphorylation of key regulators of cell proliferation and survival, suppresses Bcr-Abl activity, and induces its degradation in imatinib-sensitive and -resistant (including the T315I mutant) Bcr-Abl[+] cell lines and CML-BC progenitors, leading to growth suppression, enhanced apoptosis, restored differentiation, impaired clonogenic potential, and decreased *in vivo* leukemogenesis of imatinib-sensitive and -resistant Bcr-Abl^{+} cells [228]. As noted above, JAK2 may have a role in activating SET downstream of Bcr-Abl [221]. Fingolimod (Gilenya™, Novartis), FDA-approved for the treatment of patients with relapsing forms of multiple sclerosis [229], is a potent PP2A activator that induces apoptosis and impairs clonogenicity of TKI-sensitive and –resistant Bcr-Abl^{+} (both p210 and p190 fusion proteins) myeloid and lymphoid cell lines and CML-BC and Ph^{+} ALL progenitors, but not of normal CD34^{+} and CD34^{+}CD19^{+} BM cells [230]. Furthermore, pharmacologic doses of fingolimod profoundly suppress *in vivo* Bcr-Abl-driven leukemogenesis (both p210 and p190 and including Bcr-Abl[T315I]) [230]. PP2A activity is suppressed in CML stem cells relative to normal HSCs [231]. TKI-resistant quiescent stem cells from patients with CML exhibit increased levels of Bcr-Abl, but very low kinase activity, and Bcr-Abl overexpression, but not the kinase function of Bcr-Abl, was very recently shown to be required for activation of a JAK2/β-catenin-dependent survival/self-renewal pathway and inhibition of PP2A [231]. Fingolimod strikingly reduced survival and self-renewal of quiescent CML stem cells, but not of normal quiescent HSCs, through Bcr-Abl kinase-independent and PP2A-mediated inhibition of JAK2 and β-catenin, findings that were vindicated in xenograft models and serial transplantation assays [231]. Fingolimod also inhibits sphingosine kinase 1 (SK1), thereby inhibiting the formation of sphingosine-1-phosphate (S1P), a bioactive lipid that is a key player in a number of biological processes including cell survival, proliferation and migration and represents a novel emerging target in cancer (reviewed in ref. [232]). Alteration of the balance between S1P and ceramide, another biologically active lipid, due to overexpression of SK1 has been implicated as a mechanism of resistance to imatinib-induced apoptosis in CML cells [233]. SK1 plays a critical role in the inhibition of apoptosis in CML cells, serving both as a downstream effector of Bcr-Abl/Ras/ERK signaling and an upstream regulator of Bcl-2 family proteins [234]. Fingolimod therefore represents an attractive agent with multiple targets that needs to be explored further against CML stem cells.

SIRT1 Inhibition

SIRT1 is a multi-faceted, nicotinamide adenine dinucleotide (NAD)[+]-dependent protein deacetylase whose role in cancer may either be oncogenic or tumor-suppressive depending on the mutational status of p53 (reviewed in ref. [235]). Bcr-Abl activates SIRT1 through

STAT5 signaling, thus promoting CML cell survival and proliferation associated with deacetylation of multiple SIRT1 substrates, including FOXO1, p53, and the DNA repair enzyme Ku70 [236]. Bcr-Abl inhibition by imatinib partially reduces SIRT1 expression and SIRT1 inhibition further sensitizes CML cells to imatinib-induced apoptosis. Furthermore, knockout of *SIRT1* suppresses Bcr-Abl-induced transformation of mouse BM cells and the development of a CML-like myeloproliferative disease [236]. Inhibition of SIRT1 by small molecule inhibitors or gene knockdown blocks the acquisition of Bcr-Abl mutations ("clonal evolution") in and relapse of CML cells on TKIs [237]. Additionally, although SIRT1 can enhance the cellular DNA damage response (DDR), it interferes with the DNA repair machinery in CML cells and increases error-prone DNA damage repair [237]. Most significantly, Li *et al.* have recently shown that SIRT1 is overexpressed in human CML stem cells, and that pharmacological inhibition of SIRT1 or *SIRT1* gene knockdown increases apoptosis in CML-CP and –BC LSCs through p53 activation, reducing their growth *in vitro* and *in vivo*, effects that are potentiated by co-administration of imatinib [238].

Targeting Anti-Apoptotic Proteins of the Bcl-2 Family

A large body of evidence suggests that Bcr-Abl signaling results in blockade of apoptotic pathways [15,16]. The Bcl-2 family of pro- and anti-apoptotic proteins stands at the crossroads of cellular survival and death, controlling the intrinsic (mitochondrial) pathway of apoptosis, and regulating the two other major forms of cell death, viz. necrosis and autophagy, thus operating as nodal points at the convergence of multiple pathways with broad relevance to oncology (reviewed in ref. [239]). Bcl-2 has been shown to have a fundamental role in the causation of Bcr-Abl[+] leukemias [240,241]. The anti-apoptotic Bcl-2 family protein myeloid cell leukemia-1 (Mcl-1) has been shown to be a Bcr-Abl-dependent survival factor in CML cells, the expression of which can be down-regulated by the mitogen activated protein kinase kinase (MEK)/ERK inhibitor PD98059 [242]. Similarly, the pro-apoptotic Bcl-2 family proteins Bcl-2 interacting mediator of cell death (Bim) and Bcl-2 anatgonist of cell death (Bad) play major roles in imatinib-induced apoptosis of Bcr-Abl^{+} cells [243]. ABT-737 (Abbvie), a "BH3-mimetic" antagonist of the anti-apoptotic proteins Bcl-2 and Bcl-xL [244], can overcome resistance to imatinib caused by overexpression of Bcl-2 or loss of Bim (plus Bad) [243], or various other mechanisms, but not when leukemic cells harbor imatinib-insensitive mutations in Bcr-Abl such as T315I [245], thus invoking a role for BH3-mimetics in combinatorial strategies directed against CML stem cells. Bcl-xL, the transcription of which is activated by Bcr-Abl through STAT5 [246,247], was recently shown to be critical for disease progression in CML, and combined Bcl-xL inhibition and Bad activation markedly augmented apoptosis of CML-BC cell lines and primary CD34^{+} CML progenitors but not of those from healthy donors [248]. Inhibition of Bcl-2/Bcl-xL by ABT-737 in cells from patients with TKI-resistant CML-BC promotes apoptosis in quiescent CD34^{+} progenitor cells with an efficacy similar to that in proliferating cells [249]. Combination of ABT-737 with imatinib (which decreases Mcl-1 levels) synergistically induces death of both proliferating and quiescent CD34^{+} progenitor cells obtained from TKI-resistant CML-BC patients [249]. TKIs synergize strongly with ABT-737 to induce apoptosis in CML cell lines, including in the CD34^{+}CD38^{-} TKI-insensitive population; this is accompanied by off-target post-translational down-regulation of the X-linked inhibitor of apoptosis (XIAP) protein

[250]. Navitoclax (Abbvie), an orally bioavailable analog of ABT-737, has demonstrated robust single-agent activity in a phase I trial in patients with relapsed or refractory chronic lymphocytic leukemia (CLL) [251]. The company has since developed a highly potent, orally bioavailable, Bcl-2-selective inhibitor, ABT-199, by re-engineering of navitoclax in order to avoid thrombocytopenia due to platelet apoptosis (caused by Bcl-xL inhibition with navitoclax), while maintaining efficacy against Bcl-2-dependent malignancies [252]. While none of the aforementioned BH3-mimetics inhibits Mcl-1, a variety of approaches exist to target this short-lived anti-apoptotic protein, such as inhibition of transcription and translation, as well as promotion of degradation; these have recently been reviewed [253]. Omacetaxine mepesuccinate (Synribo™, Teva) is a semi-synthetic derivative of homoharringtonine, a natural alkaloid obtained from various *Cephalotaxus* species that exerts its antitumor activity through inhibition of protein synthesis and promotion of apoptosis, primarily due to down-regulation of Mcl-1 [254]. One study found that omacetaxine killed more than 90% of CML and Ph$^+$ ALL stem cells (including those bearing Bcr-Abl^{T315I}) *in vitro*, in contrast to < 9% and < 25% with imatinib or dasatinib, respectively [255]. Omacetaxine received approval from the FDA in 2012 for the treatment of patients in CML-CP or –AP who have failed two or more TKIs. Among 62 CML-CP patients with the Bcr-Abl T315I mutation and TKI failure treated on a phase II trial of omacetaxine at the MDACC, 14 (23%) achieved a MCyR, including CCyR in 10 (16%), and median progression-free survival (PFS) was 7.7 months [256]. Sabutoclax (Boehringer Ingelheim, Oncothyreon) is a pan-Bcl-2 inhibitor that was very recently shown to sensitize BM niche-resident CML-BC stem cells to TKIs at doses that spare normal progenitors [257].

Rac2 GTPase Inhibition

Bcr-Abl kinase activity leads to the formation of reactive oxygen species (ROS), with resultant oxidative DNA damage, genomic instability and mutations in the kinase domain that confer resistance to imatinib [258,259]. Additionally, Bcr-Abl stimulates single strand annealing, a relatively rare but very unfaithful DNA double strand break (DSB) repair mechanism, causing chromosomal aberrations [260]. Finally, Bcr-Abl directly phosphorylates the DNA DSB repair protein RAD51 on the Y315 residue to promote RAD51 recombinase-mediated unfaithful homologous recombination repair in a dosage-dependent manner, which can be prevented by a peptide aptamer mimicking the RAD51(pY315) fragment [261]. These issues are particularly relevant in CML-BP cells and very primitive CML-CP cells (LSCs), which are characterized by high levels of Bcr-Abl expression [17,141]. Gene targeting of the guanosine triphosphatases (GTPases) *Rac1* and *Rac2* significantly delays or abrogates disease development in a murine model of p210-Bcr-Abl-induced MPN, accompanied by severely diminished p210-Bcr-Abl-induced downstream signaling in primary hematopoietic cells [262]. Rac has been validated as a molecular target in both primary human CML cells *in vitro* and in a xenograft model *in vivo* using a small-molecule antagonist of Rac1, NSC23766 [262]. Rac2 GTPase alters mitochondrial membrane potential and electron flow through the mitochondrial respiratory chain complex III (MRC-cIII), thereby generating high levels of ROS in CML-CP LSCs and primitive leukemia progenitor cells (LPCs) [263]. Inhibition of Rac2 by genetic deletion or a small-molecule inhibitor and down-regulation of mitochondrial ROS by disruption of MRC-cIII, expression of mitochondria-targeted catalase, or addition of

a ROS-scavenging mitochondria-targeted peptide aptamer have been shown to reduce genomic instability, suggesting that the Rac2-MRC-cIII pathway could be targeted to prevent both relapse and disease progression of CML [263].

Interferon and Growth Factors

In the pre-imatinib era, IFN-α was first-line therapy for patients with CML ineligible for allogeneic HSCT [14]. IFN-α induces CCyR in a substantial minority of patients with CML-CP, although molecular evidence of disease persists [264]. In these patients, IFN-α may induce a situation of "tumor dormancy", where the presence of a minority of Ph$^+$ CML progenitor cells for a very long period of time is still compatible with durable remission [265]. In preclinical studies, IFN-α is more active against primitive CML progenitors, inducing G1 cell cycle arrest and terminal differentiation, than imatinib, which is more active against differentiated CML progenitors, perhaps explaining why responses to IFN-α, while slower and less dramatic than those to imatinib, can be durable even after discontinuation of the drug [266]. Myeloid growth factors augmented the anti-leukemic activity of IFN-α, and neutralizing antibodies directed against myeloid growth factors inhibited it [266]. In an intriguing study, 6 of 12 patients with CML-CP who discontinued imatinib after having been in a CMR for at least 2 years continued to have undetectable Bcr-Abl transcripts after 18 months (median) of follow-up; notably, 10 of these 12 patients had previously been treated with IFN-α [267]. Essers *et al.* reported that in mice, acute treatment with IFN-α induces dormant HSCs to enter the cell cycle and proliferate, whereas chronic activation of the IFN-α pathway in HSCs impairs their function [268], suggesting that combining IFN-α with TKI therapy may allow the latter to more effectively kill CML stem cells. A retrospective study of 76 early CML-CP patients treated with imatinib plus IFN-α and 419 early CML-CP patients treated with imatinib alone found that responses (CCyR, MMR) to the combination were more rapid, but that the response rates in the imatinib alone group "caught up" with those in the imatinib + IFN-α group with longer follow-up [269]. In the French STI571 Prospective Randomized Trial (SPIRIT) in 636 patients with previously untreated CML-CP, the rates of a "superior molecular response" (defined as a decrease in the ratio of transcripts of Bcr-Abl to transcripts of Abl of 0.01% or less, corresponding to a 4-log reduction or more from the baseline level, as assessed by a real-time Q-PCR assay) at 12 months was significantly higher (p=0.001) among patients receiving imatinib 400 mg daily plus peginterferon alfa-2a (30%) than among patients receiving 400 mg of imatinib daily alone (14%), although rates of CCyR were not significantly different between the groups [270]. IFN-α maintenance after treatment with imatinib plus IFN-α for ≥ 2 years was evaluated longitudinally in twenty patients with CML-CP with regular monitoring of peripheral blood Bcr-Abl transcripts by Q-PCR after imatinib discontinuation (due to patient request and/or imatinib intolerance) [271]. After a median follow-up of 2.4 years from imatinib discontinuation, 15 of the 20 patients remained in molecular remission, and all 5 patients who relapsed could be rescued with imatinib. Notably, the number of patients in CMR increased on IFN from two patients at baseline to five patients after 2 years [271].

Analogous to the concept of promoting killing of CML stem cells by imatinib by IFN-α-induced proliferation, intermittent (but not continuous) exposure of primitive, quiescent CD34$^+$ CML cells to G-CSF *in vitro* has been shown to promote their elimination by imatinib

[272]. Other investigators have obtained similar results, demonstrating that high growth factor concentrations significantly enhance CML proliferation with or without imatinib treatment and reduce the number of viable, quiescent CML progenitors remaining after imatinib exposure [273]. Stimulation with high growth factor concentrations before imatinib treatment further reduced the number of residual non-dividing CML CD34^{+} cells [273]. Importantly, clinically achievable concentrations of GM-CSF alone or in combination with G-CSF also significantly reduced non-dividing CML CD34^{+} cells [273]. Unfortunately, these promising preclinical findings did not translate into a significant difference in efficacy between any of the arms in a small pilot trial of continuous imatinib versus pulsed imatinib with or without G-CSF in patients with CML-CP who had achieved a CCyR (but not a CMR) on imatinib at trial entry [274]. However, the addition of GM-CSF has been shown to improve the cytogenetic response obtained with IFN-α therapy alone in patients with CML-CP [275].

Nuclear Export Inhibition

One of the mechanisms by which Bcr-Abl promotes cellular survival is by retaining the endogenous cyclin-dependent kinase (CDK) inhibitor, p21[WAF1] in a cytosolic location, thereby preventing cell cycle arrest normally induced by nuclear p21[WAF1] [276]. The nuclear export protein implicated in this process is the karyopherin chromosome maintenance protein 1 (CRM1), blocking which with small-molecule selective inhibitors of nuclear export (SINEs) has emerged as a novel therapeutic strategy to restore tumor suppressor function applicable to a number of cancers, since CRM1 is also involved in the active transport of p53 and nucleophosmin out of the nucleus [277], and in the nucleocytoplasmic shuttling of heterogenous nuclear ribonucleoproteins (hnRNPs) that are critical for the expression of factors (e.g., SET/PP2A, c-Myc, C/EBPα/miR-328 (CCAAT enhancer binding protein alpha/microRNA-328)) that regulate proliferation and survival of Ph[+] progenitors [19]. Vigneri and Wang showed that imatinib stimulates nuclear entry of the Bcr-Abl TK, which can then be trapped in the nucleus by the nuclear export inhibitor leptomycin B, with resultant activation of apoptosis [278]. The drug combination caused complete and irreversible killing of Bcr-Abl-transformed cells [278]. This strategy has been shown to be effective in killing human CML cells *ex vivo* [279] and in overcoming imatinib resistance due to Bcr-Abl amplification and clonal evolution, but not due to Bcr-Abl kinase domain mutations [280]; however, it does not eliminate CD34^{+} CML stem cells [281]. Leptomycin B may be too toxic for therapeutic use, and the novel CRM1 inhibitors KPT-185 and KPT-207 (Karyopharm) have been shown recently to selectively kill mouse Bcr-Abl^{+} cells (American Association for Cancer Research Annual meeting, Chicago, IL, April 3rd, 2012, abstract # 3839). These agents may have the potential to target CML stem cells, and deserve to be explored further.

Cyclopentenone Prostaglandins (CyPGs)

It was demonstrated recently that Δ(12)-prostaglandin J(3), a natural metabolite of the ω-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA), completely restored normal hematologic parameters, splenic histology, and enhanced survival in mice expressing Bcr-Abl in the HSC pool [282]. Furthermore, Δ(12)-prostaglandin J(3) activated p53 selectively in

LSCs, targeting LSCs for apoptosis in the spleen and BM, and completely eradicated LSCs *in vivo*, as demonstrated by the inability of donor cells from treated mice to cause leukemia in secondary transplantations [282]. These natural compounds therefore represent an exciting avenue for investigation in the search for the elusive cure for CML.

Farnesyltransferase Inhibition

Farnesyltransferase inhibitors (FTIs), originally developed to target the subcellular localization of Ras at the plasma membrane via inhibition of prenylation, are an interesting group of agents with complex biologic actions (reviewed in ref. [283]). The FTI lonafarnib (Sarasar™, Schering-Plough) exhibits single agent activity against CML cell lines *in vitro* and *in vivo*, as well as against primary human CML cells [284], augments imatinib-induced apoptosis in imatinib-sensitive cells, and overcomes imatinib resistance due to *BCR-ABL1* gene amplification [285]. Lonafarnib is also a potent inhibitor of the P-glycoprotein [286]. Lonafarnib enhances the toxicity of imatinib against K562 CML-BC cell lines and primitive quiescent CD34^{+} leukemic cells from CML-CP patients *in vitro* [287]. In a pilot study at the MDACC in 13 CML-CP (n = 6) or –AP (n = 7) patients with imatinib resistance or intolerance, it produced responses in two patients [288]. A phase I study of lonafarnib in combination with imatinib was subsequently conducted in 23 patients with CML who had failed imatinib therapy [289]. Eight (35%) patients responded; three each with CP and AP disease, and 2 with CML-BC, with one CML-CP patient attaining a CCyR [289]. BMS-214662 (Bristol-Myers Squibb), another FTI, alone or in combination with imatinib or dasatinib, potently induced apoptosis of both proliferating and quiescent CML stem/progenitor cells, while relatively sparing normal stem/progenitor cells [290]. BMS-214662 was cytotoxic against CML-BC stem/progenitor cells, particularly in combination with a TKI and equally effective in cell lines harboring wild-type versus mutant Bcr-Abl, including the T315I mutant [290]. Interestingly, the mechanism of these effects in primitive CD34^{+}CD38^{-} CML cells appeared to involve up-regulation of protein kinase C beta (PKCβ), down-regulation of E2F1, and phosphorylation of cyclin A/CDK2, resulting in apoptosis via the intrinsic, or mitochondrial pathway, rather than through Ras modulation [291]. Recently, the MEK inhibitor PD184352 was shown to increase the apoptotic effect of BMS-214662 in K562 and primary CML-CP CD34^{+} cells; this was accompanied by Mcl-1 down-regulation [292]. However, despite evidence of modest single-agent anti-leukemia activity in a phase I trial [293], there appear to be no current plans for continued clinical development of BMS-214662. A phase I trial conducted at the MDACC of the FTI tipifarnib (Zarnestra®, Johnson & Johnson) in combination with imatinib in patients with CML-CP who had failed therapy with imatinib yielded hematologic responses in 17 (68%) of 25 evaluable patients and cytogenetic responses in 9 (36%), including four with mutant forms of Bcr-Abl (one with Bcr-Abl[T315I]) [294]. As a single agent, tipifarnib yielded only transient responses in 7 of 22 patients with CML in another MDACC study [295].

Targeting BMI1

The transcriptional repressor BMI1, a polycomb group gene product that functions as an epigenetic regulator during development, is essential for proliferation, self-renewal and maintenance of normal adult HSCs as well as LSCs [296,297]. BMI1 expression increases with disease progression in CML from CP to AP and BC, and the protein is also overexpressed in CML CD34$^+$ cells compared to their normal counterparts [298]. Rizo *et al.* showed that BMI1 collaborates with Bcr-Abl in inducing a fatal, transplantable leukemia in NOD/SCID mice transplanted with transduced human CD34$^+$ cells [299]. Retroviral introduction of BMI1 in primary CD34$^+$ cells from CML-CP patients elevated their proliferative capacity and self-renewal properties [299]. Taken together, these findings identify BMI1 as a novel therapeutic target to eradicate CML at the stem cell level.

Targeting MicroRNAs

Micro-RNAs (miRs) are small (approximately 22 bases), non-protein-coding RNAs that recognize target sequences of imperfect complementarity in cognate messenger RNAs (mRNAs) and either destabilize them or inhibit protein translation [300]. Among other regulatory roles in vertebrate development, miRs modulate hematopoietic lineage differentiation [301]. In CML-BC, loss of miR-328 occurs in a Bcr-Abl dose- and kinase-dependent manner through the mitogen activated protein kinase (MAPK)-hnRNP E2 pathway [302]. In this setting, miR-328 functions as an RNA decoy to release CEBP/α mRNA from translational inhibition mediated by hnRNP E2 [302]. CML-CP CD34$^+$ cells are characterized by Bcr-Abl- and c-Myc-dependent up-regulation of the miR-17-92 polycistron [303]. Finally, genetic and epigenetic silencing of the tumor suppressor miR-203 enhances expression of the *BCR-ABL1* oncogene in CML [304]. There is thus considerable evidence of miR deregulation in CML and their manipulation for therapeutic benefit is an area of intense investigation. Small interfering RNAs (siRNAs) have been shown to inhibit Bcr-Abl oncoprotein expression in hematopoietic cells, and sensitize Bcr-Abl-overexpressing and –mutant CML cells to imatinib [305,306].

Proteasome Inhibition

NFκB activation is a requirement for Bcr-Abl-mediated transformation [307], and is the primary target of proteasome inhibitors [308]. The proteasome inhibitor bortezomib (Velcade®, Millennium) targets primitive CML cells, both alone and in combination with dasatinib, with effects on CD34$^+$38$^-$, long-term culture-initiating cells (LTC-IC) and NOD/SCID mouse repopulating cells, but also induces apoptosis in normal CD34$^+$38$^-$ cells [309]. The drug also induces apoptosis in CML cell lines expressing Bcr-Abl mutations, including T315I [309]. Interestingly, proteasome inhibitors may be able to "re-express" the pro-apoptotic "BH3-only" protein Bim, which is down-regulated by Bcr-Abl, in CML cells [310]. Bortezomib treatment leads to inhibition of Bcr-Abl-induced suppression of FoxO proteins and their pro-apoptotic targets, TRAIL and Bim in CML cell lines *in vitro* and *in vivo*, as well as in primary CML cells, including those expressing Bcr-Abl^{T315I} [311].

Bortezomib up-regulates TRAIL receptor expression on quiescent CD34$^+$ CML cells, and enhances their susceptibility to cytotoxicity by *in vitro* expanded donor natural killer (NK) cells, suggesting that donor-derived NK cell-mediated graft versus leukemia (GVL) effects may be improved by sensitizing residual quiescent CML cells to NK-cell cytotoxicity after HSCT using bortezomib [312]. Thus, bortezomib could possibly serve as an adjunct to donor lymphocyte infusions (DLIs) and pharmacologic therapy to reduce the risk of relapse in CML patients who require HSCT [312]. Another potential mechanism of therapeutic NFκB pathway blockade involves the inhibition of IκB kinase (IKK); the IKK inhibitor PS1145 (Millennium) inhibits the proliferation of CML cell lines and primary BM cells, effects that are enhanced by imatinib [313].

Targeting TNF-α

Very recently, it has been demonstrated that CML stem and progenitor cells produce tumor necrosis factor-alpha (TNF-α) in a kinase-independent manner and at higher levels compared to their normal counterparts [314]. It was found that TNF-α supports survival of CML stem and progenitor cells via activation of the NFκB/p65 pathway and expression of the β-chain receptor common to IL-3 and GM-CSF. A small-molecule inhibitor of TNF-α was shown to induce apoptosis in CML stem and progenitor cells, both alone and synergistically in combination with nilotinib [314].

Other Potential Targets in CML Stem Cells

It was recently shown that Setbp1 overexpression confers self-renewal capability on myeloid progenitors *in vitro* via direct transcriptional activation of the homeobox genes *HOXA9* and *HOXA10*, causing their immortalization, and that self-renewal after immortalization requires continuous Setbp1 expression [315]. Setbp1 was also found to promote self-renewal of myeloid progenitors *in vivo* as its co-expression with Bcr-Abl transformed primary mouse myeloid progenitors, generating aggressive leukemias in recipient mice resembling CML-BC. Increased Setbp1 mRNA levels were also detected in a subset of CML AP/BC patients with high levels of Hoxa9 and Hoxa10 expression [315]. The *Blk* (B-lymphoid kinase) gene has recently been identified as a tumor suppressor gene in CML stem cells that appears not to affect normal HSCs or hematopoiesis [316]. Blk, which is down-regulated by Bcr-Abl through c-Myc in LSCs in CML mice, suppresses LSC function through a pathway involving an upstream regulator, Pax5, and a downstream effector, the endogenous CDK inhibitor p27. Inhibition of the Blk pathway accelerates, whereas increased Blk pathway activity delays CML development in mice, and Blk suppresses proliferation of human CML stem cells [316]. CML LSCs may be particularly dependent on CD44 for BM homing and engraftment, suggesting that pharmacologic blockade of CD44 might be of therapeutic benefit [317]. The tumor suppressor phosphatase and tensin homolog (PTEN) is down-regulated by Bcr-Abl in CML LSCs and PTEN deletion causes acceleration of CML development in mice [318]. Overexpression of PTEN suppresses LSCs and induces cell cycle arrest of leukemia cells, delaying the development of CML and B-ALL via regulation of Akt and prolonging the survival of Ph$^+$ leukemia mice [318]. The hairy enhancer of split 1 (Hes1) is a key target of

Notch signaling that functions as a transcriptional repressor and is able to immortalize common myeloid progenitors (CMPs) and GMPs in the presence of IL-3; the combination of Hes1 and Bcr-Abl in CMPs and GMPs causes an acute leukemia resembling CML-BC in irradiated mice [319]. This molecule is likely to play a key role in CML progression to BC, which is characterized by deregulation and cross-talk among several key self-renewal pathways, viz., sonic Hh, Wnt, Hox and Notch [320]. Eukaryotic initiation factor 5A (eIF5A), essential for cell proliferation and the only known eukaryotic protein activated by posttranslational hypusination, can be targeted by hypusination inhibitors (HIs); HIs exert anti-proliferative effects on Bcr-Abl$^+$ cells, including primary CD34$^+$ CML cells, and synergize with imatinib [321]. Fbxw7 may play a pivotal role in maintenance of quiescence in CML LICs by down-regulating c-myc, and abrogation of quiescence in CML LICs by Fbxw7 ablation may selectively increase their elimination by imatinib [322]. Quiescence in normal HSCs, critical to their self-renewal capacity, is maintained in the hypoxic bone marrow endosteal niche through stabilization of hypoxia-inducible factor-1α (HIF-1α) [323]. Compared with normal HSCs, CML LSCs appear to be more dependent on the HIF-1α pathway, thus potentially providing another therapeutic strategy for eradicating CML stem cells [324].

Immunotherapy

The success of allogeneic HSCT and DLIs in CML attests to the efficacy of immunotherapy in this disease [325]. Allogeneic HSCT is considered to be the only potentially curative strategy at the present time, albeit with considerable attendant morbidity and mortality [326]. Recently, Jaras *et al.* reported IL-1 receptor accessory protein (IL1RAP) as being specifically expressed on the surfaces of Ph$^+$ CD34$^+$CD38$^-$ candidate stem cells in CML, potentially serving as a unique biomarker able to reliably distinguish between CML stem cells and normal HSCs, and targetable by an anti-IL1RAP antibody that induces antibody-dependent cell-mediated cytotoxicity (ADCC) [327]. There is evidence that responders to IFN-α and allogeneic HSCT develop CML-specific cytotoxic T cells that recognize PR-1, a proteinase 3-derived nonapeptide [328,329]. CML cells express human leukocyte antigen (HLA)-associated leukemia-specific immunogenic peptides derived from the joining regions of the Bcr-Abl fusion protein, such as b3a2 and b2a2, that can be recognized by cytotoxic T cells [330-333]. In a small trial of a peptide vaccine derived from the sequence p210-b3a2 plus molgramostim and Quillaja saponaria (QS-21) as adjuvants (CMLVAX100) in CML-CP patients with stable residual disease on imatinib or IFN-α, almost all patients achieved deeper cytogenetic responses [334]. Measurable peptide-specific CD4$^+$ T-cell immune responses to a Bcr-Abl-derived fusion peptide vaccine were observed in another phase II trial in 14 CML-CP patients, including some who were in molecular relapse after allogeneic transplantation, with several patients obtaining improved cytogenetic and molecular responses [335]. There is a report of enduring CMR induced by vaccination with an immunogenic 25-mer b2a2 breakpoint-derived peptide (CMLb2a2-25) in a patient who had received IFN-α for six years, obtaining both CCyR and MMR, but who had lost MMR about 2 years after stopping IFN-α therapy [336]. Vaccination of imatinib-treated CML-CP patients with Bcr-Abl peptides spanning the e14a2 fusion junction elicited T-cell responses to

the Bcr-Abl peptides in 14 of 19 patients in one study [337], and this correlated with a subsequent fall in Bcr-Abl transcript levels. Benefit was restricted to patients in MCyR at baseline and was generally delayed, consistent with effects at the LSC level [337]. In a small phase II trial in ten patients with CML-CP who had received imatinib for a median of 62 months, vaccination with a mixture of heteroclitic and native peptides derived from both the b3a2 and b2a2 sequences of Bcr-Abl led to molecular responses in 6 patients, including three who achieved a MMR [338]. However, other trials have found less encouraging responses, and it has been suggested that although it is possible to immunize patients with CML against their own breakpoint peptides, there may be insufficient processing or presentation of the appropriate peptides on the cell surface of target CML cells or too weak a T-cell response to allow for clinically relevant activity, even in the setting of minimal disease [339]. Using a slightly different approach, investigators vaccinated 19 patients with CML-CP who had achieved at least a MCyR but continued to have measurable, persistent disease after one or more years on imatinib (median 37 months) with K562/GM-CSF, a GM-CSF-producing vaccine derived from a CML cell line (K562) that expresses several CML-associated antigens [340]. Mean PCR measurements of Bcr-Abl for the group declined significantly following the vaccines (P = 0.03), and seven subjects became PCR-undetectable [340]. A study of a novel, autologous vaccine of leukocyte derived Hsp70-peptide complexes (Hsp70PC) in conjunction with imatinib in imatinib-treated CML-CP patients reported cytogenetic or molecular responses in 13 of 20 patients, and immunologic responses in 9 of 16 patients analyzed [341]. Preferentially expressed antigen of melanoma (PRAME) is aberrantly expressed in several hematologic malignancies, elicits $CD8^+$ T-cell responses and may be a useful target for immunotherapy in CML [342].

Conclusion

The evolution of our understanding of CML biology and translation of the same into effective therapy for patients has been a fascinating story. Deep molecular remissions can now be achieved and sustained for long periods with modern TKI therapy, and transformation to advanced phases of the disease substantially delayed, if not largely prevented. The vast improvement in life expectancy for patients with this disease has made the possibility of a cure somewhat realistic. Most authorities believe that this will require eradication of the elusive CML stem cell. Although formidable challenges remain in this area, the above discussion illustrates the exciting advances that have been made in elucidating survival mechanisms in CML stem cells, opening up previously unexplored avenues for rational combination therapy. Hopefully, one or more of these approaches will lead to a cure for CML in the not too distant future, and CML will once again write medical history.

References

[1]   Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heal of cancer. *Science* 2002 Jul 5;297(5578):63-64.

[2]   Weinstein IB, Joe AK. Mechanisms of disease: Oncogene addiction--a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol* 2006 Aug;3(8):448-457.

[3]   NOWELL PC, HUNGERFORD DA. Chromosome studies on normal and leukemic human leukocytes. *J Natl Cancer* Inst 1960 Jul;25:85-109.

[4]   Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973 Jun 1;243(5405):290-293.

[5]   Bartram CR, de Klein A, Hagemeijer A, van Agthoven T, Geurts van Kessel A, Bootsma D, et al. Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* 1983 Nov 17-23;306(5940):277-280.

[6]   Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 1984 Jan;36(1):93-99.

[7]   Shtivelman E, Lifshitz B, Gale RP, Roe BA, Canaani E. Alternative splicing of RNAs transcribed from the human abl gene and from the bcr-abl fused gene. *Cell* 1986 Oct 24;47(2):277-284.

[8]   Ben-Neriah Y, Daley GQ, Mes-Masson AM, Witte ON, Baltimore D. The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. *Science* 1986 Jul 11;233(4760):212-214.

[9]   McLaughlin J, Chianese E, Witte ON. In vitro transformation of immature hematopoietic cells by the P210 BCR/ABL oncogene product of the Philadelphia chromosome. *Proc Natl Acad Sci U S A* 1987 Sep;84(18):6558-6562.

[10]   Daley GQ, Baltimore D. Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. *Proc Natl Acad Sci U S A* 1988 Dec;85(23):9312-9316.

[11]   Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science 1990 Mar* 2;247(4946):1079-1082.

[12]   Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. *Science* 1990 Feb 16;247(4944):824-830.

[13]   Kelliher MA, McLaughlin J, Witte ON, Rosenberg N. Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. *Proc Natl Acad Sci U S A* 1990 Sep;87(17):6649-6653.

[14]   Sawyers CL. Chronic myeloid leukemia. *N Engl J Med* 1999 Apr 29;340(17):1330-1340.

[15]   Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer* 2005 Mar;5(3):172-183.

[16]   Quintas-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood* 2009 Feb 19;113(8):1619-1630.

[17]   Calabretta B, Perrotti D. The biology of CML blast crisis. *Blood* 2004 Jun 1;103(11):4010-4022.

[18]   Perrotti D, Jamieson C, Goldman J, Skorski T. Chronic myeloid leukemia: mechanisms of blastic transformation. *J Clin Invest* 2010 Jul;120(7):2254-2264.

[19] Melo JV, Barnes DJ. Chronic myeloid leukaemia as a model of disease evolution in human cancer. *Nat Rev Cancer* 2007 Jun;7(6):441-453.

[20] Hehlmann R. How I treat CML blast crisis. *Blood* 2012 Jul 26;120(4):737-747.

[21] Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 1996 May;2(5):561-566.

[22] Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 2001 Apr 5;344(14):1031-1037.

[23] Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C, et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. *N Engl J Med* 2002 Feb 28;346(9):645-652.

[24] Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 2001 Apr 5;344(14):1038-1042.

[25] O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib Compared with Interferon and Low-Dose Cytarabine for Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia. *N Engl J Med* 2003 03/13; 2013/05;348(11):994-1004.

[26] Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-Year Follow-up of Patients Receiving Imatinib for Chronic Myeloid Leukemia. *N Engl J Med* 2006 12/07; 2013/05;355(23):2408-2417.

[27] Hochhaus A, O'Brien SG, Guilhot F, Druker BJ, Branford S, Foroni L, et al. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia* 2009 Jun;23(6):1054-1061.

[28] Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 2001 Aug 3;293(5531):876-880.

[29] Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. *Blood* 2002 May 1;99(9):3472-3475.

[30] Hochhaus A, Kreil S, Corbin AS, La Rosee P, Muller MC, Lahaye T, et al. Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. *Leukemia* 2002 Nov;16(11):2190-2196.

[31] Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2002 Aug;2(2):117-125.

[32] Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. *Science* 2000 Sep 15;289(5486):1938-1942.

[33] Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. *Cancer Cell* 2005 Feb;7(2):129-141.

[34] Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 2004 Jul 16;305(5682):399-401.

[35] Tokarski JS, Newitt JA, Chang CY, Cheng JD, Wittekind M, Kiefer SE, et al. The structure of Dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. *Cancer Res* 2006 Jun 1;66(11):5790-5797.

[36] Weisberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. *Nat Rev Cancer* 2007 May;7(5):345-356.

[37] Saglio G, Kim D, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus Imatinib for Newly Diagnosed Chronic Myeloid Leukemia. *N Engl J Med* 2010 06/17; 2013/05;362(24):2251-2259.

[38] Kantarjian H, Shah NP, Hochhaus A, Cortes J, Shah S, Ayala M, et al. Dasatinib versus Imatinib in Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia. *N Engl J Med* 2010 06/17; 2013/05;362(24):2260-2270.

[39] O'Hare T, Walters DK, Stoffregen EP, Jia T, Manley PW, Mestan J, et al. In vitro activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res* 2005 Jun 1;65(11):4500-4505.

[40] Bradeen HA, Eide CA, O'Hare T, Johnson KJ, Willis SG, Lee FY, et al. Comparison of imatinib mesylate, dasatinib (BMS-354825), and nilotinib (AMN107) in an N-ethyl-N-nitrosourea (ENU)-based mutagenesis screen: high efficacy of drug combinations. *Blood* 2006 Oct 1;108(7):2332-2338.

[41] O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, 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 Nov 6;16(5):401-412.

[42] Cortes JE, Kim D-W, Pinilla-Ibarz, J, le Coutre P, Paquette R, Chuah C, et al. A Phase 2 Trial of Ponatinib in Philadelphia Chromosome–Positive Leukemias. *N Engl J Med* 2013 11/07; 2013/11;369(19):1783-1796.

[43] Shah NP, Skaggs BJ, Branford S, Hughes TP, Nicoll JM, Paquette RL, et al. Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J Clin Invest* 2007 Sep;117(9):2562-2569.

[44] Khorashad JS, Milojkovic D, Mehta P, Anand M, Ghorashian S, Reid AG, et al. In vivo kinetics of kinase domain mutations in CML patients treated with dasatinib after failing imatinib. *Blood* 2008 February 15;111(4):2378-2381.

[45] O'Hare T, Walters DK, Stoffregen EP, Sherbenou DW, Heinrich MC, Deininger MW, et al. Combined Abl inhibitor therapy for minimizing drug resistance in chronic myeloid leukemia: Src/Abl inhibitors are compatible with imatinib. *Clin Cancer Res* 2005 Oct 1;11(19 Pt 1):6987-6993.

[46] Weisberg E, Catley L, Wright RD, Moreno D, Banerji L, Ray A, et al. Beneficial effects of combining nilotinib and imatinib in preclinical models of BCR-ABL+ leukemias. *Blood* 2007 Mar 1;109(5):2112-2120.

[47] Goldman JM. Ponatinib for Chronic Myeloid Leukemia. *N Engl J Med* 2012 11/29; 2013/05;367(22):2148-2149.

[48] Bjorkholm M, Ohm L, Eloranta S, Derolf A, Hultcrantz M, Sjoberg J, et al. Success story of targeted therapy in chronic myeloid leukemia: a population-based study of patients diagnosed in Sweden from 1973 to 2008. *J Clin Oncol* 2011 Jun 20;29(18):2514-2520.

[49] Experts in chronic myeloid leukemia. Price of drugs for chronic myeloid leukemia (CML), reflection of the unsustainable cancer drug prices: perspective of CML Experts. *Blood* 2013 April 25.

[50] Gambacorti-Passerini C, Antolini L, Mahon FX, Guilhot F, Deininger M, Fava C, et al. Multicenter independent assessment of outcomes in chronic myeloid leukemia patients treated with imatinib. *J Natl Cancer Inst* 2011 Apr 6;103(7):553-561.

[51] Mahon FX. Is going for cure in chronic myeloid leukemia possible and justifiable? *Hematology Am Soc Hematol Educ Program* 2012;2012:122-128.

[52] Smith CC, Shah NP. Is it downhill from here? Eliminating leukemic stem cells and curing chronic myeloid leukemia. *Clin Cancer Res* 2011 Nov 1;17(21):6605-6607.

[53] Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP, et al. International Randomized Study of Interferon Vs STI571 (IRIS) 8-Year Follow up: Sustained Survival and Low Risk for Progression or Events in Patients with Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP) Treated with Imatinib. *ASH Annual Meeting Abstracts* 2009 November 20;114(22):1126.

[54] de Lavallade H, Apperley JF, Khorashad JS, Milojkovic D, Reid AG, Bua M, et al. Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis. *J Clin Oncol* 2008 Jul 10;26(20):3358-3363.

[55] Lucas CM, Wang L, Austin GM, Knight K, Watmough SJ, Shwe KH, et al. A population study of imatinib in chronic myeloid leukaemia demonstrates lower efficacy than in clinical trials. *Leukemia* 2008 Oct;22(10):1963-1966.

[56] Larson RA, Hochhaus A, Hughes TP, Clark RE, Etienne G, Kim DW, et al. Nilotinib vs imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: ENESTnd 3-year follow-up. *Leukemia* 2012 Oct;26(10):2197-2203.

[57] Kantarjian HM, Shah NP, Cortes JE, Baccarani M, Agarwal MB, Undurraga MS, et al. Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). *Blood* 2012 February 02;119(5):1123-1129.

[58] Cortes JE, Kim D, Kantarjian HM, Brümmendorf TH, Dyagil I, Griskevicius L, et al. Bosutinib Versus Imatinib in Newly Diagnosed Chronic-Phase Chronic Myeloid Leukemia: Results From the BELA Trial. *Journal of Clinical Oncology* 2012 October 01;30(28):3486-3492.

[59] Jordan CT, Guzman ML, Noble M. Cancer Stem Cells. *N Engl J Med* 2006 09/21; 2013/05;355(12):1253-1261.

[60] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001 Nov 1;414(6859):105-111.

[61] LEVIN RH, WHANG J, TJIO JH, CARBONE PP, FREI E,3rd, FREIREICH EJ. Persistent Mitosis of Transfused Homologous Leukocytes in Children Receiving Antileukemic Therapy. *Science* 1963 Dec 6;142(3597):1305-1311.

[62] Graw RG,Jr, Buckner CD, Whang-Peng J, Leventhal BG, Kruger G, Berard C, et al. Complication of bone-marrow transplantation. Graft-versus-host disease resulting from chronic-myelogenous-leukaemia leucocyte transfusions. *Lancet* 1970 Aug 15;2(7668):338-341.

[63] Deisseroth AB, Zu Z, Claxton D, Hanania EG, Fu S, Ellerson D, et al. Genetic marking shows that Ph+ cells present in autologous transplants of chronic myelogenous leukemia (CML) contribute to relapse after autologous bone marrow in CML. *Blood* 1994 May 15;83(10):3068-3076.

[64] Sirard C, Lapidot T, Vormoor J, Cashman JD, Doedens M, Murdoch B, et al. Normal and leukemic SCID-repopulating cells (SRC) coexist in the bone marrow and peripheral blood from CML patients in chronic phase, whereas leukemic SRC are detected in blast crisis. *Blood* 1996 Feb 15;87(4):1539-1548.

[65] Wang JC, Lapidot T, Cashman JD, Doedens M, Addy L, Sutherland DR, et al. High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase. *Blood* 1998 Apr 1;91(7):2406-2414.

[66] Holyoake T, Jiang X, Eaves C, Eaves A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. *Blood* 1999 Sep 15;94(6):2056-2064.

[67] Jiang X, Lopez A, Holyoake T, Eaves A, Eaves C. Autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia. *Proc Natl Acad Sci U S A* 1999 Oct 26;96(22):12804-12809.

[68] Holyoake TL, Jiang X, Jorgensen HG, Graham S, Alcorn MJ, Laird C, et al. Primitive quiescent leukemic cells from patients with chronic myeloid leukemia spontaneously initiate factor-independent growth in vitro in association with up-regulation of expression of interleukin-3. *Blood* 2001 Feb 1;97(3):720-728.

[69] Fialkow PJ, Jacobson RJ, Papayannopoulou T. Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. *Am J Med* 1977 Jul;63(1):125-130.

[70] O'Hare T, Zabriskie MS, Eiring AM, Deininger MW. Pushing the limits of targeted therapy in chronic myeloid leukaemia. *Nat Rev Cancer* 2012 Jul 24;12(8):513-526.

[71] Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N, et al. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. *Cancer Cell* 2004 Dec;6(6):587-596.

[72] Passegue E, Jochum W, Schorpp-Kistner M, Mohle-Steinlein U, Wagner EF. Chronic myeloid leukemia with increased granulocyte progenitors in mice lacking junB expression in the myeloid lineage. *Cell* 2001 Jan 12;104(1):21-32.

[73] Passegue E, Wagner EF, Weissman IL. JunB deficiency leads to a myeloproliferative disorder arising from hematopoietic stem cells. *Cell* 2004 Oct 29;119(3):431-443.

[74] Jamieson CHM, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte–Macrophage Progenitors as Candidate Leukemic Stem Cells in Blast-Crisis CML. *N Engl J Med* 2004 08/12; 2013/05;351(7):657-667.

[75] Abrahamsson AE, Geron I, Gotlib J, Dao KH, Barroga CF, Newton IG, et al. Glycogen synthase kinase 3beta missplicing contributes to leukemia stem cell generation. *Proc Natl Acad Sci U S A* 2009 Mar 10;106(10):3925-3929.

[76] Minami Y, Stuart SA, Ikawa T, Jiang Y, Banno A, Hunton IC, et al. BCR-ABL-transformed GMP as myeloid leukemic stem cells. *Proc Natl Acad Sci U S A* 2008 Nov 18;105(46):17967-17972.

[77] Bhatia R, Holtz M, Niu N, Gray R, Snyder DS, Sawyers CL, et al. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood* 2003 June 15;101(12):4701-4707.

[78] Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, et al. Frequency of Major Molecular Responses to Imatinib or Interferon Alfa plus Cytarabine in Newly Diagnosed Chronic Myeloid Leukemia. *N Engl J Med* 2003 10/09; 2013/05;349(15):1423-1432.

[79] Branford S, Seymour JF, Grigg A, Arthur C, Rudzki Z, Lynch K, et al. BCR-ABL Messenger RNA Levels Continue to Decline in Patients with Chronic Phase Chronic Myeloid Leukemia Treated with Imatinib for More Than 5 Years and Approximately Half of All First-Line Treated Patients Have Stable Undetectable BCR-ABL Using Strict Sensitivity Criteria. *Clinical Cancer Research* 2007 December 01;13(23):7080-7085.

[80] Chomel JC, Bonnet ML, Sorel N, Bertrand A, Meunier MC, Fichelson S, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. *Blood* 2011 Sep 29;118(13):3657-3660.

[81] Chu S, McDonald T, Lin A, Chakraborty S, Huang Q, Snyder DS, et al. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. *Blood* 2011 November 17;118(20):5565-5572.

[82] Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol* 2010 Nov;11(11):1029-1035.

[83] Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Yeung DT, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER Study. *Blood* 2013 May 23.

[84] Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood* 2002 Jan 1;99(1):319-325.

[85] Copland M, Hamilton A, Elrick LJ, Baird JW, Allan EK, Jordanides N, et al. Dasatinib (BMS-354825) targets an earlier progenitor population than imatinib in primary CML but does not eliminate the quiescent fraction. *Blood* 2006 Jun 1;107(11):4532-4539.

[86] Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. *Blood* 2007 May 1;109(9):4016-4019.

[87] Konig H, Holtz M, Modi H, Manley P, Holyoake TL, Forman SJ, et al. Enhanced BCR-ABL kinase inhibition does not result in increased inhibition of downstream signaling pathways or increased growth suppression in CML progenitors. *Leukemia* 2008 Apr;22(4):748-755.

[88]   Konig H, Holyoake TL, Bhatia R. Effective and selective inhibition of chronic myeloid leukemia primitive hematopoietic progenitors by the dual Src/Abl kinase inhibitor SKI-606. *Blood* 2008 Feb 15;111(4):2329-2338.

[89]   Holtz MS, Slovak ML, Zhang F, Sawyers CL, Forman SJ, Bhatia R. Imatinib mesylate (STI571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. *Blood* 2002 May 15;99(10):3792-3800.

[90]   Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. *J Clin Invest* 2011 Jan;121(1):396-409.

[91]   Hamilton A, Helgason GV, Schemionek M, Zhang B, Myssina S, Allan EK, et al. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. *Blood* 2012 Feb 9;119(6):1501-1510.

[92]   Jin L, Tabe Y, Konoplev S, Xu Y, Leysath CE, Lu H, et al. CXCR4 up-regulation by imatinib induces chronic myelogenous leukemia (CML) cell migration to bone marrow stroma and promotes survival of quiescent CML cells. *Mol Cancer Ther* 2008 Jan;7(1):48-58.

[93]   Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, et al. Dynamics of chronic myeloid leukaemia. *Nature* 2005 Jun 30;435(7046):1267-1270.

[94]   Roeder I, Horn M, Glauche I, Hochhaus A, Mueller MC, Loeffler M. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. *Nat Med* 2006 Oct;12(10):1181-1184.

[95]   Tang M, Gonen M, Quintas-Cardama A, Cortes J, Kantarjian H, Field C, et al. Dynamics of chronic myeloid leukemia response to long-term targeted therapy reveal treatment effects on leukemic stem cells. *Blood* 2011 Aug 11;118(6):1622-1631.

[96]   Goldman JM. Chronic Myeloid Leukemia Stem Cells: Now on the Run. *Journal of Clinical Oncology* 2009 January 10;27(2):313-314.

[97]   Goldman J, Gordon M. Why do chronic myelogenous leukemia stem cells survive allogeneic stem cell transplantation or imatinib: does it really matter? *Leuk Lymphoma* 2006 Jan;47(1):1-7.

[98]   Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Bartley PA, et al. Patients with chronic myeloid leukemia who maintain a complete molecular response after stopping imatinib treatment have evidence of persistent leukemia by DNA PCR. *Leukemia* 2010 Oct;24(10):1719-1724.

[99]   Stein AM, Bottino D, Modur V, Branford S, Kaeda J, Goldman JM, et al. BCR-ABL transcript dynamics support the hypothesis that leukemic stem cells are reduced during imatinib treatment. *Clin Cancer Res* 2011 Nov 1;17(21):6812-6821.

[100] Ross DM, Hughes TP, Melo JV. Do we have to kill the last CML cell? *Leukemia* 2011 Feb;25(2):193-200.

[101] Biernaux C, Loos M, Sels A, Huez G, Stryckmans P. Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. *Blood* 1995 October 15;86(8):3118-3122.

[102] Bose S, Deininger M, Gora-Tybor J, Goldman JM, Melo JV. The Presence of Typical and Atypical BCR-ABL Fusion Genes in Leukocytes of Normal Individuals: Biologic Significance and Implications for the Assessment of Minimal Residual Disease. *Blood* 1998 November 01;92(9):3362-3367.

[103] Saglio G, Kantarjian HM, Shah N, Jabbour EJ, Quintas-Cardama A, Steegmann JL, et al. Early Response (Molecular and Cytogenetic) and Long-Term Outcomes in Newly Diagnosed Chronic Myeloid Leukemia in Chronic Phase (CML-CP): Exploratory Analysis of DASISION 3-Year Data. *ASH Annual Meeting Abstracts 2012 November* 16;120(21):1675.

[104] Burgess MR, Skaggs BJ, Shah NP, Lee FY, Sawyers CL. Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. *Proc Natl Acad Sci U S A* 2005 Mar 1;102(9):3395-3400.

[105] Chan WW, Wise SC, Kaufman MD, Ahn YM, Ensinger CL, Haack T, et al. Conformational control inhibition of the BCR-ABL1 tyrosine kinase, including the gatekeeper T315I mutant, by the switch-control inhibitor DCC-2036. Cancer Cell 2011 Apr 12;19(4):556-568.

[106] Carmena M, Earnshaw WC. The cellular geography of aurora kinases. *Nat Rev Mol Cell Biol* 2003 Nov;4(11):842-854.

[107] Farag SS. The potential role of Aurora kinase inhibitors in haematological malignancies. *Br J Haematol* 2011 Dec;155(5):561-579.

[108] Moore AS, Blagg J, Linardopoulos S, Pearson AD. Aurora kinase inhibitors: novel small molecules with promising activity in acute myeloid and Philadelphia-positive leukemias. *Leukemia* 2010 Apr;24(4):671-678.

[109] Yang J, Ikezoe T, Nishioka C, Udaka K, Yokoyama A. Bcr-Abl activates AURKA and AURKB in chronic myeloid leukemia cells via AKT signaling. *Int J Cancer* 2013 Aug 12.

[110] Meulenbeld HJ, Mathijssen RH, Verweij J, de Wit R, de Jonge MJ. Danusertib, an aurora kinase inhibitor. *Expert Opin Investig Drugs* 2012 Mar;21(3):383-393.

[111] Kimura S. AT-9283, a small-molecule multi-targeted kinase inhibitor for the potential treatment of cancer. *Curr Opin Investig Drugs* 2010 Dec;11(12):1442-1449.

[112] Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, et al. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood* 2003 Jan 15;101(2):690-698.

[113] Santos FP, Kantarjian H, Cortes J, Quintas-Cardama A. Bafetinib, a dual Bcr-Abl/Lyn tyrosine kinase inhibitor for the potential treatment of leukemia. *Curr Opin Investig Drugs* 2010 Dec;11(12):1450-1465.

[114] Weisberg E, Choi HG, Ray A, Barrett R, Zhang J, Sim T, et al. Discovery of a small-molecule type II inhibitor of wild-type and gatekeeper mutants of BCR-ABL, PDGFRalpha, Kit, and Src kinases: novel type II inhibitor of gatekeeper mutants. *Blood* 2010 May 27;115(21):4206-4216.

[115] Gumireddy K, Baker SJ, Cosenza SC, John P, Kang AD, Robell KA, et al. A non-ATP-competitive inhibitor of BCR-ABL overrides imatinib resistance. *Proc Natl Acad Sci* U S A 2005 Feb 8;102(6):1992-1997.

[116] Jatiani SS, Cosenza SC, Reddy MV, Ha JH, Baker SJ, Samanta AK, et al. A Non-ATP-Competitive Dual Inhibitor of JAK2 and BCR-ABL Kinases: Elucidation of a Novel Therapeutic Spectrum Based on Substrate Competitive Inhibition. *Genes Cancer* 2010 Apr;1(4):331-345.

[117] Samanta AK, Chakraborty SN, Wang Y, Schlette E, Reddy EP, Arlinghaus RB. Destabilization of Bcr-Abl/Jak2 Network by a Jak2/Abl Kinase Inhibitor ON044580

Overcomes Drug Resistance in Blast Crisis Chronic Myelogenous Leukemia (CML). *Genes Cancer* 2010 Apr;1(4):346-359.

[118] Adrian FJ, Ding Q, Sim T, Velentza A, Sloan C, Liu Y, et al. Allosteric inhibitors of Bcr-abl-dependent cell proliferation. *Nat Chem Biol* 2006 Feb;2(2):95-102.

[119] Zhang J, Adrian FJ, Jahnke W, Cowan-Jacob SW, Li AG, Iacob RE, et al. Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. *Nature* 2010 Jan 28;463(7280):501-506.

[120] Grebien F, Hantschel O, Wojcik J, Kaupe I, Kovacic B, Wyrzucki AM, et al. Targeting the SH2-kinase interface in Bcr-Abl inhibits leukemogenesis. *Cell* 2011 Oct 14;147(2):306-319.

[121] Marin D, Bazeos A, Mahon FX, Eliasson L, Milojkovic D, Bua M, et al. Adherence is the critical factor for achieving molecular responses in patients with chronic myeloid leukemia who achieve complete cytogenetic responses on imatinib. *J Clin Oncol* 2010 May 10;28(14):2381-2388.

[122] Noens L, van Lierde MA, De Bock R, Verhoef G, Zachee P, Berneman Z, et al. Prevalence, determinants, and outcomes of nonadherence to imatinib therapy in patients with chronic myeloid leukemia: the ADAGIO study. *Blood* 2009 May 28;113(22):5401-5411.

[123] Gambacorti-Passerini C, Barni R, le Coutre P, Zucchetti M, Cabrita G, Cleris L, et al. Role of alpha1 acid glycoprotein in the in vivo resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. *J Natl Cancer Inst* 2000 Oct 18;92(20):1641-1650.

[124] Gambacorti-Passerini C, Zucchetti M, Russo D, Frapolli R, Verga M, Bungaro S, et al. Alpha1 acid glycoprotein binds to imatinib (STI571) and substantially alters its pharmacokinetics in chronic myeloid leukemia patients. *Clin Cancer Res* 2003 Feb;9(2):625-632.

[125] Green H, Skoglund K, Rommel F, Mirghani RA, Lotfi K. CYP3A activity influences imatinib response in patients with chronic myeloid leukemia: a pilot study on in vivo CYP3A activity. *Eur J Clin Pharmacol* 2010 Apr;66(4):383-386.

[126] Thomas J, Wang L, Clark RE, Pirmohamed M. Active transport of imatinib into and out of cells: implications for drug resistance. *Blood* 2004 Dec 1;104(12):3739-3745.

[127] White D, Saunders V, Lyons AB, Branford S, Grigg A, To LB, et al. In vitro sensitivity to imatinib-induced inhibition of ABL kinase activity is predictive of molecular response in patients with de novo CML. *Blood* 2005 Oct 1;106(7):2520-2526.

[128] White DL, Dang P, Engler J, Frede A, Zrim S, Osborn M, et al. Functional activity of the OCT-1 protein is predictive of long-term outcome in patients with chronic-phase chronic myeloid leukemia treated with imatinib. *J Clin Oncol* 2010 Jun 1;28(16):2761-2767.

[129] Illmer T, Schaich M, Platzbecker U, Freiberg-Richter J, Oelschlagel U, von Bonin M, et al. P-glycoprotein-mediated drug efflux is a resistance mechanism of chronic myelogenous leukemia cells to treatment with imatinib mesylate. *Leukemia* 2004 Mar;18(3):401-408.

[130] Mahon F, Belloc F, Lagarde V, Chollet C, Moreau-Gaudry F, Reiffers J, et al. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. *Blood* 2003 March 15;101(6):2368-2373.

[131] Burger H, van Tol H, Boersma AW, Brok M, Wiemer EA, Stoter G, et al. Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. *Blood* 2004 Nov 1;104(9):2940-2942.

[132] White DL, Saunders VA, Dang P, Engler J, Venables A, Zrim S, et al. Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. *Blood* 2007 December 01;110(12):4064-4072.

[133] Jorgensen HG, Elliott MA, Allan EK, Carr CE, Holyoake TL, Smith KD. Alpha1-acid glycoprotein expressed in the plasma of chronic myeloid leukemia patients does not mediate significant in vitro resistance to STI571. *Blood* 2002 Jan 15;99(2):713-715.

[134] Nakanishi T, Shiozawa K, Hassel BA, Ross DD. Complex interaction of BCRP/ABCG2 and imatinib in BCR-ABL-expressing cells: BCRP-mediated resistance to imatinib is attenuated by imatinib-induced reduction of BCRP expression. *Blood* 2006 Jul 15;108(2):678-684.

[135] Hatziieremia S, Jordanides NE, Holyoake TL, Mountford JC, Jorgensen HG. Inhibition of MDR1 does not sensitize primitive chronic myeloid leukemia CD34+ cells to imatinib. *Exp Hematol* 2009 Jun;37(6):692-700.

[136] Giannoudis A, Davies A, Lucas CM, Harris RJ, Pirmohamed M, Clark RE. Effective dasatinib uptake may occur without human organic cation transporter 1 (hOCT1): implications for the treatment of imatinib-resistant chronic myeloid leukemia. *Blood* 2008 Oct 15;112(8):3348-3354.

[137] White DL, Saunders VA, Dang P, Engler J, Zannettino AC, Cambareri AC, et al. OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): reduced OCT-1 activity is the cause of low in vitro sensitivity to imatinib. *Blood* 2006 Jul 15;108(2):697-704.

[138] Jiang X, Zhao Y, Smith C, Gasparetto M, Turhan A, Eaves A, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. *Leukemia* 2007 May;21(5):926-935.

[139] Holtz MS, Forman SJ, Bhatia R. Nonproliferating CML CD34+ progenitors are resistant to apoptosis induced by a wide range of proapoptotic stimuli. *Leukemia* 2005 Jun;19(6):1034-1041.

[140] Elrick LJ, Jorgensen HG, Mountford JC, Holyoake TL. Punish the parent not the progeny. *Blood* 2005 Mar 1;105(5):1862-1866.

[141] Savona M, Talpaz M. Getting to the stem of chronic myeloid leukaemia. *Nat Rev Cancer* 2008 May;8(5):341-350.

[142] Barnes DJ, Palaiologou D, Panousopoulou E, Schultheis B, Yong AS, Wong A, et al. Bcr-Abl expression levels determine the rate of development of resistance to imatinib mesylate in chronic myeloid leukemia. *Cancer Res* 2005 Oct 1;65(19):8912-8919.

[143] Donato NJ, Wu JY, Stapley J, Lin H, Arlinghaus R, Aggarwal BB, et al. Imatinib mesylate resistance through BCR-ABL independence in chronic myelogenous leukemia. *Cancer Res* 2004 Jan 15;64(2):672-677.

[144] Hu Y, Swerdlow S, Duffy TM, Weinmann R, Lee FY, Li S. Targeting multiple kinase pathways in leukemic progenitors and stem cells is essential for improved treatment of Ph+ leukemia in mice. *Proc Natl Acad Sci U S A* 2006 Nov 7;103(45):16870-16875.

[145] Kumano K, Arai S, Hosoi M, Taoka K, Takayama N, Otsu M, et al. Generation of induced pluripotent stem cells from primary chronic myelogenous leukemia patient samples. *Blood* 2012 Jun 28;119(26):6234-6242.

[146] Kaelin WG,Jr. The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer 2005* Sep;5(9):689-698.

[147] Cortes J, Kantarjian H. New targeted approaches in chronic myeloid leukemia. *J Clin Oncol* 2005 Sep 10;23(26):6316-6324.

[148] Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med* 2013 Feb 14;368(7):651-662.

[149] Bellodi C, Lidonnici MR, Hamilton A, Helgason GV, Soliera AR, Ronchetti M, et al. Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells. *J Clin Invest* 2009 May;119(5):1109-1123.

[150] Maclean KH, Dorsey FC, Cleveland JL, Kastan MB. Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. *J Clin Invest* 2008 Jan;118(1):79-88.

[151] Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005 Apr 14;434(7035):843-850.

[152] Radich JP, Dai H, Mao M, Oehler V, Schelter J, Druker B, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. *Proc Natl Acad Sci U S A* 2006 Feb 21;103(8):2794-2799.

[153] Zhao C, Blum J, Chen A, Kwon HY, Jung SH, Cook JM, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell* 2007 Dec;12(6):528-541.

[154] Hu Y, Chen Y, Douglas L, Li S. beta-Catenin is essential for survival of leukemic stem cells insensitive to kinase inhibition in mice with BCR-ABL-induced chronic myeloid leukemia. *Leukemia* 2009 Jan;23(1):109-116.

[155] Heidel FH, Bullinger L, Feng Z, Wang Z, Neff TA, Stein L, et al. Genetic and pharmacologic inhibition of beta-catenin targets imatinib-resistant leukemia stem cells in CML. *Cell Stem Cell* 2012 Apr 6;10(4):412-424.

[156] Nagao R, Ashihara E, Kimura S, Strovel JW, Yao H, Takeuchi M, et al. Growth inhibition of imatinib-resistant CML cells with the T315I mutation and hypoxia-adaptation by AV65--a novel Wnt/beta-catenin signaling inhibitor. *Cancer Lett* 2011 Dec 15;312(1):91-100.

[157] Schurch C, Riether C, Matter MS, Tzankov A, Ochsenbein AF. CD27 signaling on chronic myelogenous leukemia stem cells activates Wnt target genes and promotes disease progression. *J Clin Invest 2012* Feb 1;122(2):624-638.

[158] Reddiconto G, Toto C, Palama I, De Leo S, de Luca E, De Matteis S, et al. Targeting of GSK3beta promotes imatinib-mediated apoptosis in quiescent CD34+ chronic myeloid leukemia progenitors, preserving normal stem cells. *Blood* 2012 Mar 8;119(10):2335-2345.

[159] Gregory MA, Phang TL, Neviani P, Alvarez-Calderon F, Eide CA, O'Hare T, et al. Wnt/Ca2+/NFAT signaling maintains survival of Ph+ leukemia cells upon inhibition of Bcr-Abl. *Cancer Cell* 2010 Jul 13;18(1):74-87.

[160] Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. *Nat Rev Cancer* 2003 Dec;3(12):903-911.

[161] Dierks C, Beigi R, Guo GR, Zirlik K, Stegert MR, Manley P, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. *Cancer Cell* 2008 Sep 9;14(3):238-249.

[162] Zhao C, Chen A, Jamieson CH, Fereshteh M, Abrahamsson A, Blum J, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. *Nature* 2009 Apr 9;458(7239):776-779.

[163] Sekulic A, Migden MR, Oro AE, Dirix L, Lewis KD, Hainsworth JD, et al. Efficacy and Safety of Vismodegib in Advanced Basal-Cell Carcinoma. *N Engl J Med* 2012 06/07; 2013/06;366(23):2171-2179.

[164] Gao J, Graves S, Koch U, Liu S, Jankovic V, Buonamici S, et al. Hedgehog signaling is dispensable for adult hematopoietic stem cell function. *Cell Stem Cell* 2009 Jun 5;4(6):548-558.

[165] Chen Y, Hu Y, Zhang H, Peng C, Li S. Loss of the Alox5 gene impairs leukemia stem cells and prevents chronic myeloid leukemia. *Nat Genet* 2009 Jul;41(7):783-792.

[166] Peters-Golden M, Henderson WR. Leukotrienes. *N Engl J Med* 2007 11/01; 2013/06;357(18):1841-1854.

[167] Zhang H, Li H, Ho N, Li D, Li S. Scd1 plays a tumor-suppressive role in survival of leukemia stem cells and the development of chronic myeloid leukemia. *Mol Cell Biol* 2012 May;32(10):1776-1787.

[168] Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov* 2006 Sep;5(9):769-784.

[169] Burgess A, Ruefli A, Beamish H, Warrener R, Saunders N, Johnstone R, et al. Histone deacetylase inhibitors specifically kill nonproliferating tumour cells. *Oncogene* 2004 Sep 2;23(40):6693-6701.

[170] Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, et al. Inhibition of histone deacetylase 6 acetylates and disrupts the chaperone function of heat shock protein 90: a novel basis for antileukemia activity of histone deacetylase inhibitors. *J Biol Chem* 2005 Jul 22;280(29):26729-26734.

[171] An WG, Schulte TW, Neckers LM. The heat shock protein 90 antagonist geldanamycin alters chaperone association with p210bcr-abl and v-src proteins before their degradation by the proteasome. *Cell Growth Differ* 2000 Jul;11(7):355-360.

[172] Weisberg E, Catley L, Kujawa J, Atadja P, Remiszewski S, Fuerst P, et al. Histone deacetylase inhibitor NVP-LAQ824 has significant activity against myeloid leukemia cells in vitro and in vivo. *Leukemia* 2004 Dec;18(12):1951-1963.

[173] Nimmanapalli R, Fuino L, Bali P, Gasparetto M, Glozak M, Tao J, et al. Histone deacetylase inhibitor LAQ824 both lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of imatinib mesylate-sensitive or -refractory chronic myelogenous leukemia-blast crisis cells. *Cancer Res* 2003 Aug 15;63(16):5126-5135.

[174] Nimmanapalli R, Fuino L, Stobaugh C, Richon V, Bhalla K. Cotreatment with the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) enhances imatinib-induced apoptosis of Bcr-Abl-positive human acute leukemia cells. *Blood* 2003 Apr 15;101(8):3236-3239.

[175] Fiskus W, Pranpat M, Balasis M, Bali P, Estrella V, Kumaraswamy S, et al. Cotreatment with vorinostat (suberoylanilide hydroxamic acid) enhances activity of dasatinib (BMS-354825) against imatinib mesylate-sensitive or imatinib mesylate-

resistant chronic myelogenous leukemia cells. *Clin Cancer Res* 2006 Oct 1;12(19):5869-5878.

[176] Yu C, Rahmani M, Almenara J, Subler M, Krystal G, Conrad D, et al. Histone deacetylase inhibitors promote STI571-mediated apoptosis in STI571-sensitive and -resistant Bcr/Abl+ human myeloid leukemia cells. *Cancer Res* 2003 May 1;63(9):2118-2126.

[177] Fiskus W, Pranpat M, Bali P, Balasis M, Kumaraswamy S, Boyapalle S, et al. Combined effects of novel tyrosine kinase inhibitor AMN107 and histone deacetylase inhibitor LBH589 against Bcr-Abl-expressing human leukemia cells. *Blood* 2006 Jul 15;108(2):645-652.

[178] Zhang B, Strauss AC, Chu S, Li M, Ho Y, Shiang KD, et al. Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. *Cancer Cell* 2010 May 18;17(5):427-442.

[179] Isaacs JS, Xu W, Neckers L. Heat shock protein 90 as a molecular target for cancer therapeutics. *Cancer Cell* 2003 Mar;3(3):213-217.

[180] Nimmanapalli R, O'Bryan E, Bhalla K. Geldanamycin and its analogue 17-allylamino-17-demethoxygeldanamycin lowers Bcr-Abl levels and induces apoptosis and differentiation of Bcr-Abl-positive human leukemic blasts. *Cancer Res* 2001 Mar 1;61(5):1799-1804.

[181] Gorre ME, Ellwood-Yen K, Chiosis G, Rosen N, Sawyers CL. BCR-ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR-ABL chaperone heat shock protein 90. *Blood* 2002 Oct 15;100(8):3041-3044.

[182] Radujkovic A, Schad M, Topaly J, Veldwijk MR, Laufs S, Schultheis BS, et al. Synergistic activity of imatinib and 17-AAG in imatinib-resistant CML cells overexpressing BCR-ABL--Inhibition of P-glycoprotein function by 17-AAG. *Leukemia* 2005 Jul;19(7):1198-1206.

[183] Peng C, Brain J, Hu Y, Goodrich A, Kong L, Grayzel D, et al. Inhibition of heat shock protein 90 prolongs survival of mice with BCR-ABL-T315I-induced leukemia and suppresses leukemic stem cells. *Blood* 2007 Jul 15;110(2):678-685.

[184] Tauchi T, Okabe S, Ashihara E, Kimura S, Maekawa T, Ohyashiki K. Combined effects of novel heat shock protein 90 inhibitor NVP-AUY922 and nilotinib in a random mutagenesis screen. *Oncogene* 2011 Jun 16;30(24):2789-2797.

[185] George P, Bali P, Annavarapu S, Scuto A, Fiskus W, Guo F, et al. Combination of the histone deacetylase inhibitor LBH589 and the hsp90 inhibitor 17-AAG is highly active against human CML-BC cells and AML cells with activating mutation of FLT-3. *Blood* 2005 Feb 15;105(4):1768-1776.

[186] Salomoni P, Pandolfi PP. The role of PML in tumor suppression. *Cell* 2002 Jan 25;108(2):165-170.

[187] Ito K, Bernardi R, Morotti A, Matsuoka S, Saglio G, Ikeda Y, et al. PML targeting eradicates quiescent leukaemia-initiating cells. *Nature* 2008 Jun 19;453(7198):1072-1078.

[188] Tallman MS, Altman JK. How I treat acute promyelocytic leukemia. *Blood* 2009 Dec 10;114(25):5126-5135.

[189] Goussetis DJ, Gounaris E, Wu EJ, Vakana E, Sharma B, Bogyo M, et al. Autophagic degradation of the BCR-ABL oncoprotein and generation of antileukemic responses by arsenic trioxide. *Blood* 2012 Oct 25;120(17):3555-3562.

[190] Zhang QY, Mao JH, Liu P, Huang QH, Lu J, Xie YY, et al. A systems biology understanding of the synergistic effects of arsenic sulfide and Imatinib in BCR/ABL-associated leukemia. *Proc Natl Acad Sci* U S A 2009 Mar 3;106(9):3378-3383.

[191] Nie Y, Han YC, Zou YR. CXCR4 is required for the quiescence of primitive hematopoietic cells. *J Exp Med* 2008 Apr 14;205(4):777-783.

[192] Vianello F, Villanova F, Tisato V, Lymperi S, Ho KK, Gomes AR, et al. Bone marrow mesenchymal stromal cells non-selectively protect chronic myeloid leukemia cells from imatinib-induced apoptosis via the CXCR4/CXCL12 axis. *Haematologica* 2010 Jul;95(7):1081-1089.

[193] Zhang B, Ho YW, Huang Q, Maeda T, Lin A, Lee SU, et al. Altered microenvironmental regulation of leukemic and normal stem cells in chronic myelogenous leukemia. *Cancer Cell* 2012 Apr 17;21(4):577-592.

[194] Flomenberg N, Devine SM, Dipersio JF, Liesveld JL, McCarty JM, Rowley SD, et al. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood* 2005 Sep 1;106(5):1867-1874.

[195] Weisberg E, Azab AK, Manley PW, Kung AL, Christie AL, Bronson R, et al. Inhibition of CXCR4 in CML cells disrupts their interaction with the bone marrow microenvironment and sensitizes them to nilotinib. *Leukemia* 2012 May;26(5):985-990.

[196] Bader AG, Kang S, Zhao L, Vogt PK. Oncogenic PI3K deregulates transcription and translation. *Nat Rev Cancer* 2005 Dec;5(12):921-929.

[197] Riobo NA, Lu K, Ai X, Haines GM, Emerson CP,Jr. Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling. *Proc Natl Acad Sci* U S A 2006 Mar 21;103(12):4505-4510.

[198] Burchert A, Wang Y, Cai D, von Bubnoff N, Paschka P, Muller-Brusselbach S, et al. Compensatory PI3-kinase/Akt/mTor activation regulates imatinib resistance development. *Leukemia* 2005 Oct;19(10):1774-1782.

[199] Mohi MG, Boulton C, Gu TL, Sternberg DW, Neuberg D, Griffin JD, et al. Combination of rapamycin and protein tyrosine kinase (PTK) inhibitors for the treatment of leukemias caused by oncogenic PTKs. *Proc Natl Acad Sci* U S A 2004 Mar 2;101(9):3130-3135.

[200] Mancini M, Petta S, Martinelli G, Barbieri E, Santucci MA. RAD 001 (everolimus) prevents mTOR and Akt late re-activation in response to imatinib in chronic myeloid leukemia. *J Cell Biochem* 2010 Feb 1;109(2):320-328.

[201] Carayol N, Vakana E, Sassano A, Kaur S, Goussetis DJ, Glaser H, et al. Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells. *Proc Natl Acad Sci* U S A 2010 Jul 13;107(28):12469-12474.

[202] Kharas MG, Janes MR, Scarfone VM, Lilly MB, Knight ZA, Shokat KM, et al. Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR-ABL+ leukemia cells. *J Clin Invest* 2008 Sep;118(9):3038-3050.

[203] Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 1999 Mar 19;96(6):857-868.

[204] Ghaffari S, Jagani Z, Kitidis C, Lodish HF, Khosravi-Far R. Cytokines and BCR-ABL mediate suppression of TRAIL-induced apoptosis through inhibition of forkhead FOXO3a transcription factor. *Proc Natl Acad Sci* U S A 2003 May 27;100(11):6523-6528.

[205] Komatsu N, Watanabe T, Uchida M, Mori M, Kirito K, Kikuchi S, et al. A member of Forkhead transcription factor FKHRL1 is a downstream effector of STI571-induced cell cycle arrest in BCR-ABL-expressing cells. *J Biol Chem* 2003 Feb 21;278(8):6411-6419.

[206] Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* 2007 Jun 7;1(1):101-112.

[207] Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 2007 Jan 26;128(2):325-339.

[208] Naka K, Hoshii T, Muraguchi T, Tadokoro Y, Ooshio T, Kondo Y, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature* 2010 Feb 4;463(7281):676-680.

[209] Hurtz C, Hatzi K, Cerchietti L, Braig M, Park E, Kim YM, et al. BCL6-mediated repression of p53 is critical for leukemia stem cell survival in chronic myeloid leukemia. *J Exp Med* 2011 Oct 24;208(11):2163-2174.

[210] Duy C, Hurtz C, Shojaee S, Cerchietti L, Geng H, Swaminathan S, et al. BCL6 enables Ph+ acute lymphoblastic leukaemia cells to survive BCR-ABL1 kinase inhibition. *Nature* 2011 May 19;473(7347):384-388.

[211] Akhurst RJ, Hata A. Targeting the TGFbeta signalling pathway in disease. *Nat Rev Drug Discov* 2012 Oct;11(10):790-811.

[212] Moller GM, Frost V, Melo JV, Chantry A. Upregulation of the TGFbeta signalling pathway by Bcr-Abl: implications for haemopoietic cell growth and chronic myeloid leukaemia. *FEBS Lett* 2007 Apr 3;581(7):1329-1334.

[213] Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. *Cell* 1998 May 1;93(3):397-409.

[214] Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, et al. Jak2 is essential for signaling through a variety of cytokine receptors. *Cell* 1998 May 1;93(3):385-395.

[215] Levine RL, Pardanani A, Tefferi A, Gilliland DG. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. *Nat Rev Cancer* 2007 Sep;7(9):673-683.

[216] Hiwase DK, White DL, Powell JA, Saunders VA, Zrim SA, Frede AK, et al. Blocking cytokine signaling along with intense Bcr-Abl kinase inhibition induces apoptosis in primary CML progenitors. *Leukemia* 2010 Apr;24(4):771-778.

[217] Wang Y, Cai D, Brendel C, Barett C, Erben P, Manley PW, et al. Adaptive secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF) mediates imatinib and nilotinib resistance in BCR/ABL+ progenitors via JAK-2/STAT-5 pathway activation. *Blood* 2007 Mar 1;109(5):2147-2155.

[218] Samanta A, Perazzona B, Chakraborty S, Sun X, Modi H, Bhatia R, et al. Janus kinase 2 regulates Bcr-Abl signaling in chronic myeloid leukemia. *Leukemia* 2011 Mar;25(3):463-472.

[219] Zhou LL, Zhao Y, Ringrose A, DeGeer D, Kennah E, Lin AE, et al. AHI-1 interacts with BCR-ABL and modulates BCR-ABL transforming activity and imatinib response of CML stem/progenitor cells. *J Exp Med* 2008 Oct 27;205(11):2657-2671.

[220] Samanta AK, Lin H, Sun T, Kantarjian H, Arlinghaus RB. Janus kinase 2: a critical target in chronic myelogenous leukemia. *Cancer Res* 2006 Jul 1;66(13):6468-6472.

[221] Samanta AK, Chakraborty SN, Wang Y, Kantarjian H, Sun X, Hood J, et al. Jak2 inhibition deactivates Lyn kinase through the SET-PP2A-SHP1 pathway, causing apoptosis in drug-resistant cells from chronic myelogenous leukemia patients. *Oncogene* 2009 Apr 9;28(14):1669-1681.

[222] Hantschel O, Warsch W, Eckelhart E, Kaupe I, Grebien F, Wagner KU, et al. BCR-ABL uncouples canonical JAK2-STAT5 signaling in chronic myeloid leukemia. *Nat Chem Biol* 2012 Jan 29;8(3):285-293.

[223] Nam S, Scuto A, Yang F, Chen W, Park S, Yoo HS, et al. Indirubin derivatives induce apoptosis of chronic myelogenous leukemia cells involving inhibition of Stat5 signaling. *Mol Oncol* 2012 Jun;6(3):276-283.

[224] Traer E, MacKenzie R, Snead J, Agarwal A, Eiring AM, O'Hare T, et al. Blockade of JAK2-mediated extrinsic survival signals restores sensitivity of CML cells to ABL inhibitors. *Leukemia* 2012 May;26(5):1140-1143.

[225] Chen M, Gallipoli P, DeGeer D, Sloma I, Forrest DL, Chan M, et al. Targeting primitive chronic myeloid leukemia cells by effective inhibition of a new AHI-1-BCR-ABL-JAK2 complex. *J Natl Cancer Inst* 2013 Mar 20;105(6):405-423.

[226] Harrison C, Kiladjian J, Al-Ali H, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis. N *Engl J Med* 2012 03/01; 2013/06;366(9):787-798.

[227] Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A Double-Blind, Placebo-Controlled Trial of Ruxolitinib for Myelofibrosis. *N Engl J Med* 2012 03/01; 2013/06;366(9):799-807.

[228] Neviani P, Santhanam R, Trotta R, Notari M, Blaser BW, Liu S, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. *Cancer Cell* 2005 Nov;8(5):355-368.

[229] Cohen JA, Barkhof F, Comi G, Hartung HP, Khatri BO, Montalban X, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* 2010 Feb 4;362(5):402-415.

[230] Neviani P, Santhanam R, Oaks JJ, Eiring AM, Notari M, Blaser BW, et al. FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. *J Clin Invest* 2007 Sep;117(9):2408-2421.

[231] Neviani P, Harb JG, Oaks JJ, Santhanam R, Walker CJ, Ellis JJ, et al. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. *J Clin Invest* 2013 Oct 1;123(10):4144-4157.

[232] Pyne S, Bittman R, Pyne NJ. Sphingosine kinase inhibitors and cancer: seeking the golden sword of Hercules. *Cancer Res* 2011 Nov 1;71(21):6576-6582.

[233] Baran Y, Salas A, Senkal CE, Gunduz U, Bielawski J, Obeid LM, et al. Alterations of ceramide/sphingosine 1-phosphate rheostat involved in the regulation of resistance to imatinib-induced apoptosis in K562 human chronic myeloid leukemia cells. *J Biol Chem* 2007 Apr 13;282(15):10922-10934.

[234] Bonhoure E, Lauret A, Barnes DJ, Martin C, Malavaud B, Kohama T, et al. Sphingosine kinase-1 is a downstream regulator of imatinib-induced apoptosis in chronic myeloid leukemia cells. *Leukemia* 2008 May;22(5):971-979.

[235] Brooks CL, Gu W. How does SIRT1 affect metabolism, senescence and cancer? *Nat Rev Cancer* 2009 Feb;9(2):123-128.

[236] Yuan H, Wang Z, Li L, Zhang H, Modi H, Horne D, et al. Activation of stress response gene SIRT1 by BCR-ABL promotes leukemogenesis. *Blood* 2012 Feb 23;119(8):1904-1914.

[237] Wang Z, Yuan H, Roth M, Stark JM, Bhatia R, Chen WY. SIRT1 deacetylase promotes acquisition of genetic mutations for drug resistance in CML cells. *Oncogene* 2013 Jan 31;32(5):589-598.

[238] Li L, Wang L, Li L, Wang Z, Ho Y, McDonald T, et al. Activation of p53 by SIRT1 inhibition enhances elimination of CML leukemia stem cells in combination with imatinib. *Cancer Cell* 2012 Feb 14;21(2):266-281.

[239] Yip KW, Reed JC. Bcl-2 family proteins and cancer. *Oncogene* 2008 Oct 27;27(50):6398-6406.

[240] Sanchez-Garcia I, Grutz G. Tumorigenic activity of the BCR-ABL oncogenes is mediated by BCL2. *Proc Natl Acad Sci* U S A 1995 Jun 6;92(12):5287-5291.

[241] Jaiswal S, Traver D, Miyamoto T, Akashi K, Lagasse E, Weissman IL. Expression of BCR/ABL and BCL-2 in myeloid progenitors leads to myeloid leukemias. *Proc Natl Acad Sci* U S A 2003 Aug 19;100(17):10002-10007.

[242] Aichberger KJ, Mayerhofer M, Krauth MT, Skvara H, Florian S, Sonneck K, et al. Identification of mcl-1 as a BCR/ABL-dependent target in chronic myeloid leukemia (CML): evidence for cooperative antileukemic effects of imatinib and mcl-1 antisense oligonucleotides. *Blood* 2005 Apr 15;105(8):3303-3311.

[243] Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. *Proc Natl Acad Sci* U S A 2006 Oct 3;103(40):14907-14912.

[244] Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* 2005 Jun 2;435(7042):677-681.

[245] Kuroda J, Kimura S, Andreeff M, Ashihara E, Kamitsuji Y, Yokota A, et al. ABT-737 is a useful component of combinatory chemotherapies for chronic myeloid leukaemias with diverse drug-resistance mechanisms. *Br J Haematol* 2008 Jan;140(2):181-190.

[246] Gesbert F, Griffin JD. Bcr/Abl activates transcription of the Bcl-X gene through STAT5. *Blood* 2000 Sep 15;96(6):2269-2276.

[247] Horita M, Andreu EJ, Benito A, Arbona C, Sanz C, Benet I, et al. Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xL. *J Exp Med* 2000 Mar 20;191(6):977-984.

[248] Harb JG, Neviani P, Chyla BJ, Ellis JE, Ferenchak GJ, Oaks JJ, et al. Bcl-xL anti-apoptotic network is dispensable for development and maintenance of CML but is required for disease progression where it represents a new therapeutic target. *Leukemia* 2013 May 14.

[249] Mak DH, Wang RY, Schober WD, Konopleva M, Cortes J, Kantarjian H, et al. Activation of apoptosis signaling eliminates CD34+ progenitor cells in blast crisis CML independent of response to tyrosine kinase inhibitors. *Leukemia* 2012 Apr;26(4):788-794.

[250] Airiau K, Mahon FX, Josselin M, Jeanneteau M, Turcq B, Belloc F. ABT-737 increases tyrosine kinase inhibitor-induced apoptosis in chronic myeloid leukemia cells through XIAP downregulation and sensitizes CD34(+) CD38(-) population to imatinib. *Exp Hematol* 2012 May;40(5):367-78.e2.

[251] Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J Clin Oncol* 2012 Feb 10;30(5):488-496.

[252] Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat Med* 2013 Feb;19(2):202-208.

[253] Quinn BA, Dash R, Azab B, Sarkar S, Das SK, Kumar S, et al. Targeting Mcl-1 for the therapy of cancer. *Expert Opin Investig Drugs* 2011 Oct;20(10):1397-1411.

[254] Quintas-Cardama A, Kantarjian H, Cortes J. Homoharringtonine, omacetaxine mepesuccinate, and chronic myeloid leukemia circa 2009. *Cancer* 2009 Dec 1;115(23):5382-5393.

[255] Chen Y, Hu Y, Michaels S, Segal D, Brown D, Li S. Inhibitory effects of omacetaxine on leukemic stem cells and BCR-ABL-induced chronic myeloid leukemia and acute lymphoblastic leukemia in mice. *Leukemia* 2009 Aug;23(8):1446-1454.

[256] Cortes J, Lipton JH, Rea D, Digumarti R, Chuah C, Nanda N, et al. Phase 2 study of subcutaneous omacetaxine mepesuccinate after TKI failure in patients with chronic-phase CML with T315I mutation. *Blood* 2012 Sep 27;120(13):2573-2580.

[257] Goff DJ, Recart AC, Sadarangani A, Chun HJ, Barrett CL, Krajewska M, et al. A Pan-BCL2 inhibitor renders bone-marrow-resident human leukemia stem cells sensitive to tyrosine kinase inhibition. *Cell Stem Cell* 2013 Mar 7;12(3):316-328.

[258] Koptyra M, Falinski R, Nowicki MO, Stoklosa T, Majsterek I, Nieborowska-Skorska M, et al. BCR/ABL kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. *Blood* 2006 Jul 1;108(1):319-327.

[259] Koptyra M, Cramer K, Slupianek A, Richardson C, Skorski T. BCR/ABL promotes accumulation of chromosomal aberrations induced by oxidative and genotoxic stress. *Leukemia* 2008 Oct;22(10):1969-1972.

[260] Cramer K, Nieborowska-Skorska M, Koptyra M, Slupianek A, Penserga ET, Eaves CJ, et al. BCR/ABL and other kinases from chronic myeloproliferative disorders stimulate single-strand annealing, an unfaithful DNA double-strand break repair. *Cancer Res* 2008 Sep 1;68(17):6884-6888.

[261] Slupianek A, Dasgupta Y, Ren SY, Gurdek E, Donlin M, Nieborowska-Skorska M, et al. Targeting RAD51 phosphotyrosine-315 to prevent unfaithful recombination repair in BCR-ABL1 leukemia. *Blood* 2011 Jul 28;118(4):1062-1068.

[262] Thomas EK, Cancelas JA, Chae HD, Cox AD, Keller PJ, Perrotti D, et al. Rac guanosine triphosphatases represent integrating molecular therapeutic targets for BCR-ABL-induced myeloproliferative disease. *Cancer Cell* 2007 Nov;12(5):467-478.

[263] Nieborowska-Skorska M, Kopinski PK, Ray R, Hoser G, Ngaba D, Flis S, et al. Rac2-MRC-cIII-generated ROS cause genomic instability in chronic myeloid leukemia stem cells and primitive progenitors. *Blood* 2012 May 3;119(18):4253-4263.

[264] Hochhaus A, Reiter A, Saussele S, Reichert A, Emig M, Kaeda J, et al. Molecular heterogeneity in complete cytogenetic responders after interferon-alpha therapy for chronic myelogenous leukemia: low levels of minimal residual disease are associated with continuing remission. German CML Study Group and the UK MRC CML Study Group. *Blood* 2000 Jan 1;95(1):62-66.

[265] Talpaz M, Estrov Z, Kantarjian H, Ku S, Foteh A, Kurzrock R. Persistence of dormant leukemic progenitors during interferon-induced remission in chronic myelogenous leukemia. Analysis by polymerase chain reaction of individual colonies. *J Clin Invest* 1994 Oct;94(4):1383-1389.

[266] Angstreich GR, Matsui W, Huff CA, Vala MS, Barber J, Hawkins AL, et al. Effects of imatinib and interferon on primitive chronic myeloid leukaemia progenitors. *Br J Haematol* 2005 Aug;130(3):373-381.

[267] Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood* 2007 Jan 1;109(1):58-60.

[268] Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA, et al. IFNalpha activates dormant haematopoietic stem cells in vivo. *Nature* 2009 Apr 16;458(7240):904-908.

[269] Palandri F, Castagnetti F, Iacobucci I, Martinelli G, Amabile M, Gugliotta G, et al. The response to imatinib and interferon-alpha is more rapid than the response to imatinib alone: a retrospective analysis of 495 Philadelphia-positive chronic myeloid leukemia patients in early chronic phase. *Haematologica* 2010 Aug;95(8):1415-1419.

[270] Preudhomme C, Guilhot J, Nicolini FE, Guerci-Bresler A, Rigal-Huguet F, Maloisel F, et al. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. *N Engl J Med* 2010 Dec 23;363(26):2511-2521.

[271] Burchert A, Muller MC, Kostrewa P, Erben P, Bostel T, Liebler S, et al. Sustained molecular response with interferon alfa maintenance after induction therapy with imatinib plus interferon alfa in patients with chronic myeloid leukemia. *J Clin Oncol* 2010 Mar 10;28(8):1429-1435.

[272] Jorgensen HG, Copland M, Allan EK, Jiang X, Eaves A, Eaves C, et al. Intermittent exposure of primitive quiescent chronic myeloid leukemia cells to granulocyte-colony stimulating factor in vitro promotes their elimination by imatinib mesylate. *Clin Cancer Res* 2006 Jan 15;12(2):626-633.

[273] Holtz M, Forman SJ, Bhatia R. Growth factor stimulation reduces residual quiescent chronic myelogenous leukemia progenitors remaining after imatinib treatment. *Cancer Res* 2007 Feb 1;67(3):1113-1120.

[274] Drummond MW, Heaney N, Kaeda J, Nicolini FE, Clark RE, Wilson G, et al. A pilot study of continuous imatinib vs pulsed imatinib with or without G-CSF in CML patients who have achieved a complete cytogenetic response. *Leukemia* 2009 Jun;23(6):1199-1201.

[275] Cortes J, Kantarjian H, O'Brien S, Kurzrock R, Keating M, Talpaz M. GM-CSF can improve the cytogenetic response obtained with interferon-alpha therapy in patients with chronic myelogenous leukemia. *Leukemia* 1998 Jun;12(6):860-864.

[276] Keeshan K, Cotter TG, McKenna SL. Bcr-Abl upregulates cytosolic p21WAF-1/CIP-1 by a phosphoinositide-3-kinase (PI3K)-independent pathway. *Br J Haematol* 2003 Oct;123(1):34-44.

[277] Ranganathan P, Yu X, Na C, Santhanam R, Shacham S, Kauffman M, et al. Preclinical activity of a novel CRM1 inhibitor in acute myeloid leukemia. *Blood* 2012 Aug 30;120(9):1765-1773.

[278] Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat Med* 2001 Feb;7(2):228-234.

[279] Aloisi A, Di Gregorio S, Stagno F, Guglielmo P, Mannino F, Sormani MP, et al. BCR-ABL nuclear entrapment kills human CML cells: ex vivo study on 35 patients with the combination of imatinib mesylate and leptomycin B. *Blood* 2006 Feb 15;107(4):1591-1598.

[280] Kancha RK, von Bubnoff N, Miething C, Peschel C, Gotze KS, Duyster J. Imatinib and leptomycin B are effective in overcoming imatinib-resistance due to Bcr-Abl amplification and clonal evolution but not due to Bcr-Abl kinase domain mutation. *Haematologica* 2008 Nov;93(11):1718-1722.

[281] Allan EK, Hamilton A, Hatziieremia S, Zhou P, Jorgensen HG, Vigneri P, et al. Nuclear entrapment of BCR-ABL by combining imatinib mesylate with leptomycin B does not eliminate CD34+ chronic myeloid leukaemia cells. *Leukemia* 2009 May;23(5):1006-1008.

[282] Hegde S, Kaushal N, Ravindra KC, Chiaro C, Hafer KT, Gandhi UH, et al. Delta12-prostaglandin J3, an omega-3 fatty acid-derived metabolite, selectively ablates leukemia stem cells in mice. *Blood* 2011 Dec 22;118(26):6909-6919.

[283] Brunner TB, Hahn SM, Gupta AK, Muschel RJ, McKenna WG, Bernhard EJ. Farnesyltransferase inhibitors: an overview of the results of preclinical and clinical investigations. *Cancer Res* 2003 Sep 15;63(18):5656-5668.

[284] Peters DG, Hoover RR, Gerlach MJ, Koh EY, Zhang H, Choe K, et al. Activity of the farnesyl protein transferase inhibitor SCH66336 against BCR/ABL-induced murine leukemia and primary cells from patients with chronic myeloid leukemia. *Blood* 2001 Mar 1;97(5):1404-1412.

[285] Hoover RR, Mahon FX, Melo JV, Daley GQ. Overcoming STI571 resistance with the farnesyl transferase inhibitor SCH66336. *Blood* 2002 Aug 1;100(3):1068-1071.

[286] Wang E, Casciano CN, Clement RP, Johnson WW. The farnesyl protein transferase inhibitor SCH66336 is a potent inhibitor of MDR1 product P-glycoprotein. *Cancer Res* 2001 Oct 15;61(20):7525-7529.

[287] Jorgensen HG, Allan EK, Graham SM, Godden JL, Richmond L, Elliott MA, et al. Lonafarnib reduces the resistance of primitive quiescent CML cells to imatinib mesylate in vitro. *Leukemia* 2005 Jul;19(7):1184-1191.

[288] Borthakur G, Kantarjian H, Daley G, Talpaz M, O'Brien S, Garcia-Manero G, et al. Pilot study of lonafarnib, a farnesyl transferase inhibitor, in patients with chronic myeloid leukemia in the chronic or accelerated phase that is resistant or refractory to imatinib therapy. *Cancer* 2006 Jan 15;106(2):346-352.

[289] Cortes J, Jabbour E, Daley GQ, O'Brien S, Verstovsek S, Ferrajoli A, et al. Phase 1 study of lonafarnib (SCH 66336) and imatinib mesylate in patients with chronic myeloid leukemia who have failed prior single-agent therapy with imatinib. *Cancer* 2007 Sep 15;110(6):1295-1302.

[290] Copland M, Pellicano F, Richmond L, Allan EK, Hamilton A, Lee FY, et al. BMS-214662 potently induces apoptosis of chronic myeloid leukemia stem and progenitor cells and synergizes with tyrosine kinase inhibitors. *Blood* 2008 Mar 1;111(5):2843-2853.

[291] Pellicano F, Copland M, Jorgensen HG, Mountford J, Leber B, Holyoake TL. BMS-214662 induces mitochondrial apoptosis in chronic myeloid leukemia (CML) stem/progenitor cells, including CD34+38- cells, through activation of protein kinase Cbeta. *Blood* 2009 Nov 5;114(19):4186-4196.

[292] Pellicano F, Simara P, Sinclair A, Helgason GV, Copland M, Grant S, et al. The MEK inhibitor PD184352 enhances BMS-214662-induced apoptosis in CD34+ CML stem/progenitor cells. *Leukemia* 2011 Jul;25(7):1159-1167.

[293] Cortes J, Faderl S, Estey E, Kurzrock R, Thomas D, Beran M, et al. Phase I study of BMS-214662, a farnesyl transferase inhibitor in patients with acute leukemias and high-risk myelodysplastic syndromes. *J Clin Oncol* 2005 Apr 20;23(12):2805-2812.

[294] Cortes J, Quintas-Cardama A, Garcia-Manero G, O'Brien S, Jones D, Faderl S, et al. Phase 1 study of tipifarnib in combination with imatinib for patients with chronic myelogenous leukemia in chronic phase after imatinib failure. *Cancer* 2007 Nov 1;110(9):2000-2006.

[295] Cortes J, Albitar M, Thomas D, Giles F, Kurzrock R, Thibault A, et al. Efficacy of the farnesyl transferase inhibitor R115777 in chronic myeloid leukemia and other hematologic malignancies. *Blood* 2003 Mar 1;101(5):1692-1697.

[296] Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* 2003 May 15;423(6937):255-260.

[297] Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 2003 May 15;423(6937):302-305.

[298] Mohty M, Yong AS, Szydlo RM, Apperley JF, Melo JV. The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. *Blood* 2007 Jul 1;110(1):380-383.

[299] Rizo A, Horton SJ, Olthof S, Dontje B, Ausema A, van Os R, et al. BMI1 collaborates with BCR-ABL in leukemic transformation of human CD34+ cells. *Blood* 2010 Nov 25;116(22):4621-4630.

[300] Shivdasani RA. MicroRNAs: regulators of gene expression and cell differentiation. *Blood* 2006 Dec 1;108(12):3646-3653.

[301] Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004 Jan 2;303(5654):83-86.

[302] Eiring AM, Harb JG, Neviani P, Garton C, Oaks JJ, Spizzo R, et al. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. *Cell* 2010 Mar 5;140(5):652-665.

[303] Venturini L, Battmer K, Castoldi M, Schultheis B, Hochhaus A, Muckenthaler MU, et al. Expression of the miR-17-92 polycistron in chronic myeloid leukemia (CML) CD34+ cells. *Blood* 2007 May 15;109(10):4399-4405.

[304] Bueno MJ, Perez de Castro I, Gomez de Cedron M, Santos J, Calin GA, Cigudosa JC, et al. Genetic and epigenetic silencing of microRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression. *Cancer Cell* 2008 Jun;13(6):496-506.

[305] Scherr M, Battmer K, Winkler T, Heidenreich O, Ganser A, Eder M. Specific inhibition of bcr-abl gene expression by small interfering RNA. *Blood* 2003 Feb 15;101(4):1566-1569.

[306] Wohlbold L, van der Kuip H, Miething C, Vornlocher HP, Knabbe C, Duyster J, et al. Inhibition of bcr-abl gene expression by small interfering RNA sensitizes for imatinib mesylate (STI571). *Blood* 2003 Sep 15;102(6):2236-2239.

[307] Reuther JY, Reuther GW, Cortez D, Pendergast AM, Baldwin AS,Jr. A requirement for NF-kappaB activation in Bcr-Abl-mediated transformation. *Genes Dev* 1998 Apr 1;12(7):968-981.

[308] Rajkumar SV, Richardson PG, Hideshima T, Anderson KC. Proteasome inhibition as a novel therapeutic target in human cancer. *J Clin Oncol* 2005 Jan 20;23(3):630-639.

[309] Heaney NB, Pellicano F, Zhang B, Crawford L, Chu S, Kazmi SM, et al. Bortezomib induces apoptosis in primitive chronic myeloid leukemia cells including LTC-IC and NOD/SCID repopulating cells. *Blood* 2010 Mar 18;115(11):2241-2250.

[310] Aichberger KJ, Mayerhofer M, Krauth MT, Vales A, Kondo R, Derdak S, et al. Low-level expression of proapoptotic Bcl-2-interacting mediator in leukemic cells in patients with chronic myeloid leukemia: role of BCR/ABL, characterization of underlying signaling pathways, and reexpression by novel pharmacologic compounds. *Cancer Res* 2005 Oct 15;65(20):9436-9444.

[311] Jagani Z, Song K, Kutok JL, Dewar MR, Melet A, Santos T, et al. Proteasome inhibition causes regression of leukemia and abrogates BCR-ABL-induced evasion of apoptosis in part through regulation of forkhead tumor suppressors. *Cancer Res* 2009 Aug 15;69(16):6546-6555.

[312] Yong AS, Keyvanfar K, Hensel N, Eniafe R, Savani BN, Berg M, et al. Primitive quiescent CD34+ cells in chronic myeloid leukemia are targeted by in vitro expanded natural killer cells, which are functionally enhanced by bortezomib. *Blood* 2009 Jan 22;113(4):875-882.

[313] Cilloni D, Messa F, Arruga F, Defilippi I, Morotti A, Messa E, et al. The NF-kappaB pathway blockade by the IKK inhibitor PS1145 can overcome imatinib resistance. *Leukemia* 2006 Jan;20(1):61-67.

[314] Gallipoli P, Pellicano F, Morrison H, Laidlaw K, Allan EK, Bhatia R, et al. Autocrine TNF-alpha production supports CML stem and progenitor cell survival and enhances their proliferation. *Blood* 2013 Sep 16.

[315] Oakley K, Han Y, Vishwakarma BA, Chu S, Bhatia R, Gudmundsson KO, et al. Setbp1 promotes the self-renewal of murine myeloid progenitors via activation of Hoxa9 and Hoxa10. *Blood* 2012 Jun 21;119(25):6099-6108.

[316] Zhang H, Peng C, Hu Y, Li H, Sheng Z, Chen Y, et al. The Blk pathway functions as a tumor suppressor in chronic myeloid leukemia stem cells. *Nat Genet* 2012 Jul 15;44(8):861-871.

[317] Krause DS, Lazarides K, von Andrian UH, Van Etten RA. Requirement for CD44 in homing and engraftment of BCR-ABL-expressing leukemic stem cells. *Nat Med* 2006 Oct;12(10):1175-1180.

[318] Peng C, Chen Y, Yang Z, Zhang H, Osterby L, Rosmarin AG, et al. PTEN is a tumor suppressor in CML stem cells and BCR-ABL-induced leukemias in mice. *Blood* 2010 Jan 21;115(3):626-635.

[319] Nakahara F, Sakata-Yanagimoto M, Komeno Y, Kato N, Uchida T, Haraguchi K, et al. Hes1 immortalizes committed progenitors and plays a role in blast crisis transition in chronic myelogenous leukemia. *Blood* 2010 Apr 8;115(14):2872-2881.

[320] Sengupta A, Banerjee D, Chandra S, Banerji SK, Ghosh R, Roy R, et al. Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. *Leukemia* 2007 May;21(5):949-955.

[321] Balabanov S, Gontarewicz A, Ziegler P, Hartmann U, Kammer W, Copland M, et al. Hypusination of eukaryotic initiation factor 5A (eIF5A): a novel therapeutic target in BCR-ABL-positive leukemias identified by a proteomics approach. *Blood* 2007 Feb 15;109(4):1701-1711.

[322] Takeishi S, Matsumoto A, Onoyama I, Naka K, Hirao A, Nakayama KI. Ablation of Fbxw7 eliminates leukemia-initiating cells by preventing quiescence. *Cancer Cell* 2013 Mar 18;23(3):347-361.

[323] Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. *Cell Stem Cell* 2010 Sep 3;7(3):391-402.

[324] Zhang H, Li H, Xi HS, Li S. HIF1alpha is required for survival maintenance of chronic myeloid leukemia stem cells. *Blood* 2012 Mar 15;119(11):2595-2607.

[325] Pavlu J, Szydlo RM, Goldman JM, Apperley JF. Three decades of transplantation for chronic myeloid leukemia: what have we learned? *Blood* 2011 Jan 20;117(3):755-763.

[326] Goldman JM, Majhail NS, Klein JP, Wang Z, Sobocinski KA, Arora M, et al. Relapse and late mortality in 5-year survivors of myeloablative allogeneic hematopoietic cell transplantation for chronic myeloid leukemia in first chronic phase. *J Clin Oncol* 2010 Apr 10;28(11):1888-1895.

[327] Jaras M, Johnels P, Hansen N, Agerstam H, Tsapogas P, Rissler M, et al. Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein. *Proc Natl Acad Sci* U S A 2010 Sep 14;107(37):16280-16285.

[328] Molldrem JJ, Lee PP, Wang C, Felio K, Kantarjian HM, Champlin RE, et al. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. *Nat Med* 2000 Sep;6(9):1018-1023.

[329] Burchert A, Wolfl S, Schmidt M, Brendel C, Denecke B, Cai D, et al. Interferon-alpha, but not the ABL-kinase inhibitor imatinib (STI571), induces expression of myeloblastin and a specific T-cell response in chronic myeloid leukemia. *Blood* 2003 Jan 1;101(1):259-264.

[330] Chen W, Peace DJ, Rovira DK, You SG, Cheever MA. T-cell immunity to the joining region of p210BCR-ABL protein. *Proc Natl Acad Sci* U S A 1992 Feb 15;89(4):1468-1472.

[331] Cullis JO, Barrett AJ, Goldman JM, Lechler RI. Binding of BCR/ABL junctional peptides to major histocompatibility complex (MHC) class I molecules: studies in antigen-processing defective cell lines. *Leukemia* 1994 Jan;8(1):165-170.

[332] Yotnda P, Firat H, Garcia-Pons F, Garcia Z, Gourru G, Vernant JP, et al. Cytotoxic T cell response against the chimeric p210 BCR-ABL protein in patients with chronic myelogenous leukemia. *J Clin Invest* 1998 May 15;101(10):2290-2296.

[333] Clark RE, Dodi IA, Hill SC, Lill JR, Aubert G, Macintyre AR, et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. *Blood* 2001 Nov 15;98(10):2887-2893.

[334] Bocchia M, Gentili S, Abruzzese E, Fanelli A, Iuliano F, Tabilio A, et al. Effect of a p210 multipeptide vaccine associated with imatinib or interferon in patients with chronic myeloid leukaemia and persistent residual disease: a multicentre observational trial. *Lancet* 2005 Feb 19-25;365(9460):657-662.

[335] Cathcart K, Pinilla-Ibarz J, Korontsvit T, Schwartz J, Zakhaleva V, Papadopoulos EB, et al. A multivalent bcr-abl fusion peptide vaccination trial in patients with chronic myeloid leukemia. *Blood* 2004 Feb 1;103(3):1037-1042.

[336] Bocchia M, Defina M, Aprile L, Ippoliti M, Crupi R, Rondoni M, et al. Complete molecular response in CML after p210 BCR-ABL1-derived peptide vaccination. *Nat Rev Clin Oncol* 2010 Oct;7(10):600-603.

[337] Rojas JM, Knight K, Wang L, Clark RE. Clinical evaluation of BCR-ABL peptide immunisation in chronic myeloid leukaemia: results of the EPIC study. *Leukemia* 2007 Nov;21(11):2287-2295.

[338] Jain N, Reuben JM, Kantarjian H, Li C, Gao H, Lee BN, et al. Synthetic tumor-specific breakpoint peptide vaccine in patients with chronic myeloid leukemia and minimal residual disease: a phase 2 trial. *Cancer* 2009 Sep 1;115(17):3924-3934.

[339] Maslak PG, Dao T, Gomez M, Chanel S, Packin J, Korontsvit T, et al. A pilot vaccination trial of synthetic analog peptides derived from the BCR-ABL breakpoints in CML patients with minimal disease. *Leukemia* 2008 Aug;22(8):1613-1616.

[340] Smith BD, Kasamon YL, Kowalski J, Gocke C, Murphy K, Miller CB, et al. K562/GM-CSF immunotherapy reduces tumor burden in chronic myeloid leukemia patients with residual disease on imatinib mesylate. *Clin Cancer Res* 2010 Jan 1;16(1):338-347.

[341] Li Z, Qiao Y, Liu B, Laska EJ, Chakravarthi P, Kulko JM, et al. Combination of imatinib mesylate with autologous leukocyte-derived heat shock protein and chronic myelogenous leukemia. *Clin Cancer Res 2005* Jun 15;11(12):4460-4468.

[342] Rezvani K, Yong AS, Tawab A, Jafarpour B, Eniafe R, Mielke S, et al. Ex vivo characterization of polyclonal memory CD8+ T-cell responses to PRAME-specific peptides in patients with acute lymphoblastic leukemia and acute and chronic myeloid leukemia. *Blood* 2009 Mar 5;113(10):2245-2255.