Mechanisms of Disease Progression and Resistance to Tyrosine Kinase Inhibitor Therapy in Chronic Myeloid Leukemia: An Update

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Abstract: Chronic myeloid leukemia (CML) is characterized by the presence of the BCR-ABL1 fusion gene, which encodes a constitutive active tyrosine kinase considered to be the pathogenic driver capable of initiating and maintaining the disease. Despite the remarkable efficacy of tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1, some patients may not respond (primary resistance) or may relapse after an initial response (secondary resistance). In a small proportion of cases, development of resistance is accompanied or shortly followed by progression from chronic to blastic phase (BP), characterized by a dismal prognosis. Evolution from CP into BP is a multifactorial and probably multistep phenomenon. Increase in BCR-ABL1 transcript levels is thought to promote the onset of secondary chromosomal or genetic defects, induce differentiation arrest, perturb RNA transcription, editing and translation that together with epigenetic and metabolic changes may ultimately lead to the expansion of highly proliferating, differentiation-arrested malignant cells. A multitude of studies over the past two decades have investigated the mechanisms underlying the closely intertwined phenomena of drug resistance and disease progression. Here, we provide an update on what is currently known on the mechanisms underlying progression and present the latest acquisitions on BCR-ABL1-independent resistance and leukemia stem cell persistence.

Keywords: chronic myeloid leukemia; blastic phase; BCR-ABL1; tyrosine kinase inhibitors; resistance; persistence

1. Introduction

Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder arising from a multipotent hematopoietic stem cell (HSC). CML is characterized by a unique cytogenetic abnormality involving the long arms of chromosomes 9 and 22, the t(9;22)(q34;q11) translocation, resulting in a derivative 22q- traditionally known as the Philadelphia chromosome [1]. The translocation juxtaposes the c-ABL oncogene 1 (ABL1) gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22, causing the formation of the BCR-ABL1 fusion gene which encodes an oncoprotein with enhanced tyrosine kinase activity. It is believed that the acquisition of this fusion gene occurs in a single HSC that gains a proliferative advantage and/or aberrant differentiation capacity with consequent expansion of the myeloid compartment [2]. CML is characterized by a triphasic clinical course. More than 90% of CML cases are diagnosed in an early phase known as chronic phase (CP). Up to 50% of patients are asymptomatic at this stage and the disease is diagnosed by routine blood tests. Common findings at the time of diagnosis are fatigue, weight loss, abdominal fullness, bleeding, purpura, splenomegaly, leukocytosis, anemia, and thrombocytosis [1]. In this initial stage,
mature granulocytes are still produced but there is an increased pool of myeloid progenitor cells in the peripheral blood (PB). If not effectively treated, CML may progress to an accelerated phase (AP), that is variable in duration and may be followed by a blastic phase (BP), which resembles morphologically acute leukemia: myeloid and/or lymphoid differentiation stops and immature blasts accumulate in the bone marrow (BM) spreading then to tissues and organs. Twenty to 25% of patients may develop BP without going through the intermediate AP. There is currently no consensus between the European Leukemia Net (ELN) and the World Health Organization (WHO) as to the percentage of blasts concurring to the definition of BP. According to the former, BP is defined by ≥30% blasts in the bone marrow or peripheral blood or extramedullary disease apart from spleen. The WHO instead sets the percentage of blasts required for BP definition at 20% [3]. Before the advent of ABL1 tyrosine kinase inhibitors (TKIs), blasts were usually of myeloid phenotype in approximately 70% of CML-BP patients and of B-Lymphoid phenotype in approximately 30% of patients; rarely, mixed phenotypes were found. In the TKI-era, the blastic transformation of CML with mixed phenotype is getting even more rare. In the myeloid phenotype, blasts can be neutrophilic, eosinophilic, basophilic, monocytic, megakaryocytic, or erythroid [4–6]. Rarely, cases of T-lymphoid and promyelocytic-BP have been documented [7,8] and they are even more rare during TKI treatment [9,10]. Looking at gene expression profiling during the evolution, the progression from CP to advanced phases has been described as a two-step rather than a three-step process. Gene expression patterns resulted to be very similar between accelerated and blast phases and crucial steps in progression show up at the transition of CP to AP when a block of differentiation and apoptosis, alterations in cell adhesion, activation of alternative signaling pathways, and a shift toward turning on expression of genes involved in the nucleosome are observed [11].

CP CML patients can successfully be treated with ABL1 TKIs. The first one to be introduced was imatinib mesylate, which showed a high rate of responses and an acceptable side effect profile when evaluated as initial therapy for newly diagnosed CP CML in the pivotal IRIS study. However, some patients may not respond to imatinib (primary resistance) and others relapse after an initial response (secondary resistance). In a small proportion of cases, development of resistance is accompanied or shortly followed by progression to BP. The problem of resistance has been the main trigger for the development of second-generation TKIs (2G-TKIs)—first approved for imatinib-resistant patients and later as frontline therapy. Today three 2G-TKIs (bosutinib, dasatinib, and nilotinib) are available and significant clinical benefit as compared to imatinib has been demonstrated by randomized clinical trials [12–14]. A third generation TKI (ponatinib) is also available for patients with a peculiar TKI-resistant mutation in the BCR-ABL1 gene (the T315I, against whom imatinib and 2G-TKIs are ineffective; discussed in detail below) or for whom no other TKI is indicated. However, TKIs have only modestly improved survival of BP patients. Indeed, in a cohort study of 477 BP-CML patients, Jain et al. observed an overall survival (OS) of 12 months [15]. Therefore, preventing rather than counteracting disease progression is essential in an attempt to improve outcomes as well as healthcare resource utilization [15]. For almost two decades, a multitude of research studies have investigated the mechanisms underlying the closely intertwined phenomena of drug resistance and disease progression, with the aim to identify dysregulated molecules, validate new druggable targets and optimize treatment decision algorithms. This review provides a comprehensive overview of what is currently known and discusses what would deserve further investigation in an attempt to optimize patient outcomes.

2. Overview of Known Mechanisms Underlying Progression to BP

The BCR-ABL1 oncogene arises in a primitive cell named leukemia stem cell (LSC), not yet committed to either myeloid or lymphoid differentiation. The blastic clone may originate either at the level of the multipotent LSC (CD34+CD38-Lin-) or at the level of a more committed granulocyte-macrophage progenitor cell, where the activation of β-catenin [16,17] and of hairy enhancer of split 1 (Hes1) [18] appear to be among the key events responsible for the re-acquisition of self-renewal capacity and enhancement of leukemic potential.
Since the BCR-ABL1 oncoprotein is found in the cytoplasm [19], it can interact with many cellular substrates leading to abnormal pathways activation. By now, the oncoprotein is recognized to enhance proliferation activating Ras-mitogen-activated protein kinase (MAPK), to increase the transcriptional activity perturbing the JAK-STAT pathway, to decrease the apoptosis activating phosphatidylinositol 3-kinase (PI3K)/AKT pathway and to alter the expression of several genes/molecules implicated in cell adhesion and motility like L-selectin, ICAM-1, and CCR7. BCR-ABL1 is necessary for malignant transformation but not sufficient to sustain BP. As matter of fact, the expression levels of BCR-ABL1 (both at the mRNA and protein level) increase with disease progression [20,21], promoting the onset of secondary molecular and chromosomal “hits” which ultimately lead to the expansion of highly proliferating differentiation-arrested malignant cell clones. Once these “hits” have been acquired, inhibiting BCR-ABL1 alone often fails, as testified by the low efficacy of TKI treatment in the advanced phases of the disease. However, although BCR-ABL1 is likely to be the primum movens responsible for the acquisition of many of the additional hits that have been implicated in disease progression, the fact that some patients suddenly progress while on TKI treatment suggests that disease progression may also involve BCR-ABL1-independent mechanisms. Evolution from CP into BP is a multifactorial and probably multistep phenomenon, and although hundreds of studies have been performed to dissect progression mechanisms, some pieces of the puzzle are still missing. It is believed that disease progression may be triggered by a series of distinct but functionally equivalent events in different patients, whereby the increase in BCR-ABL1 transcript levels cooperate with BCR-ABL1-dependent and –independent genomic instability to determine the accumulation of key events at the DNA, RNA and protein level that converge to subvert cell cycle control, differentiation, apoptosis (Figure 1).

**Figure 1.** Overview of BCR-ABL1-dependent mechanisms. High BCR-ABL1 expression and activity in chronic myeloid leukemia (CML) implicate alteration of the normal cellular and genetic characteristics leading to transformation and progression to advanced phases.

### 3. BCR-ABL1 and Genomic Instability

The frequency of numerical and structural chromosomal changes results to be much higher in BP-CML compared with CP-CML. Studies in pediatric and adult CML patients showed that copy number alterations (CNAs) are rare or absent in CML-CP but increase upon disease progression, a sign of the increasing genomic instability [22–27].

BCR-ABL1 is considered a direct cause of genomic instability since its expression and activity are responsible for the generation of reactive oxygen species (ROS), disruption of DNA repair pathways and inhibition of DNA damage-induced apoptosis that may result in aneuploidy, chromosomal
alterations, DNA deletions and insertions, and point mutations (Figure 2). The involvement of ROS in genomic instability and CML progression has been extensively reviewed [28]. Controlling the levels of intracellular ROS is one of the mechanisms required to guarantee genomic integrity, because ROS can damage both the nucleobases and the deoxyribose incorporated in DNA as well as the free nucleotides, leading to oxidized bases and DNA double-strand breaks (DSBs). BCR-ABL1 kinase activity has been found to elevate intracellular ROS levels [29], and this was markedly more evident in BP CML cells, exhibiting higher BCR-ABL1 levels, than in CP CML cells [30]. If, on one hand, ROS levels and exogenous factors such as radiations or genotoxic compounds may enhance oxidative DNA damage, on the other hand the DNA repair machinery are deregulated by loss or gain of function in BCR-ABL1-positive cells [31]. In human cells, DSBs are preferentially repaired by homologous recombination (HR) or non-homologous end joining (NHEJ), but sometimes the highly unfaithful single-strand annealing (SSA) mechanism may operate [32]. Novicki et al. have demonstrated an enhanced activity of HR and NHEJ in the repair of ROS-mediated DSBs in BCR-ABL1 cells, where these mechanisms resulted to be unfaithful, causing mutations and large deletions. As matter of fact, it has later been demonstrated that BCR-ABL1 (non mutated and T315I mutant) may bind and phosphorylate RAD51, as well as its paralog, RAD51B, promoting unfaithful homologous HR in a dosage-dependent manner.

![Figure 2](image_url)

**Figure 2.** The role of BCR-ABL1 in the genomic instability. High levels of BCR-ABL1 are responsible for the generation of reactive oxygen species (ROS) and stimulate unfaithful DNA repair mechanisms (HRR = homologous recombination repair, NHEJ = non-homologous end-joining, SSA = single-cell annealing) thus leading to increased DNA damage.

Another key player in HR, BRCA1, has been found to be downregulated in cell lines and primary samples from patients with CP and BP [33]. Reduced BRCA1 protein levels in CML has been ascribed to BCR-ABL1-dependent inhibition of BRCA1 mRNA translation [34] and to BCR-ABL1-dependent downregulation of the BRCA1-associated protein 1 (BAP1) deubiquitinase [35].

Since 2002 an increased error-prone repair by NHEJ had been remarked in myeloid leukemia cells. The “canonical” NHEJ pathway in human cells is triggered by the recognition and binding of DSBs by Ku70 and Ku80, which recruit the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to form the functional DNA-dependent protein kinase (DNA-PK). DNA-PK serves as a bridge to mediate the ligation of DNA ends by the DNA ligase IV (LIG4) complex and activates the Artemis nuclease which is required, together with DNA polymerases mu and lambda (Pol μ and Pol λ),
to create ligatable ends [36]. However, an alternative ("backup") NHEJ, slow and error-prone, may be carried out by a different subset of proteins—including poly (ADP-ribose) polymerase (PARP) and DNA ligase IIIa (LIG3a). In CML, Artemis and LIG4 have been found to be downregulated, whereas LIG3a and the Werner helicase (WRN) are upregulated and form a novel complex acting on DSBs in BCR-ABL1-positive cells [37]. BCR-ABL1 has later been found to induce WRN mRNA and protein expression by c-MYC-dependent activation of transcription and BCL-Xl-dependent inhibition of caspase cleavage, respectively [38]. BCR-ABL1 can complex with and phosphorylate WRN, stimulating its helicase and hexonuclease activities [38] increasing the infidelity of NHEJ and enhancing the activity of the more unfaithful HR and SSA. SSA mediates the annealing of complementary single strands formed after extensive resection at DSBs until repeated sequences are detected. BCR-ABL1 has been reported to stimulate SSA by upregulating RAD52 and ERCC1, which may also result in an accumulation of chromosomal aberrations such as translocations, partial deletions (e.g tumor suppressor genes) and duplications.

The mismatch repair (MMR) system has the role to maintain genomic stability by detecting misincorporated nucleotides, by acting an excision repair before point mutations emerge and by inducing cell apoptosis in case of unrepairable DNA damage. Following the observation that a certain degree of microsatellite instability can be observed at multiple loci in BP-CML differently to CP-CML [39], Stoklosa et al. have demonstrated that the BCR-ABL1 kinase is responsible for reduced MMR activity. The efficacy of MMR was reduced ~2-fold in BCR-ABL1-positive cell lines and in CD34+ CML cells as compared to normal counterparts. Impaired MMR activity in leukemia cells was associated with accumulation of p53 but not of p73, and lack of activation of caspase 3 after treatment with DNA damage-inducing agents. This resulted in a 15-fold increase in mutation rate in BCR-ABL1-positive cells. The resulting "mutator phenotype" may be the primary responsible for the accumulation of point mutations in the BCR-ABL1 kinase domain (KD) sequence and in other key genes, causing TKI-resistance, and promoting the disease progression. Defective base excision repair (BER) may also contribute to the accumulation of point mutations. BER is primarily responsible for the correction of DNA damage by oxidation, alkylation, and deamination processes. In the stem/progenitor cell compartment, BCR-ABL1 has been found to inhibit in a kinase-dependent manner the activity of uracil-N-glycosylase 2 (UNG2), one of the key enzymes that initiates BER by recognizing and eliminating the damaged DNA bases [40].

4. Additional Chromosomal Abnormalities

In Philadelphia-positive clones, the classical t(9;22)(q34;q11) can be found in combination with secondary chromosomal aberrations known as additional chromosomal abnormalities (ACAs) [41]. The presence of ACAs can be observed at diagnosis in ~5% or less of CP CML patients as demonstrated by several studies [42–44]. The European LeukemiaNet recommendations suggest that the presence of ACAs at diagnosis should be considered a "warning" sign for patients in early CP, requiring a closer monitoring of this subset of patients. The appearance of ACAs on treatment is well known as clonal evolution (CE) and it has been associated with disease evolution since ACAs frequency is higher in advanced phases, AP (~30%) and BP (~80%) [45–47]. However, ACAs constitute a heterogeneous collection of karyotype abnormalities with different frequency and different prognostic significance. Mitelman classified them into “major” and “minor” route chromosomal abnormalities according to the frequency of the pathways of cytogenetic evolution followed by CML cells during disease progression. The most common ACAs involved in karyotype evolution are trisomy 8 (the most frequent), a second Ph chromosome, an isochromosome 17 (i(17q)) and trisomy 19. These aberrations are called “major route changes” [48,49]. At least one of these four major karyotypic changes is found in 71% of CML cases with cytogenetic abnormalities in addition to the Ph chromosome [49]. Some among the infrequent cytogenetic changes are trisomies of chromosomes 17 and 21, monosomies of chromosomes 7, 17 and 21, translocations t(3;12), t(4;6), t(2;16), and t(1;21). They are known as “minor route changes” and at least one of them occurs in 15% of all Ph-positive CML patients with additional
cytogenetic abnormalities [49]. The lack of the Y chromosome, observed in 5% of Ph+ patients [49], has also been recognized as a minor route ACA. The prognostic significance of ACAs in CP CML patients at diagnosis is still debated. Whereas some studies associated the presence of ACAs in general [50–52] or major route abnormalities to worse progression-free survival and/or overall survival [53–55] others failed to find any significantly association [8,56].

5. Gene Mutations and Submicroscopic Genetic Abnormalities

The most frequently mutated gene in CML is BCR-ABL1 itself. Point mutations within the ABL1 KD may indeed be selected during TKI therapy, leading to treatment failure. Mutations disrupt critical contact residues between the TKIs and their target, or induce a shift to a conformation that TKIs may be unable to bind (e.g., from the closed to the open conformation of the kinase, that imatinib and nilotinib cannot recognize). The frequency of BCR-ABL1 KD mutations [57] is relatively low (25% to 30%) in CP patients on first-line therapy but increases up to 70%–80% in BP patients [58,59]. BP patients, much more frequently than CP patients, may accumulate multiple BCR-ABL1 KD mutations as a result of sequential therapy with TKIs. Multiple mutations arising in the same molecule (compound mutations) can be observed in more than 30% of BP patients and are particularly challenging [9,60–63]. In vitro studies [64] have shown that they are highly resistant to 2GTKIs and in some cases even to ponatinib. Beyond BCR-ABL1 KD point mutations, other genetic lesions have been described with different patterns in myeloblastic and lymphoblastic transformations. The most common detectable gene alterations “historically” reported in myeloid BP cases (20% to 30%) before the advent of high throughput technologies, involved the tumor suppressor gene TP53, mapping at 17p13.1 [65]. These alterations usually consist in the loss of one TP53 allele (e.g., as a consequence of structural alterations like i(17p)) and in point mutations in the coding sequence of the remaining allele [66]. The p53 tumor suppressor is the master regulator of the DNA damage checkpoint. Mice injected with p53-deficient BCR-ABL1-expressing bone marrow cells developed a more aggressive disease than those injected with the wild-type p53 counterpart [67]. Blastic transformation was also observed in a transgenic model in which mice expressing BCR-ABL1 under the control of the Tec promoter were crossed with p53-heterozygous (p53+/-) mice [68]. Functional p53 has been involved in imatinib-induced apoptosis [69]. RB1 inactivating mutations or deletions are the second most frequent genetic abnormality detected in myeloid BP (20% of cases) [70,71]. The RB tumor suppressor protein restricts cell cycle progression at the G1/S checkpoint until a cell is ready to divide. Point mutations within the DNA-binding region of the RUNX1 gene were first described in association with trisomy 21 in some myeloid BP [72]. A later study in a large cohort of Chinese CML-AP and CML-BP patients identified missense, nonsense, and frameshift RUNX1 mutations displaying a reduced transactivation activity and/or a dominant-negative function over the wild-type RUNX1 in 12% of the cases [73]. The RUNX1 gene, located on chromosome 21q22, encodes the major subunit of the heterodimeric core binding factor (CBF) complex, a transcription factor essential for myeloid differentiation. In a mouse model, runx1 mutations were found to perturb myeloid differentiation and induce an AP or BP-like phenotype [73]. Chromosomal translocations involving the RUNX1 gene have also been reported in BP-CML. The t(3;21)(q26;q22) translocation can be observed in BP CML patients and also in therapy-related AML and myelodysplasia (MDS) [74–78]. Consequently to this translocation, RUNX1 resulted to be fused with EAP, EVII and MDS, or both MDS1 and EVII. EVII (also known as MECOM or PRDM3) plays an important role as regulator of self-renewal in hematopoietic stem cells [79]. A study in a transgenic zebrafish model showed the capability of this fusion gene to inhibit myeloid cell differentiation and to induce resistance to apoptosis [80].

In lymphoid BP, one of the most frequent abnormalities (detected in approximately 50% of cases), known even before the advent of genome-wide technologies [57,81,82] is represented by monoallelic or biallelic deletions of the CDKN2A/B locus, encoding the tumor suppressors and cell cycle regulators INK4A, INK4B, and ARF. The CDKN2A and CDKN2B genes encode p16INK4A and p15INK4B, which are inhibitors of cyclin-dependent kinases. In addition, transcription of an alternate reading frame
of the CDKN2A locus produces p14ARF, which antagonizes the p53 ubiquitin ligase, MDM2. Thus, the INK4-ARF locus regulates both RB and p53 function to prevent an inappropriate progenitor cell self-renewal and to eliminate cells driven by oncogenic signaling [83].

The recent advent of high throughput technologies like DNA microarrays and next generation sequencing (NGS) has contributed to further unravel the genetic/genomic complexity of BP cells. Array-CGH and SNP-arrays [22–26,84,85]; have highlighted the presence of multiple recurrent submicroscopic genomic lesions in myeloid and lymphoid BP. In the latter, as well as in acute lymphoblastic leukemias, such methodologies have enabled the characterization of IKZF1 loss [86]. The IKZF1 gene on chromosome 7 encodes IKAROS, a transcription factor essential for lymphoid development. Detected early in hematopoietic development, IKAROS expression plays a key role in shutting down stem-cell programs and driving cells towards lymphoid development [87,88]. Monoallelic and bi-allelic deletions of the whole gene or focal in-frame deletions of exons 4 to 7, creating a dominant negative isoform (Ik6) lacking the DNA-binding domain have been reported [86,89–91].

NGS has also contributed to pinpoint sequence and structural alterations associated with TKI-resistance and disease progression. An IDH2 mutation was detected by whole transcriptome sequencing in a CML case who had progressed to lymphoid BP while on nilotinib treatment and subsequently reported in 3/75 myeloid BP and 1/31 lymphoid BP patients [92]. No IDH1 mutation emerged in that study. Using a targeted NGS approach to screen for mutations 12 candidate genes in 39 BP cases, Grossman et al. reported mutations in RUNX1, IKZF1, ASXL1, WT1, TET2, CBL, IDH1, NRAS, KRAS, and TP53 overall occurring in 76.9% of the patients. RUNX1, ASXL1 and IKZF1 had the highest mutation rate [93]. Makishima et al. screened Janus kinase (JAK)-2, CBL, CBLB, TET2, ASXL1, IDHI, and IDH2 coding sequences in 26 BP patients, identifying four TET2, two ASXL1, two IDH1/2, one CBL and one CBLB mutations in myeloid BP [94]. A whole exome sequencing analysis of 10 matched CP-BP samples has more recently uncovered that UBE2A mutations may recurrently (16.7% of cases in the validation cohort) be acquired at the time of progression [95]. Functional studies have revealed that mutations abrogate UBE2A activity, leading to an impairment of myeloid differentiation [95]. At present, the largest study taking advantage of an integrated approach of whole-exome sequencing, copy-number variation (CNV) analysis and RNA sequencing in CML patients has been performed by Branford et al. [96]. This study has revealed one or multiple mutations in all BP patients tested, CNVs in 90% of cases and gene fusions other than BCR-ABL1 in 42% of cases [96]. Genes affected by missense, nonsense, frameshift, or splice site mutations included BCR-ABL1, ASXL1, RUNX1, IKZF1, BCOR, BCorL1, GATA2, PHF6, SETD1B, SETD2, U2AF1, IDH1/2, KMT2D, and XPO1. Among CNVs, a novel recurrent 60-Kb deletion comprising exons 1 to 2 of the HBS1L gene and an intergenic sequence between HBS1L and MYB was detected in 3 of 10 patients with lymphoid BP. Known and novel fusions were also detected by RNA-seq. MLL (KMT2A) fusions (some of whom were cytogenetically cryptic) were the most frequent. The other known fusions were CBFB-MYH11 and PAX5-ZCCHC7. Novel fusions resulting from intra-or inter-chromosomal rearrangements and mostly cytogenetically cryptic involved cancer genes implicated in hematologic malignancy: RUNX1, IKZF1, MECOM, and MSI2. They included RUNX1-MXI, MBNL1-MECOM, and MSI2-PRMT2. When matched CP and BP samples from the same patient could be analyzed, some gene mutations like ASXL1, IKZF1 and SETD1B were found to precede disease progression [96].

6. Non Genomic Loss of Tumor-Suppressor Function

In BP-CML, loss of function of key tumor suppressors may also be mediated by events not acting at the genomic level. The best example is represented by protein phosphatase A (PP2A). PP2A is one of the major cellular serine-threonine phosphatases and is directly involved in a multitude of cellular processes by dephosphorylating a wide variety of key target proteins (MYC, BCL-2, MEK/ERK, AKT, RB, JAK2, and β-catenin), hence providing a negative feedback to signals triggers and sustained by protein kinases [97]. As such, it has been shown to negatively regulate G2 to M transition, inhibit mitogenic signals, promote apoptosis, and interfere with WNT signaling. Inactivation of PP2A at the genetic or
functional level has been shown to play a crucial role in cancer progression. In CML, PP2A inhibition is accomplished mainly through the formation of an inhibitory complex with the phosphoprotein SET. Neviani et al. have shown that, in CML BP progenitors, SET expression is markedly upregulated by BCR-ABL1 in a dose and kinase-dependent manner via induction of hnRNP A1 [98]. Direct phosphorylation of one of PP2A subunits by BCR-ABL1 itself or by other kinases like JAK2 or SRC may also contribute to the suppression of its phosphatase activity. BCR-ABL1 and PP2A share several targets. Among these, MYC, STAT5, MAPK, AKT, BAD and RB are essential for BCR-ABL1-mediated leukemogenesis and have been found altered in BP. Indeed, in BCR-ABL1-expressing myeloid progenitor 32Dc13 cells, inhibition of SET expression or forced expression of PP2A decreased MYC expression, increased the levels of proapoptotic BAD and led to inhibition of MAPK, STAT5 and AKT phosphorylation thus inducing growth suppression, enhancing apoptosis and restoring differentiation. Additionally, PP2A-induced activation of the SHP-1 tyrosine phosphatase has been shown to promote BCR-ABL1 tyrosine dephosphorylation (inactivation) which, in turn, triggers its proteasome-dependent degradation in CML BP progenitor patient cells [98].

7. Differentiation Arrest

In CML, the transition from CP to BP is characterized by a progressively increasing impairment of myeloid blast differentiation which determines the stack of myeloid progenitors. Both BCR-ABL1-dependent and BCR-ABL1-independent mechanisms interfering with differentiation pathways have been described. For example, the marked downregulation of the lineage-specific transcription factor CCAAT-enhancer binding protein alpha (C/EBPα) induced by high BCR-ABL1 levels plays a crucial role in the arrest of myeloid cell development [99]. Indeed, C/EBPα expression rises with the commitment of multipotential precursors to myeloid differentiation: its upregulation drives the granulopoiesis while its downregulation contributes to monopoiesis [100]. As a result of genetic and epigenetic alterations, C/EBPα activity may be repressed at different levels in myeloid leukemias. As matter of fact, the increase in BCR-ABL1 expression, marking the transition from CP to BP, is associated with suppression of C/EBPα expression at the translational level. Perrotti et al. showed that in myeloid precursor 32Dc13 cells transfected with BCR-ABL1 and in primary bone marrow cells from individuals with CML-BP, suppression of C/EBPα protein expression is due to the interaction between CEBPA mRNA with the RNA-binding protein hnRNP E2 [99]. hnRNP E2 and C/EBPα expressions resulted to be inversely correlated: the first one was undetectable in normal marrow and in CML-CP mononuclear cells, which regularly express C/EBPα. Conversely, hnRNP E2 expression was high in CML-BP samples lacking C/EBPα [99]. hnRNP-E2 is post-translationally induced by BCR-ABL1 in a dose and kinase-dependent manner through constitutive activation. ERK1/2 phosphorylate hnRNP-E2, increasing protein stability [101,102]. Following the observation of an inverse correlation between miR-328 and hnRNP E2 expression and between miR-328 and BCR-ABL1 expression and activity in CML-BP progenitors, it was later found that the BCR-ABL1/MAPK/hnRNP-E2/C/EBPα network also involves miR-328 [103]. miR-328 was found to compete with C/EBPα mRNA for binding to hnRNP-E2, thus interfering with hnRNP-E2-mediated inhibition of C/EBPα translation, and to silence the expression of the pro-survival PIM1 kinase through the interaction with its 3′-UTR. Additionally, C/EBPα was found to bind miR-328 promoter, thus enhancing its transcription. It was thus demonstrated that BCR-ABL1 uses the MAPK/hnRNP-E2/C/EBPα pathway to suppress both C/EBPα protein expression and miR-328 transcription [103]. Additional mechanisms acting at the genetic level may contribute to the arrest of myeloid or lymphoid development. As detailed in the chapters above, many recurrent mutations and copy number alterations both in myeloid and in lymphoid BP involve transcription factors regulating myeloid (GATA2, UBE2A, RUNX1) or lymphoid (CDKN2A, 1karos) differentiation.
8. Methylation Changes

Epigenetic changes are known to cooperate with genetic events in human tumorigenesis. Although aberrant hypermethylation in CML had previously been described [104–109], the role of altered DNA methylation in CML progression is still to be deeply investigated. There is a strong evidence that methylation of CpG islands (CGIs) is associated with transcriptional gene silencing. In this regard, Jelinek et al. studied the methylation status of promoter-associated CpG island of 10 genes (ABL1, CDH13, DPYS, CDKN2B, OSCIP1, PGR-A, PGR-B, TFAP2E, NPM2, PDLIM4) in CML patients observing a remarkable methylation increase underlying the progression from AP to BP [110]. A correlation between an increased methylation status of these genes and resistance or intolerance to imatinib was also found [110]. Hypermethylation of the tumor suppressor PDLIM4 was found to be a negative prognostic factor independent from imatinib response and from CML phase [110]. Thank to the high throughput of NGS, Heller et al. [111] investigated the methylome of CML patients in CP, AP, and BP as compared to controls. CpG site methylation was found to increase dramatically (up to 58,691 differentially methylated CpG sites) during progression from CP to AP/BP. Interestingly, they were able to localize the CpG sites associated with a higher methylation status in the advanced CML stages in or around CGIs, but also in the binding sites of specific transcription factors. Taking advantage of RNA-seq, they were also able to identify the genes that are transcriptionally regulated by methylation in BP samples. Some of these genes were known to be implicated in the pathogenesis of various malignancies, but their exact role in CML progression remains to be further investigated.

9. Perturbations in RNA Transcription, Editing and Translation

The increased BCR-ABL1 expression observed in BP CML has also been linked to an aberrant regulation of processing, nuclear export and translation of mRNA. Aberrant expression of various RNA-binding proteins (RBPs) with post-transcriptional and translational regulatory roles has been observed in mouse models and primary blasts from CML patients. RNA-binding proteins regulate transcription, maturation, nucleocytoplasmic transport, RNA stability and translation; some of them are general regulators while others recognize given mRNAs in a sequence-specific manner. Besides the already mentioned interplays between hnRNP E2 and C/EBPα, and between hnRNP-A1 and SET/PP2A, other RBPs that have been implicated in CML progression are: HNRPK, which binds the internal ribosome entry site (IRES) element of MYC mRNA and enhances its translation in a BCR-ABL1 and MAPK-dependent manner [101]; La/SSB, which enhances MDM2 translation contributing to p53 inactivation [112]; FUS/TLS, which binds the granulocyte colony-stimulating factor receptor (G-CSFR) pre-mRNA in the nucleus and interferes with its processing and export [113]; and CUGBP1, whose loss results in a reduced translation of C/EBPβ [114]. hnRNP-A1 has also been found to bind the mRNA encoding the E2F3 transcription factor [115] leading to its upregulation in BP CML progenitors [116,117]. Different BCR-ABL1-dependent mechanisms underlie the aberrant expression and/or function of RBPs, some of whom involve phosphorylation events by PI-3K/AKT, ERK or PKC. Overall, these phenomena contribute to differentiation arrest and resistance to apoptosis of BP-CML progenitors by either the loss of function of tumor suppressors, upregulation of oncogenes, or altered expression of regulatory factors essential in differentiation processes.

There is also evidence that BCR-ABL1 interferes with the efficiency of the basal machinery responsible for mRNA translation. The latter is accomplished via regulation of the mTOR and S6 kinase pathway and of the EIF4E e EIF4E-BP translation factors [116,117].

More recently, a massively parallel RNA sequencing approach has revealed an increased expression of the adenosine deaminase acting on dsRNA-1 (ADAR-1) in progenitor cells obtained from primary BP CML patient samples. ADAR-1 performs adenosine to inosine (A to I) RNA editing of double strand RNA hairpins formed between Alu repetitive elements, thus altering mRNA structure, generating or abolishing donor and acceptor splice sites and introducing sequence alterations. Functional studies demonstrated that ADAR-1-mediated RNA editing plays a role in the malignant reprogramming of myeloid progenitors into LSC that drive CML progression, by promoting the expression of the myeloid...
transcription factor PU.1 and producing misspliced isoforms of GSK3β [118]. The increased expression and activity of ADAR-1 was found to be mediated by inflammatory cytokine-activated JAK/STAT signaling as well as by BCR-ABL1 signaling [119]. Other ADAR-1 targets were found to be MDM2, APOBEC3D, GLI1 and AZIN [120] and the miRNA Let-7 [119]. In particular, ADAR-1 hyper-editing of the 3′-UTR of MDM2 prevents miRNA binding, thus resulting in increased MDM2 expression and repression of the p53 tumor suppressor [118].

10. The Role of MicroRNAs

MicroRNAs (miRNAs) are known to play an essential role in tumorigenesis [121–123] by post-transcriptional regulation of gene expression. Aberrant expression of several miRNAs has been described in CML, in association with stem cell survival and self-renewal, sensitivity or resistance to TKI therapy, and disease progression. In the latter context, a microarray analysis revealed differential expression profiles of several miRNAs—namely, the up-regulation of miR-19a, miR-19b, miR-17, miR-20a, miR-92a, miR-221, miR-222, miR-126, miR-146a, miR-181a, miR-181b, let-7c, and miR-155 and the down-regulation of miR-150, miR-452, miR-103, and miR-144—in BP samples [124]. The association between reduced miR-150 level, myeloid differentiation block and resistance to TKIs was later found to be due to a novel mechanism whereby BCR-ABL1 recruits MYC to bind and inhibit miR-150 expression [125].

As previously mentioned, another key miRNA is miR-328, that is involved in C/EBPα downregulation and in the subsequent block of myeloid differentiation [99,103].

11. Metabolic Changes

All cancer cells develop an altered metabolism to support their growth and survival. Recently, it has been suggested that increased branched chain amino acid (BCAAs; valine, leucine, and isoleucine) metabolism may contribute to disease progression in CML. Following the observation that mice with BP CML display higher levels of proline and BCAAs, Hattori et al. found a significant upregulation of branched chain amino acid aminotransferase 1 (BCAT1) at the mRNA and protein levels in BP-CML as compared to CP-CML or healthy mice [126]. Similarly, patients with BP-CML showed higher BCAT1 expression as compared to CP CML patients. BCAT1 is a cytosolic aminotransferase that may generate BCAAs from branched chain ketoacids. BCAAs, particularly leucine, activate the mTORC1 pathway, which promotes cell growth as a result of the integration of nutrient sensing, energy status, stress and growth factors [127]. BCAT1 upregulation was found to result from association with Musashi 2 (Msi2), an oncogenic RNA binding protein that post-transcriptionally regulates gene expression and that was already known to be upregulated and to cooperate in disease progression via repression of the cell fate regulator Numb [128,129].

12. TKI Resistance and LSC Persistence: Two Sides of the Same Coin?

Three levels of response to therapy are routinely defined in CML: hematological response, cytogenetic response (reduction in the percentage of BM metaphases positive for the Philadelphia chromosome) and molecular response (reduction in BCR-ABL1 transcript levels, measured in terms of logarithmic reduction from a standardized baseline) [30,130]. Nowadays, the term “resistance” is used to label a wide and heterogeneous spectrum of “non optimal” levels of response to TKI therapy ranging from failure to achieve a major molecular response (MMR; defined as BCR-ABL1 transcript levels at or below 0.1%) or increase in BCR-ABL1 transcript levels leading to a loss of MMR, to a frank loss of hematological response. This is essentially due to the fact that patients’ and physicians’ expectations regarding treatment endpoints have increased greatly since the advent of TKIs. If, in the early days of the imatinib era, the endpoints were still ‘limited’ to improving the overall survival and delaying disease progression, it was soon realized that molecular responses could be achieved in a significant proportion of patients and correlated with superior outcomes (hence the definition of MMR as “safe haven”) [131]. More recently, the endpoint has become even more ambitious—deep molecular
response (DMR; defined as BCR-ABL1 transcript levels at or below 0.01%), because it correlates with optimal long term survival outcomes and because it is the pre-requisite for treatment discontinuation. The fact that very low levels of residual disease may still be detected after many years of therapy in a not negligible proportion of patients, and that even when molecularly undetectable the disease may often re-emerge after TKI discontinuation is referred to as “persistence”. The mechanisms responsible for resistance are likely to be at least in part different from those underlying disease persistence at the molecular level. Resistance mechanisms have extensively been reviewed elsewhere [132–134]. In an attempt to fill the gaps in knowledge about BCR-ABL1-independent mechanisms that may enable Ph+ cells to survive despite TKI-mediated BCR-ABL1 inhibition, recent research studies have highlighted a number of pathways/molecules that might also be therapeutically targeted (summarized in Table 1).

Table 1. Overview of the molecules and pathways that have been implicated in BCR-ABL1-independent resistance.

| Gene/Pathway         | Ref                      | Druggable?                  |
|----------------------|--------------------------|----------------------------|
| PI3K/AKT/mTOR        | Burchert, Leukemia 2005 [135] | PI3K/mTOR inhibitors       |
| Lyn                  | Wu et al., Blood 2008 [136] | Src inhibitors             |
| Autophagy            | Bellodi et al., J Clin Invest 2009 [137] | hydroxychloroquine |
| SHP-1                | Esposito et al., Blood 2011 [138] | X                          |
| SIRT1                | Wang et al., Oncogene 2013 [139] | selisistat                 |
| PRKCH                | Ma et al., Sci Transl Med 2014 [140] | MEK inh (trametinib) + Imatinib |
| STAT3                | Eiring et al., Leukemia 2015 [141] | BP-5-087                   |
| CRM1/XPO1/RAN        | Khorashad et al., Blood 2015 [142] | selinexor                  |
| JAK2                 | Chakraborty et al., Genes Cancer 2016 [144] | ruxolitinib               |
| FOXO1                | Wagle et al., Leukemia 2016 [145] | PI3K inhibitors           |
| EZH2                 | Scott et al., Cancer Discov 2016 [146] | EZH2 inhibitors           |
| Wnt/β-catenin        | Eiring et al., Leukemia 2015 [141], Zhou et al., Leukemia 2017 [147] | C82                        |
| MS4A3                | Eiring et al., ASH 2017 [148] | X                          |
| PFKFB3               | Zhu et al., Oncogene 2018 [149] | PFK-158                    |
| Various miRNAs       |                          | X                          |

Persistence is thought to be mainly attributable to the fact that TKIs may eliminate differentiated and progenitor cells, but spare LSCs. BCR-ABL1-positive primitive cells are indeed detectable in the BM in patients with MMR and even DMR [150–154]. The fact that LSCs are intrinsically insensitive to TKIs was observed early after the introduction of first and second-generation TKIs [150,155–157]. Later on, a series of experiments in cell lines and mouse models suggested that LSC are not dependent from BCR-ABL1 kinase activity for their survival, and that BCR-ABL1 may possess non kinase-mediated functions influencing signaling pathways responsible for LSC survival [157–160]. It has also been hypothesized that while BCR-ABL1 expression is high in pre-therapy CD34+ cells, TKIs select for CML precursors with low BCR-ABL1 expression and signaling, hence less oncogene-addicted [161,162]. This has sparked searches for LSC-selective BCR-ABL1 kinase-independent pathways. To date, a number of cell- intrinsic and cell extrinsic pathways and mechanisms have been suggested to contribute to the TKI-resistant LSC phenotype, and many of them are potentially druggable. Among the cell-intrinsic mechanisms, Foxo [163,164], Sonic Hedgehog [165,166] and Wnt/β-catenin [167,168] pathways are the most extensively investigated ones. However, interaction with the BM microenvironment is thought to be equally critical for LSC survival. CD44/E-selectin [169,170], galectin-3 (Gal-3) [171], CXCR4/CXCL12 [172] have all been suggested to enhance LSC self-renewal. Last but not least, it has been hypothesized that modulation of host immune surveillance in the BM microenvironment may have a role in preventing LSC eradication. In this regard, cytotoxic T lymphocytes exhaustion via interaction of the PD-1 receptor expressed on cytotoxic T lymphocytes with its inhibitory ligand PD-L1 expressed on CML cells has been observed [173,174]. Cytokine-mediated downregulation of
Major Histocompatibility Complex class II (MHC-II) molecule expression has been reported to be an alternative way for LSCs to evade immune surveillance [175]. All these mechanisms are summarized in Figure 3.

![Figure 3. Persistence of LSCs: BCR-ABL1 kinase-dependent and -independent pathways.](image)

Schematic representation of the key pathways and molecules known to be implicated in LSC persistence in CML. a) Foxo3a plays a key role in the TGF-β signalling pathway driving the survival of leukemia initiating cells (LICs) during TKI treatment. In LICs, despite BCR-ABL1 expression in all CML cells, Akt is inactivated and as consequence, the Foxo proteins are retained in an active form in the nucleus inducing transcription and maintenance of LSCs. TGF-β signalling is activated in CML LICs, where it controls Foxo localization.

b) In the Sonic/Hedgehog pathway, Smo is upregulated in BCR-ABL1-positive LSCs. On the other hand, it is not necessary for the long-term regeneration of LSCs.

c) BCR-ABL1-positive LSCs are more dependent from an higher expression of CD44 for homing and engraftment as compared to the HSCs.

d) Reduced CXCL12 expression in BM stromal cells determines an impaired LSC homing and BM retention.

e) High levels of Gal-3 expression in BM support disease maintanence, multidrug resistance to TKIs and long-term BM lodgment of CML cells.

f) Expression of β-catenin in LSCs determines an increased growth and a reduced differentiation.

g) The interaction between the PD-1 receptor expressed on CTLs and the inhibitory ligand PD-L1 present on CML cells enables the latter to escape from immune surveillance.

h) The antitumor-associated immune response exercised by CD4+ T helper cells is prevented by the downregulation of the MHCII. Abbrevations: LSC (leukemia stem cell), OB (osteoblast), OC (osteoclast), SC (staminal cell), HSC (hematopoietic stem cell), CTL (cytotoxic T lymphocyte), LIC (leukemia initiating cell).

13. Conclusions and Future Perspectives

Though rare, resistance to TKI therapy and disease progression remain a concern for physicians and patients. Whenever resistance occurs, reactivation of BCR-ABL1 kinase activity represents a “fire under the ashes” that may ultimately lead to the evolution from CP to BP—which represents a clinical emergence even in the era of TKIs. The lack of a unifying path of disease progression and the profound molecular heterogeneity of BP CML patients limit the effectiveness of therapeutic options once BP is diagnosed—which strongly underline the importance of preventing rather than treating
progression. High risk patients may be identified using scores like Sokal or EUTOS [3]. Major route ACAs may also help identify patients at greater risk of progression [53]. Some biological factors have also been proposed in single studies (e.g., germline variants in ASXL1 and BIM genes [176,177]; CIP2A protein levels at diagnosis [178]). So far, however, no validated prediction algorithm has ever been derived that may soundly predict who will develop BP. Thus, strict MR monitoring and thoughtful TKI therapy reassessment in case of failure to achieve the key response milestones are currently the only way to minimize the risk of progression.

Even in patients achieving deep molecular responses to TKI therapy, LSCs have been reported to persist, constituting a dangerous reservoir that may feed disease recurrence in case of treatment discontinuation. Twenty years of research have resulted in a wealth of biological acquisitions. However, very few clinical trials evaluating the combination of TKIs with agents aimed at selectively targeting LSC are underway [179]. Hence it is currently difficult to predict whether a successful and well-tolerated eradication strategy will ever be defined.

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References
1. Faderl, S.; Talpaz, M.; Estrov, Z.; O’Brien, S.; Kurzrock, R.; Kantarjian, H.M. The biology of chronic myeloid leukemia. N. Engl. J. Med. 1999, 341, 164–172. [CrossRef] [PubMed]
2. Bruns, I.; Czibere, A.; Fischer, J.C.; Roels, F.; Cadeddu, R.P.; Buest, S.; Bruennert, D.; Huenerlituerkoglu, A.N.; Stoecklein, N.H.; Singh, R. The hematopoietic stem cell in chronic phase CML is characterized by a transcriptional profile resembling normal myeloid progenitor cells and reflecting loss of quiescence. Leukemia 2009, 23, 892. [CrossRef] [PubMed]
3. Baccarani, M.; Deininger, M.W.; Rosti, G.; Hochhaus, A.; Soverini, S.; Apperley, J.F.; Cervantes, F.; Clark, R.E.; Cortes, J.E.; Guilhot, F. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood 2013, 122, 872–884. [CrossRef] [PubMed]
4. Ch’ang, H.-J.; Tien, H.-F.; Wang, C.-H.; Chuang, S.-M.; Chen, Y.C.; Shen, M.-C.; Lin, D.-T.; Lin, K.-H. Comparison of clinical and biologic features between myeloid and lymphoid transformation of Philadelphia chromosome positive chronic myeloid leukemia. Cancer Genet. Cytogenet. 1993, 71, 87–93. [CrossRef]
5. Jaffe, E.S.; Harris., N.L.; Stein, H.; Vardiman, J.W. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues, WHO Classification of Tumours, 3rd ed.; IARC Press: Lyon, France, 2001; Volume 3.
6. Reid, A.G.; De Melo, V.A.; Elderfield, K.; Clark, I.; Marin, D.; Apperley, J.; Naresh, K.N. Phenotype of blasts in chronic myeloid leukemia in blastic phase—Analysis of bone marrow trephine biopsies and correlation with cytogenetics. Leuk. Res. 2009, 33, 418–425. [CrossRef] [PubMed]
7. Dorfman, D.M.; Longtine, J.A.; Fox, E.A.; Weinberg, D.S.; Pinkus, G.S. T-cell blast crisis in chronic myelogenous leukemia: Immunophenotypic and molecular biologic findings. Am. J. Clin. Pathol. 1997, 107, 168–176. [CrossRef] [PubMed]
8. Schoch, C.; Haferlach, T.; Kern, W.; Schnittger, S.; Berger, U.; Hehlmann, R.; Hiddemann, W.; Hochhaus, A. Occurrence of additional chromosome aberrations in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia 2003, 17, 461. [CrossRef]
9. Gozzetti, A.; Bocchia, M.; Calabrese, S.; Pirrotta, M.T.; Crupi, R.; Raspadori, D.; Lauria, F. Promyelocytic blast crisis of chronic myelogenous leukemia during imatinib treatment. Acta Haematol. 2007, 117, 236–237. [CrossRef]
10. Angriman, F.; Gutierrez Acevedo, M.N.; Rossi, M.S.; Gimenez Conca, A.D.; Otero, V.; Arbelbide, J.A.; Michelangelo, H. Promyelocytic Blast Crisis in Chronic Myeloid Leukemia during Imatinib Treatment. Turk. J. Haematol. 2015, 32, 193–194. [CrossRef]
11. Radich, J.P.; Dai, H.; Mao, M.; Oehler, V.; Schelter, J.; Druker, B.; Sawyers, C.; Shah, N.; Stock, W.; Willman, C.L. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc. Natl. Acad. Sci. USA 2006, 103, 2794–2799. [CrossRef]
12. Cortes, J.E.; Saglio, G.; Kantarjian, H.M.; Baccarani, M.; Mayer, J.; Boqué, C.; Shah, N.P.; Chuah, C.; Casanova, L.; Bradley-Garelik, B. Final 5-year study results of DASISION: The dasatinib versus imatinib
study in treatment-naïve chronic myeloid leukemia patients trial. J. Clin. Oncol. 2016, 34, 2333. [CrossRef] [PubMed]

13. Hochhaus, A.; Saglio, G.; Hughes, T.P.; Larson, R.A.; Kim, D.W.; Issaragrisil, S.; Le Coutre, P.D.; Etienne, G.; Dörhöf-Llacar, P.E.; Clark, R.E. Long-term benefits and risks of frontline nilotinib vs imatinib for chronic myeloid leukemia in chronic phase: 5-year update of the randomized ENESTnd trial. Leukemia 2016, 30, 1044. [CrossRef] [PubMed]

14. Cortes, J.E.; Gambacorti-Passerini, C.; Deininger, M.W.; Mauro, M.J.; Chuah, C.; Kim, D.-W.; Dyagil, I.; Glushko, N.; Miloškovic, D.; le Coutre, P. Bosutinib versus imatinib for newly diagnosed chronic myeloid leukemia: Results from the randomised BORIDE trial. J. Clin. Oncol. 2018, 36, 231. [CrossRef] [PubMed]

15. Jain, P.; Kantarjian, H.M.; Ghorab, A.; Sasaki, K.; Jabbour, E.J.; Nogueras Gonzalez, G.; Kanagal-Shamanna, R.; Issa, G.C.; Garcia-Manero, G.; Delassala, S.; et al. Prognostic factors and survival outcomes in patients with chronic myeloid leukemia in blast phase in the tyrosine kinase inhibitor era: Cohort study of 477 patients. Cancer 2017, 123, 4391–4402. [CrossRef] [PubMed]

16. Jamieson, C.H.M.; Ailles, L.E.; Dylla, S.J.; Muijtjens, M.; Jones, C.; Zehnder, J.L.; Gotlib, J.; Li, K.; Manz, M.G.; Keating, A. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N. Engl. J. Med. 2004, 351, 657–667. [CrossRef] [PubMed]

17. Minami, Y.; Stuart, S.A.; Ikawa, T.; Jiang, Y.; Banno, A.; Hunton, I.C.; Young, D.J.; Naoe, T.; Murre, C.; Jamieson, C.H.M. BCR-ABL-transformed GMP as myeloid leukemic stem cells. Proc. Natl. Acad. Sci. USA 2008, 105, 17967–17972. [CrossRef]

18. Nakahara, F.; Sakata-Yanagimoto, M.; Komeno, Y.; Kato, N.; Uchida, T.; Haraguchi, K.; Kumano, K.; Harada, Y.; Harada, H.; Kitaura, J. Hes1 immortalizes committed progenitors and plays a role in blast crisis transition in chronic myelogenous leukemia. Blood 2010, 115, 2872–2881. [CrossRef]

19. Wetzler, M.; Talpaz, M.; Van Etten, R.A.; Hirsh-Ginsberg, C.; Beran, M.; Kurzrock, R. Subcellular localization of Bcr, Abl, and Bcr-Abl proteins in normal and leukemic cells and correlation with expression of myeloid differentiation. J. Clin. Invest. 1993, 92, 1925–1939. [CrossRef]

20. Gaiger, A.; Henn, T.; Horth, E.; Geissler, K.; Mitterbauer, G.; Maier-Dobersberger, T.; Greinix, H.; Mannhalter, C.; Haas, O.A.; Lechner, K. Increase of bcr-abl chimeric mRNA expression in tumor cells of patients with chronic myeloid leukemia precodes disease progression. Blood 1995, 86, 2371–2378. [CrossRef]

21. Elmaaeglci, A.H.; Beelen, D.W.; Opalka, B.; Seeber, S.; Schaefer, U.W. The amount of BCR-ABL fusion transcripts detected by the real-time quantitative polymerase chain reaction method in patients with Philadelphia chromosome positive chronic myeloid leukemia correlates with the disease stage. Ann. Hematol. 2000, 79, 424–431. [CrossRef]

22. Hosoya, N.; Sanada, M.; Nannya, Y.; Nakazaki, K.; Wang, L.; Hangaiishi, A.; Kurokawa, M.; Chiba, S.; Ogawa, S. Genomewide screening of DNA copy number changes in chronic myelogenous leukemia with the use of high-resolution array-based comparative genomic hybridization. Genes Chromosom. Cancer 2006, 45, 482–494. [CrossRef] [PubMed]

23. Brazma, D.; Grace, C.; Howard, J.; Melo, J.V.; Holyoke, T.; Apperley, J.F.; Nacheva, E.P. Genomic profile of chronic myelogenous leukemia: Imbalances associated with disease progression. Genes Chromosom. Cancer 2007, 46, 1039–1050. [CrossRef] [PubMed]

24. Nowak, D.; Ogawa, S.; Münch, M.; Kato, M.; Kawamata, N.; Meixel, A.; Nowak, V.; Kim, H.S.; Kang, S.; Paquette, R. SNP array analysis of tyrosine kinase inhibitor-resistant chronic myeloid leukemia identifies heterogeneous secondary genomic alterations. Blood 2010, 115, 1049–1053. [CrossRef] [PubMed]

25. Nadarajan, V.S.; Phan, C.-L.; Ang, C.-H.; Liang, K.-L.; Gan, G.-G.; Bee, P.-C.; Zakaria, Z. Identification of copy number alterations by array comparative genomic hybridization in patients with late chronic or accelerated phase chronic myeloid leukemia treated with imatinib mesylate. Int. J. Hematol. 2011, 93, 465–473. [CrossRef] [PubMed]

26. Lee, S.-T.; Ji, Y.; Kim, H.-J.; Ki, C.-S.; Jung, C.W.; Kim, J.-W.; Kim, S.-H. Sequential array comparative genomic hybridization analysis identifies copy number changes during blastic transformation of chronic myeloid leukemia. Leuk. Res. 2012, 36, 418–421. [CrossRef]

27. van der Sligte, N.E.; Krumbholz, M.; Pastoreczak, A.; Scheijen, B.; Tauer, J.T.; Nowasz, C.; Sonneveld, E.; de Bock, G.H.; Meeuwsen-de Boer, T.G.J.; van Reijmersdal, S. DNA copy number alterations mark disease progression in paediatric chronic myeloid myeloid leukaemia. Br. J. Haematol. 2014, 166, 250–253. [CrossRef]
28. Antoszewska-Smith, J.; Pawlowska, E.; Blasiak, J. Reactive oxygen species in BCR-ABL1-expressing cells—relevance to chronic myeloid leukemia. *Acta Biochim. Pol.* 2017, 64, 1–10. [CrossRef]

29. Satzler, M.; Verma, S.; Shrikhande, G.; Byrne, C.H.; Pride, Y.B.; Winkler, T.; Greenfield, E.A.; Salgia, R.; Griffin, J.D. The BCR/ABL tyrosine kinase induces production of reactive oxygen species in hematopoietic cells. *J. Biol. Chem.* 2000, 275, 24273–24278. [CrossRef]

30. Koptyra, M.; Falinski, R.; Nowicki, M.O.; Stoklosa, T.; Majsterek, I.; Nieborowska-Skorska, M.; Blasiak, J.; Antoszewska-Smith, J.; Pawlowska, E.; Błasiak, J. Reactive oxygen species in BCR-ABL1-expressing cells. *Int. J. Mol. Sci.* 2019.

31. Nowicki, M.O.; Falinski, R.; Koptyra, M.; Slupianek, A.; Stoklosa, T.; Gloc, E.; Nieborowska-Skorska, M.; Blasiak, J.; Skorski, T. BCR/ABL1 kinase induces self-mutagenesis via reactive oxygen species to encode imatinib resistance. *Blood* 2006, 108, 319–327. [CrossRef]

32. Podsywalo-Bartnicka, P.; Wolczyk, M.; Skowronek, K.; Nowicki, M.O.; Nieborowska-Skorska, M.; Blasiak, J.; Skorski, T. BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species–dependent DNA double-strand breaks. *Blood* 2004, 104, 3746–3753. [CrossRef]

33. Deutsch, E.; Jarrousse, S.; Buet, D.; Dugray, A.; Bonnet, M.-L.; Vozenin-Brotons, M.-C.; Guilhot, F.; Turhan, A.G.; Sokal, J.E.; Gomez, G.A.; Baccarani, M.; Tura, S.; Clarkson, B.D.; Cervantes, F.; Rozman, C.; Carbonell, F.; Baccarani, M.; Tura, S.; Clarkson, B.D.; Cervantes, F.; Rozman, C.; Carbonell, F.; Pastink, A.; Eeken, J.C.J.; Lohman, P.H.M. Genomic integrity and the repair of double-strand DNA breaks. *Mutat. Res. Mol. Mech. Mutagen.* 2001, 480, 37–50. [CrossRef]

34. Dkhissi, F.; Aggoune, D.; Pontis, J.; Sorel, N.; Piccirilli, N.; LeCorf, A.; Guilhot, F.; Chevillet, J.-C.; Ait-Si-Ali, S.; Turhan, A.G. The downregulation of BAP1 expression by BCR-ABL reduces the stability of BRCA1 in chronic myeloid leukemia. *Exp. Hematol.* 2015, 43, 775–780. [CrossRef] [PubMed]

35. Pannunzio, N.R.; Watanabe, G.; Lieber, M.R. Nonhomologous DNA end-joining for repair of DNA double-strand breaks. *J. Biol. Chem.* 2018, 293, 10512–10523. [CrossRef] [PubMed]

36. Sallmyr, A.; Tomkinson, A.E.; Rassool, F.V. Up-regulation of WRN and DNA ligase IIIα in chronic myeloid leukemia: Consequences for the repair of DNA double-strand breaks. *Blood* 2008, 112, 1413–1423. [CrossRef]

37. Slupianek, A.; Poplawski, T.; Jozwikowski, S.K.; Cramer, K.; Pytel, D.; Stoczynska, E.; Nowicki, M.O.; Blasiak, J.; Skorski, T. BCR/ABL1 leukemia cells depend on stress-triggered TIAR-mediated suppression of translation. *Cell Cycle* 2014, 13, 3727–3741. [CrossRef] [PubMed]

38. Bellacosa, A. BCR-ABL1 kinase inhibits uracil DNA glycosylase UNG2 to enhance oxidative DNA damage and stimulate genomic instability. *Blood* 2013, 121, 842–851. [CrossRef]

39. Wada, C.; Shionoya, S.; Fujino, Y.; Tokuhiro, H.; Akahoshi, T.; Uchida, T.; Ohtani, H. Genomic instability of microsatellite repeats and its association with the evolution of chronic myelogenous leukemia [see comments]. *Blood* 1994, 83, 3449–3456. [CrossRef]

40. Slupianek, A.; Falinski, R.; Znojek, P.; Stoklosa, T.; Flis, S.; Doneddu, V.; Pytel, D.; Synowiec, E.; Blasiak, J.; Bellacosa, A. BCR-ABL1 kinase inhibits uracil DNA glycosylase UNG2 to enhance oxidative DNA damage and stimulate genomic instability. *Leukemia* 2013, 27, 629. [CrossRef]

41. Alhurairi, A.; Kantarjian, H.; Boddu, P.; Ravandi, F.; Borthakur, G.; DíNardo, C.; Daver, N.; Kadia, T.; Gribben, J.; Pierce, S. Prognostic significance of additional chromosomal abnormalities at the time of diagnosis in patients with chronic myeloid leukemia treated with frontline tyrosine kinase inhibitors. *Am. J. Hematol.* 2018, 93, 84–90. [CrossRef]

42. Kantarjian, H.M.; Smith, T.L.; McCredie, K.B.; Keating, M.J.; Walters, R.S.; Talpaz, M.; Hester, J.P.; Bligham, G.; Gehan, E.; Freireich, E.J. Chronic myelogenous leukemia: A multivariate analysis of the associations of patient characteristics and therapy with survival. *Blood* 1985, 66, 1326–1335. [CrossRef]

43. Sokal, J.E.; Gomez, G.A.; Baccarani, M.; Tura, S.; Clarkson, B.D.; Cervantes, F.; Rozman, C.; Carbonell, F.; Anger, B.; Heimpel, H. Prognostic significance of additional cytogenetic abnormalities at diagnosis of Philadelphia chromosome-positive chronic granulocytic leukemia. *Blood* 1988, 72, 294–298. [CrossRef]

44. Marktel, S.; Marin, D.; Foot, N.; Szydlo, R.; Bua, M.; Karadimitris, A.; De Melo, V.; Kotzampaltiris, P.; Dazzi, F.; Rahemtulla, A. Chronic myeloid leukemia in chronic phase responding to imatinib: The occurrence of additional cytogenetic abnormalities predicts disease progression. *Cancer Genet. Cytogenet.* 2003, 159, 260–267. [CrossRef]

45. Ishihara, T.; Sasaki, M.; Oshima, M.; Kamada, N.; Yamada, K.; Okada, M.; Sakurai, M.; Sugiyama, T.; Shiraiishi, Y.; Kohno, S.; et al. A summary of cytogenetic studies on 534 cases of chronic myelocytic leukemia in Japan. *Cancer Genet. Cytogenet.* 1983, 9, 81–91. [CrossRef]
46. Swolin, B.; Weinfield, A.; Westin, J.; Waldenström, J.; Magnusson, B. Karyotypic evolution in Ph-positive chronic myeloid leukemia in relation to management and disease progression. *Cancer Genet. Cytogenet.* 1985, 18, 65–79. [CrossRef]

47. Krulik, M.; Smadja, N.; De Gramont, A.; Gonzalez-Canali, G.; Audebert, A.A.; Dray, C.; Brissaud, P.; Debray, J. Sequential karyotype study on Ph-positive chronic myelocytic leukaemia. Significance of additional chromosomal abnormalities during disease evolution. *Cancer Genet. Cytogenet.* 1987, 60, 974–979. [CrossRef]

48. Mitelman, F.; Levan, G.; Nilsson, P.G.; Brandt, L. Non-random karyotypic evolution in chronic myeloid leukemia. *Int. J. Cancer* 1976, 18, 24–30. [CrossRef]

49. Mitelman, F. The cytogenetic scenario of chronic myeloid leukemia. *Leuk. Lymphoma* 1993, 11, 11–15. [CrossRef]

50. Cortes, J.E.; Talpaz, M.; Giles, F.; O’Brien, S.; Rios, M.B.; Shan, J.; Garcia-Manero, G.; Faderl, S.; Thomas, D.A.; Wierda, W. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. *Blood* 2003, 101, 3794–3800. [CrossRef]

51. Lippert, E.; Etienne, G.; Mozziiconacci, M.-J.; Laibe, S.; Gervais, C.; Girault, S.; Gachard, N.; Tigaud, I.; Dastugue, N.; Huguet, F. Loss of the Y chromosome in Philadelphia-positive cells predicts a poor response of chronic myeloid leukemia patients to imatinib mesylate therapy. *Haematologica* 2010, 95, 1604–1607. [CrossRef]

52. Luatti, S.; Castagnetti, F.; Marzocchi, G.; Baldazzi, C.; Gugliotta, G.; Iacobucci, I.; Specchia, G.; Zanatta, L.; Rege-Cambrin, G.; Mancini, M. Additional chromosomal abnormalities in Philadelphia-positive clone: Adverse prognostic influence on frontline imatinib therapy: A GIMEMA Working Party on CML analysis. *Blood* 2012, 120, 761–767. [CrossRef] [PubMed]

53. Fabarius, A.; Leitner, A.; Hochhaus, A.; Müller, M.C.; Hanfstein, B.; Haferlach, C.; Göhring, G.; Schlegelberger, B.; Jotterand, M.; Reiter, A. Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: Long-term observation of 1151 patients from the randomized CML Study IV. *Blood* 2011, 118, 6760–6768. [CrossRef] [PubMed]

54. Wang, W.; Cortes, J.E.; Tang, G.; Khouyr, J.D.; Wang, S.; Bueso-Ramos, C.E.; DiGiuseppe, J.A.; Chen, Z.; Kantarjian, H.M.; Medeiros, L.J. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. *Blood* 2016, 127, 2742–2750. [CrossRef] [PubMed]

55. Chandran, R.K.; Geetha, N.; Sukthivel, K.M.; Aswathy, C.G.; Gopinath, P.; Raj, T.V.A.; Priya, G.; Nair, J.K.K.M.; Sreedharan, H. Genomic amplification of BCR-ABL1 fusion gene and its impact on the disease progression mechanism in patients with chronic myelogenous leukemia. *Gente* 2019, 686, 85–91. [CrossRef] [PubMed]

56. Johansson, B.; Fioretos, T.; Billström, R.; Mitelman, F. Aberrant cytogenetic evolution pattern of Philadelphia-positive chronic myeloid leukemia treated with interferon-α. *Leukemia* 1996, 10, 1134–1138.

57. Serra, A.; Gottardi, E.; Della Ragione, F.; Saglio, G.; Iolascon, A. Involvement of the cyclin-dependent kinase-4 (CDKN2) gene in the pathogenesis of lymphoid blast crisis of chronic myelogenous leukemia. *Br. J. Haematol.* 1995, 91, 625–629. [CrossRef]

58. Jabbour, E.; Kantarjian, H.; Jones, D.; Talpaz, M.; Bekele, N.; O’Brien, S.; Zhou, X.; Luthra, R.; Garcia-Manero, G.; Giles, F. Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. *Leukemia* 2006, 20, 1767. [CrossRef]

59. Soverini, S.; Colorossi, S.; Gnani, A.; Rosti, G.; Castagnetti, F.; Poerio, A.; Iacobucci, I.; Amabile, M.; Abruzzese, E.; Orlandi, E. Contribution of ABL kinase domain mutations to imatinib resistance in diifferent subsets of Philadelphia-positive patients: By the GIMEMA Working Party on Chronic Myeloid Leukemia. *Clin. Cancer Res.* 2006, 12, 7374–7379. [CrossRef]

60. Shah, N.P.; Skaggs, B.J.; Branford, S.; Hughes, T.P.; Nicoll, J.M.; Paquette, R.L.; Sawyers, C.L. Sequential ABL kinase inhibitor therapy selects for compound drug-resistant BCR-ABL mutations with altered oncogenic potency. *J. Clin. Invest.* 2007, 117, 2562–2569. [CrossRef]

61. Stagno, F.; Stella, S.; Berretta, S.; Massimino, M.; Antolini, A.; Giustolisi, R.; Messina, A.; Di Raimondo, F.; Vigneri, P. Sequential mutations causing resistance to both Imatinib Mesylate and Dasatinib in a chronic myeloid leukemia patient progressing to lymphoid blast crisis. *Leuk. Res.* 2008, 32, 673–674. [CrossRef]
63. Soverini, S.; Bavaro, L.; Martelli, M.; De Benedittis, C.; Iurlo, A.; Orofino, N.; Pagano, L.; Criscuolo, M.; Bonifacio, M.; Scaffidi, L. Compound BCR-ABL1 Kinase Domain Mutants: Prevalence, Spectrum and Correlation with Tyrosine Kinase Inhibitor Resistance in a Prospective Series of Philadelphia Chromosome-Positive Leukemia Patients Analyzed By Next Generation Sequencing. *Blood* 2018, 132, 789. [CrossRef]

64. Zabriskie, M.S.; Eide, C.A.; Tantravahi, S.K.; Vellore, N.A.; Estrada, J.; Nicollin, F.E.; Khoury, H.J.; Larson, R.A.; Konepleva, M.; Cortes, J.E. BCR-ABL1 compound mutations combining key kinase domain positions confer clinical resistance to ponatinib in Ph chromosome-positive leukemia. *Cancer Cell* 2014, 26, 428–442. [CrossRef] [PubMed]

65. Ahuja, H.; Bar-Eli, M.; Advani, S.H.; Benchimol, S.; Cline, M.J. Alterations in the p53 gene and the clonal evolution of the blast crisis of chronic myelocytic leukemia. *Proc. Natl. Acad. Sci. USA* 1989, 86, 6783–6787. [CrossRef] [PubMed]

66. Feinstein, E.; Cimino, G.; Gale, R.P.; Alimena, G.; Berthier, R.; Kishi, K.; Goldman, J.; Zaccaria, A.; Berrebi, A.; Cannaani, E. p53 in chronic myelogenous leukemia in acute phase. *Proc. Natl. Acad. Sci. USA* 1991, 88, 6293–6297. [CrossRef] [PubMed]

67. Skorski, T.; Nieborowska-Skorska, M.; Wlodarski, P.; Perrotti, D.; Martinez, R.; Wasik, M.A.; Calabretta, B. Blastic transformation of p53-deficient bone marrow cells by p210bcr/abl tyrosine kinase. *Proc. Natl. Acad. Sci. USA* 1996, 93, 13137–13142. [CrossRef]

68. Honda, H.; Ushijima, T.; Wakazono, K.; Oda, H.; Tanaka, Y.; Aizawa, S.; Ishikawa, T.; Yazaki, Y.; Hirai, H. Acquired loss of p53 induces blastic transformation in p210 bcr/abl-expressing hematopoietic cells: A transgenic study for blast crisis of human CML. *Blood* 2000, 95, 1144–1150. [CrossRef]

69. Wendel, H.-G.; de Stanchina, E.; Cepero, E.; Ray, S.; Emig, M.; Fridman, J.S.; Veach, D.R.; Bornmann, W.G.; Clarkson, B.; McCombie, W.R. Loss of p53 impedes the antileukemic response to BCR-ABL inhibition. *Proc. Natl. Acad. Sci. USA* 2006, 103, 7444–7449. [CrossRef] [PubMed]

70. Ahuja, H.G.; Jat, P.S.; Foti, A.; Bar-Eli, M.; Cline, M.J. Abnormalities of the retinoblastoma gene in the pathogenesis of acute leukemia. *Blood* 1991, 78, 3259–3268. [CrossRef]

71. Beck, Z.; Kiss, A.; Tóth, F.D.; Szabó, J.; Bácsi, A.; Balogh, E.; Borbély, Á.; Telek, B.; Kovács, E.; Oláh, É. Alterations of P53 and RB genes and the evolution of the accelerated phase of chronic myeloid leukemia. *Leuk. Lymphoma* 2000, 38, 587–597. [CrossRef]

72. Roche-Leстиене, C.; Deluche, L.; Corm, S.; Tigaud, I.; Joha, S.; Philippe, N.; Geffroy, S.; Laï, J.-L.; Nicollin, F.-E.; Preudhomme, C. RUNX1 DNA-binding mutations and RUNX1-PRDM16 cryptic fusions in BCR-ABL+ leukemias are frequently associated with secondary trisomy 21 and may contribute to clonal evolution and imatinib resistance. *Blood* 2008, 111, 3735–3741. [CrossRef] [PubMed]

73. Zhao, L.-J.; Wang, Y.-Y.; Li, G.; Ma, L.-Y.; Xiong, S.-M.; Zhang, W.-N.; Wu, B.; Chen, Z.; Chen, S.-J. Functional features of RUNX1 mutants in acute transformation of chronic myeloid leukemia and their contribution to inducing murine full-bloomed leukemia. *Blood* 2012, 119, 2873–2882. [CrossRef] [PubMed]

74. Rubin, C.M.; Larson, R.A.; Anastasi, J.; Winter, J.N.; Thangavelu, M.; Rowley, J.D.; Le Beau, M.M. t (3; 21)(q26; q22): A recurring chromosomal abnormality in therapy-related myelodysplastic syndrome and acute myeloid leukemia. *Blood* 1990, 76, 2594–2598. [CrossRef] [PubMed]

75. Nucifora, G.; Begy, C.R.; Kobayashi, H.; Roulston, D.; Claxton, D.; Pedersen-Bjergaard, J.; Parganas, E.; Ihle, J.N.; Rowley, J.D. Consistent intergenic splicing and production of multiple transcripts between AML1 and the 8; 21 and 3; 21 translocations in acute and chronic myeloid leukemia. *J. Clin. Oncol.* 2010, 28, 3890–3898. [CrossRef]

76. Nukina, A.; Kagoya, Y.; Watanabe-Okochi, N.; Arai, S.; Ueda, K.; Yoshimi, A.; Nannya, Y.; Kurokawa, M. Single-cell gene expression analysis reveals clonal architecture of blast-phase chronic myeloid leukemia. *Br. J. Haematol.* 2014, 165, 414–416. [CrossRef]
Goyama, S.; Yamamoto, G.; Shimabe, M.; Sato, T.; Ichikawa, M.; Ogawa, S.; Chiba, S.; Kurokawa, M. Evi-1 is a critical regulator for hematopoietic stem cells and transformed leukemic cells. *Cell Stem Cell* 2008, 3, 207–220. [CrossRef]

Shen, L.; Zhu, J.; Chen, F.; Lin, W.; Cai, J.; Zhong, J.; Zhong, H. RUNX1-Evi-1 fusion gene inhibited differentiation and apoptosis in myeloid leukemia: An in vivo study. *BMC Cancer* 2015, 15, 970. [CrossRef]

Sill, H.; Goldman, J.M.; Cross, N.C. Homozygous deletions of the p16 tumor-suppressor gene are associated with lymphoid transformation of chronic myeloid leukemia. *Blood* 1995, 85, 2013–2016. [CrossRef]

Hernández-Boluda, J.-C.; Cervantes, F.; Colomer, D.; Vela, M.-C.; Costa, D.; Paz, M.-F.; Esteller, M.; Montserrat, E. Genomic p16 abnormalities in the progression of chronic myeloid leukemia into blast crisis: A sequential study in 42 patients. *Exp. Hematol.* 2003, 31, 204–210. [CrossRef]

Williams, R.T.; Sherr, C.J. The INK4-ARF (CDKN2A) locus in hematopoiesis and BCR-ABL-induced leukemias. In *Cold Spring Harbor Symposia on Quantitative Biology; Cold Spring Harbor Laboratory Press*; Woodbury, NY, USA, 2008; Volume 73, pp. 461–467.

Nacheva, E.P.; Brazma, D.; Virgili, A.; Howard-Reeves, J.; Chanalaris, A.; Gancheva, K.; Apostolova, M.; Valgañon, M.; Mazzullo, H.; Grace, C. Deletions of immunoglobulin heavy chain and T cell receptor gene regions are uniquely associated with lymphoid blast transformation of chronic myeloid leukemia. *BMC Genomics* 2010, 11, 41. [CrossRef] [PubMed]

Huh, J.; Jung, C.W.; Kim, J.W.; Kim, H.-J.; Kim, S.-H.; Shin, M.G.; Kim, Y.K.; Kim, H.J.; Suh, J.S.; Moon, J.H. Genome-wide high density single-nucleotide polymorphism array-based karyotyping improves detection of clonal aberrations including der (9) deletion, but does not predict treatment outcomes after imatinib therapy in chronic myeloid leukemia. *Ann. Hematol.* 2011, 90, 1255–1264. [CrossRef] [PubMed]

Mullighan, C.G.; Miller, C.B.; Radtke, I.; Phillips, L.A.; Dalton, J.; Ma, J.; White, D.; Hughes, T.P.; Le Beau, M.M.; Pui, C.-H. BCR–ABL1 lymphoblastic leukemia is characterized by the deletion of Ikaros. *Nature* 2008, 453, 110. [CrossRef]

Georgopoulos, K.; Moore, D.D.; Derfler, B. Ikaros, an early lymphoid-specific transcription factor and a putative mediator for T cell commitment. *Science* 1992, 258, 808–812. [CrossRef]

Molnár, Á.; Wu, P.; Large Spada, D.A.; Vortkamp, A.; Scherer, S.; Copeland, N.G.; Jenkins, N.A.; Bruns, G.; Georgopoulos, K. The Ikaros gene encodes a family of lymphocyte-restricted zinc finger DNA binding proteins, highly conserved in human and mouse. *J. Immunol.* 1996, 156, 585–592.

Sun, L.; Goodman, P.A.; Wood, C.M.; Crotty, M.L.; Sensel, M.; Sather, H.; Nachman, J.; Steinherz, P.G.; Gaynon, P.S. Expression of aberrantly spliced oncogenic ikaros isoforms in childhood acute lymphoblastic leukemia. *J. Clin. Oncol.* 1999, 17, 3753–3766. [CrossRef]

Iacobucci, I.; Lonetti, A.; Messa, F.; Cilloni, D.; Arruga, F.; Ottaviani, E.; Paolini, S.; Papayannidis, C.; Piccaluga, P.P.; Giannoula, P. Expression of spliced oncogenic Ikaros isoforms in Philadelphia-positive acute lymphoblastic leukemia patients treated with tyrosine kinase inhibitors: Implications for a new mechanism of resistance. *Blood* 2008, 112, 3847–3855. [CrossRef]

Wang, L.; Howarth, A.; Clark, R.E. Ikaros transcripts Ik6/10 and levels of full-length transcript are critical for chronic myeloid leukaemia blast crisis transformation. *Leukemia* 2014, 28, 1745. [CrossRef]

Soverini, S.; Score, J.; Iacobucci, I.; Poerio, A.; Lonetti, A.; Gnani, A.; Colarossi, S.; Ferrari, A.; Castagnetti, F.; Rosti, G. IDH2 somatic mutations in chronic myeloid leukemia patients in blast crisis. *Leukemia* 2011, 25, 178. [CrossRef]

Grossmann, V.; Kohlmann, A.; Zenger, M.; Schindela, S.; Eder, C.; Weissmann, S.; Schnittger, S.; Kern, W.; Müller, M.C.; Hochhaus, A. A deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. *Leukemia* 2011, 25, 557. [CrossRef] [PubMed]

Makishima, H.; Jankowska, A.M.; McDevitt, M.A.; O’Keefe, C.; Dujardin, S.; Cazzolli, H.; Przychodzen, B.; Prince, C.; Nicoll, J.; Siddaiah, H. CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. *Blood* 2011, 117, e198–e206. [CrossRef] [PubMed]

Magistroni, V.; Mauri, M.; D’Aliberti, D.; Mezzatesta, C.; Crespiatico, I.; Nava, M.; Fontana, D.; Sharma, N.; Parker, W.; Schreiber, A. De novo UBE2A mutations are recurrently acquired during chronic myeloid leukemia progression and interfere with myeloid differentiation pathways. *Haematologica* 2019, 2017, 179937. [CrossRef] [PubMed]
96. Branford, S.; Wang, P.; Yeung, D.T.; Thomson, D.; Purins, A.; Wadham, C.; Shahrin, N.H.; Marum, J.E.; Nataren, N.; Parker, W.T. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. *Blood* **2018**, *132*, 948–961. [CrossRef]

97. Janssens, V.; Goris, J. Protein phosphatase 2A: A highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *Biochem. J.* **2001**, *353*, 417–439. [CrossRef]

98. Neviani, P.; Santhanam, R.; Trotta, R.; Notari, M.; Blaser, B.W.; Liu, S.; Mao, H.; Chang, J.S.; Galietta, A.; Uttam, A. 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**, *8*, 355–368. [CrossRef]

99. Perrotti, D.; Cesi, V.; Trotta, R.; Guerzoni, C.; Santilli, G.; Campbell, K.; Iervoliti, A.; Condorelli, F.; Gambacorti-Passerini, C.; Caligiuri, M.A. BCR-ABL suppresses C/EBPα expression through inhibitory action of hnRNP E2. *Nat. Genet.* **2002**, *30*, 48. [CrossRef]

100. Radomska, H.S.; Huetttner, C.S.; Zhang, P.U.; Cheng, T.A.O.; Scadden, D.T.; Tenen, D.G. CCAAT/enhancer binding protein α is a regulatory switch sufficient for induction of granulocytic development from bipotential myeloid progenitors. *Mol. Cell. Biol.* **1998**, *18*, 4301–4314. [CrossRef]

101. Notari, M.; Neviani, P.; Santhanam, R.; Blaser, B.W.; Chang, J.-S.; Galietta, A.; Willis, A.E.; Roy, D.C.; Caligiuri, M.A.; Marucci, G. A MAPK/HNRPK pathway controls BCR/ABL oncogenic potential by regulating MYC mRNA translation. *Blood* **2006**, *107*, 2507–2516. [CrossRef]

102. Chang, J.S.; Santhanam, R.; Trotta, R.; Neviani, P.; Eiring, A.M.; Briercheck, E.; Ronchetti, M.; Roy, D.C.; Calabretta, B.; Caligiuri, M.A. High levels of the BCR/ABL oncoprotein are required for the MAPK-hnRNP-E2–dependent suppression of C/EBPα-driven myeloid differentiation. *Blood* **2007**, *110*, 994–1003. [CrossRef]

103. Eiring, A.M.; Habr, J.G.; Neviani, P.; Garton, C.; Oaks, J.J.; Spizzo, R.; Liu, S.; Schwind, S.; Santhanam, R.; Hickey, C.J. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. *Cell 2010*, *140*, 652–665. [CrossRef] [PubMed]

104. Nelkin, B.D.; Przepiorka, D.; Burke, P.J.; Thomas, E.D.; Baylin, S.B. Abnormal methylation of the calcitonin gene marks progression of chronic myelogenous leukemia. *Blood* **1991**, *77*, 2431–2434. [CrossRef] [PubMed]

105. Issa, J.-P.; Zehnbauer, B.A.; Cinvin, C.I.; Collector, M.I.; Sharkis, S.J.; Davidson, N.E.; Kaufmann, S.H.; Baylin, S.B. The estrogen receptor CpG island is methylated in most hematopoietic neoplasms. *Cancer Res.* **1996**, *56*, 973–977. [PubMed]

106. Issa, J.-P.; Zehnbauer, B.A.; Kaufmann, S.H.; Biel, M.A.; Baylin, S.B. HIC1 hypermethylation is a late event in hematopoietic neoplasms. *Cancer Res.* **1997**, *57*, 1678–1681.

107. Nguyen, T.T.; Mohrbacher, A.F.; Tsai, Y.C.; Groffen, J.; Heisterkamp, N.; Nichols, P.W.; Mimi, C.Y.; Lübbert, M.; Jones, P.A. Quantitative measure of c-abl and p15 methylation in chronic myelogenous leukemia: Biological implications. *Blood* **2000**, *95*, 2990–2992. [CrossRef]

108. Roman-Gomez, J.; Castillejo, J.A.; Jimenez, A.; Cervantes, F.; Boque, C.; Hermosin, L.; Leon, A.; Grañena, A.; Colomer, D.; Heiniger, A. Cadherin-13, a mediator of calcium-dependent cell-cell adhesion, is silenced by methylation in chronic myeloid leukemia and correlates with pretreatment risk profile and cytogenetic response to interferon alfa. *J. Clin. Oncol.* **2003**, *21*, 1472–1479. [CrossRef]

109. Strathdee, G.; Holyoke, T.L.; Sim, A.; Parker, A.; Oscier, D.G.; Melo, J.V.; Meyer, S.; Eden, T.; Dickinson, A.M.; Mountford, J.C. Inactivation of HOXA genes by hypermethylation in myeloid and lymphoid malignancy is frequent and associated with poor prognosis. *Clin. Cancer Res.* **2007**, *13*, 5048–5055. [CrossRef]

110. Jelinek, J.; Gharibyan, V.; Estecio, M.R.H.; Kondo, K.; He, R.; Chung, W.; Lu, Y.; Zhang, N.; Liang, S.; Kantarjian, H.M. aberrant DNA methylation is associated with disease progression, resistance to imatinib and shortened survival in chronic myelogenous leukemia. *PLoS ONE* **2011**, *6*, e22110. [CrossRef]

111. Heller, G.; Topakian, T.; Altenberger, C.; Cerny-Reiterer, S.; Herdlhofer, S.; Ziegler, B.; Datlinger, C.; Byrgazov, K.; Bock, C.; Mannhalter, C. Next-generation sequencing identifies major DNA methylation changes during progression of Ph+ chronic myeloid leukemia. *Leukemia* **2016**, *30*, 1861. [CrossRef]

112. Trotta, R.; Vignudelli, T.; Candini, O.; Intine, R.V.; Pecorari, L.; Guerzoni, C.; Santilli, G.; Byrom, M.W.; Goldoni, S.; Ford, L.P. BCR/ABL activates mdm2 mRNA translation via the La antigen. *Cancer Cell* **2003**, *3*, 145–160. [CrossRef]

113. Perrotti, D.; Bonatti, S.; Trotta, R.; Martinez, R.; Skorski, T.; Salomoni, P.; Grassilli, E.; Iozzo, R.V.; Cooper, D.R.; Calabretta, B. TLS/FUS, a pro-oncogene involved in multiple chromosomal translocations, is a novel regulator of BCR/ABL-mediated leukemogenesis. *EMBO J.* **1998**, *17*, 4442–4455. [CrossRef] [PubMed]
114. Guerzoni, C.; Bardini, M.; Mariani, S.A.; Ferrari-Amorotti, G.; Neviani, P.; Panno, M.L.; Zhang, Y.; Martinez, R.; Perrotti, D.; Calabretta, B. Inducible activation of CEBPB, a gene negatively regulated by BCR/ABL, inhibits proliferation and promotes differentiation of BCR/ABL-expressing cells. Blood 2006, 107, 4080–4089. [CrossRef] [PubMed]

115. Eiring, A.M.; Neviani, P.; Santhanam, R.; Oakes, J.J.; Chang, J.S.; Notari, M.; Willis, W.; Gambacorti-Passerini, C.; Volinia, S.; Marcucci, G. Identification of novel posttranscriptional targets of the BCR/ABL oncoprotein by ribonomics: Requirement of E2F3 for BCR/ABL leukemogenesis. Blood 2008, 111, 816–828. [CrossRef] [PubMed]

116. Ly, C.; Arechiga, A.F.; Melo, J.V.; Walsh, C.M.; Ong, S.T. Bcr-Abl kinase modulates the translation regulators ribosomal protein S6 and 4E-BP1 in chronic myelogenous leukemia cells via the mammalian target of rapamycin. Cancer Res. 2003, 63, 5716–5722.

117. Perrotti, D.; Turturro, F.; Neviani, P. BCR/ABL, mRNA translation and apoptosis. Cell Death Differ. 2005, 12, 534–540. [CrossRef]

118. Jiang, Q.; Crews, L.A.; Barrett, C.L.; Chun, H.-J.; Isquith, J.M.; Zipeto, M.A.; Goff, D.J.; Minden, M.; Sadarangani, A.; Ruset, J.M. ADAR1 promotes malignant progenitor reprogramming in chronic myeloid leukemia. Proc. Natl. Acad. Sci. USA 2013, 110, 1041–1046. [CrossRef]

119. Zipeto, M.A.; Court, A.C.; Sadarangani, A.; Santos, N.P.D.; Balaian, L.; Chun, H.-J.; Pineda, G.; Morris, S.R.; Mason, C.N.; Geron, I. ADAR1 activation drives leukemia stem cell self-renewal by impairing Let-7 biogenesis. Cell Stem Cell 2016, 19, 177–191. [CrossRef]

120. Crews, L.A.; Jiang, Q.; Zipeto, M.A.; Lazzari, E.; Ali, S.; Barrett, C.L.; Frazer, K.A.; Jamieson, C.H.M. An RNA editing fingerprint of cancer stem cell reprogramming. J. Transl. Med. 2015, 13, 52. [CrossRef]

121. Garzon, R.; Calin, G.A.; Croce, C.M. MicroRNAs in cancer. Annu. Rev. Med. 2009, 60, 167–179. [CrossRef]

122. Garzon, R.; Fabbri, M.; Cimmino, A.; Calin, G.A.; Croce, C.M. MicroRNA expression and function in cancer. Annu. Rev. Med. 2009, 60, 167–179. [CrossRef]

123. Sriutova, K.; Curik, N.; Burda, P.; Savvulidi, F.; Silvestri, G.; Trotta, R.; Klamova, H.; Pecherkova, P.; Sovova, Z.; Koblihova, J. BCR-ABL1 mediated miR-150 downregulation through MYC contributed to myeloid differentiation block and drug resistance in chronic myeloid leukemia. Haematologica 2018, 103, 2016–2025. [CrossRef] [PubMed]

124. Hattori, A.; Tsunoda, M.; Konuma, T.; Kobayashi, M.; Nagy, T.; Glushka, J.; Tayyari, F.; McSkimming, D.; Kannan, N.; Tojo, A. Cancer progression by reprogrammed BCAA metabolism in myeloid leukemia. Nature 2017, 545, 500. [CrossRef] [PubMed]

125. Srutova, K.; Curik, N.; Burda, P.; Savvulidi, F.; Silvestri, G.; Trotta, R.; Klamova, H.; Pecherkova, P.; Sovova, Z.; Koblihova, J. BCR-ABL1 mediated miR-150 downregulation through MYC contributed to myeloid differentiation block and drug resistance in chronic myeloid leukemia. Haematologica 2018, 103, 2016–2025. [CrossRef] [PubMed]

126. Hattori, A.; Tsunoda, M.; Konuma, T.; Kobayashi, M.; Nagy, T.; Glushka, J.; Tayyari, F.; McSkimming, D.; Kannan, N.; Tojo, A. Cancer progression by reprogrammed BCAA metabolism in myeloid leukemia. Nature 2017, 545, 500. [CrossRef] [PubMed]

127. Bar-Peled, L.; Sabatini, D.M. Regulation of mTORC1 by amino acids. Trends Cell Biol. 2014, 24, 400–406. [CrossRef] [PubMed]

128. Ito, T.; Kwon, H.Y.; Zimdahl, B.; Congdon, K.L.; Blum, J.; Lento, W.E.; Zhao, C.; Lagoa, A.; Gerrard, G.; Foroni, L. Regulation of myeloid leukaemia by the cell-fate determinant Musashi. Nature 2010, 466, 765. [CrossRef]

129. Kharas, M.G.; Lengner, C.J.; Al-Shahrour, F.; Bullinger, L.; Ball, B.; Zaidi, S.; Morgan, K.; Tam, W.; Paktinat, M.; Okabe, R. Musashi-2 regulates normal hematopoiesis and promotes aggressive myeloid leukemia. Nat. Med. 2010, 16, 903. [CrossRef]

130. Hughes, T.; Deininger, M.; Hochhaus, A.; Branford, S.; Radich, J.; Kaeda, J.; Baccarani, M.; Cortes, J.; Druker, B.J. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: Review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood 2006, 108, 28–37. [CrossRef]

131. Hughes, T.P.; Kaeda, J.; Branford, S.; Rudzki, Z.; Hochhaus, A.; Hensley, M.L.; Gathmann, I.; Bolton, A.E.; Van Hoomissen, I.C.; Goldman, J.M. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N. Engl. J. Med. 2003, 349, 1423–1432. [CrossRef]
132. Patel, A.B.; O’Hare, T.; Deininger, M.W. Mechanisms of resistance to ABL kinase inhibition in chronic myeloid leukemia and the development of next generation ABL kinase inhibitors. *Hematol. Clin.* **2017**, *31*, 589–612. [CrossRef]

133. Soverini, S.; Mancini, M.; Bavaro, L.; Cavo, M.; Martinelli, G. Chronic myeloid leukemia: The paradigm of targeting oncogenic tyrosine kinase signaling and counteracting resistance for successful cancer therapy. *Mol. Cancer* **2018**, *17*, 49. [CrossRef] [PubMed]

134. Sundaram, D.N.M.; Jiang, X.; Brandwein, J.M.; Valencia-Serna, J.; Remant, K.C.; Uludag, H. Current outlook on drug resistance in chronic myeloid leukemia (CML) and potential therapeutic options. *Drug Discov. Today* **2019**.

135. Burchert, A.; Wang, Y.; Cai, D.; Von Bubnoff, N.; Paschka, P.; Müller-Brüsselbach, S.; Ottmann, O.G.; Duyster, J.; Hochhaus, A.; Neubauer, A. Compensatory PI3-kinase/Akt/mTor activation regulates imatinib resistance development. *Leukemia* **2005**, *19*, 1774. [CrossRef] [PubMed]

136. Wu, J.; Meng, F.; Lu, H.; Kong, L.; Bornmann, W.; Peng, Z.; Talpaz, M.; Donato, N.J. Lyn regulates BCR-ABL and Gab2 tyrosine phosphorylation and c-Cbl protein stability in imatinib-resistant chronic myelogenous leukemia cells. *Blood* **2008**, *111*, 3821–3829. [CrossRef]

137. Bellodi, C.; Lidonnici, M.R.; Hamilton, A.; Helgason, G.V.; Soliera, A.R.; Ronchetti, M.; Galavotti, S.; Young, K.W.; Selmü, T.; Yacobi, R. Targeting autophagy potentiates tyrosine kinase inhibitor–induced cell death in Philadelphia chromosome–positive cells, including primary CML stem cells. *J. Clin. Invest.* **2009**, *119*, 1109–1123. [CrossRef]

138. Esposito, N.; Colavita, I.; Quintarelli, C.; Sica, A.R.; Peluso, A.L.; Luciano, L.; Picardi, M.; Del Vecchio, L.; Buonomo, T.; Hughes, T.P. SHP-1 expression accounts for resistance to imatinib treatment in Philadelphia chromosome–positive cells derived from patients with chronic myeloid leukemia. *Blood* **2011**, *118*, 3634–3644. [CrossRef]

139. Wang, Z.; Yuan, H.; Roth, M.; Stark, J.M.; Bhatia, R.; Chen, W. SIRT1 deacetylase promotes acquisition of genetic mutations for drug resistance in CML cells. *Oncogene* **2013**, *32*, 589. [CrossRef]

140. Ma, L.; Shan, Y.; Bai, R.; Xue, L.; Eide, C.A.; Ou, J.; Zhu, L.J.; Hutchinson, L.; Cerny, J.; Khoury, H.J. A therapeutically targetable mechanism of BCR-ABL–independent imatinib resistance in chronic myeloid leukemia. *Sci. Transl. Med.* **2014**, *6*, 252ra121. [CrossRef]

141. Eiring, A.M.; Page, B.D.G.; Kraft, I.L.; Mason, C.C.; Vellore, N.A.; Resetca, D.; Zabriskie, M.S.; Zhang, T.Y.; Khorashad, J.S.; Engar, A.J. Combined STAT3 and BCR-ABL1 inhibition induces synthetic lethality in therapy-resistant chronic myeloid leukemia. *Leukemia* **2015**, *29*, 586. [CrossRef]

142. Khorashad, J.S.; Eiring, A.M.; Mason, C.C.; Gantz, K.C.; Bowler, A.D.; Redwine, H.M.; Yu, F.; Kraft, I.L.; Pomicter, A.D.; Reynolds, K.R. shRNA library screening identifies nucleocytoplasmic transport as a mediator of BCR-ABL1 kinase-independent resistance. *Blood* **2015**, *125*, 1772–1781. [CrossRef]

143. Walker, C.J.; Joshua, J.O.; Ramasamy, S.; Paolo, N.; J., G.H.; Gregory, F.; Justin, J.E.; Yosef, L.; Ann-Kathrin, E.; Nash, Y.G. Preclinical and Clinical Efficacy of XPO1/CRM1 Inhibition by the Karyopherin Inhibitor KPT-330 in Ph+ Leukemias. *Blood* **2013**, *122*, 3034–3044. [CrossRef] [PubMed]

144. Chakraborty, S.N.; Leng, X.; Perazzona, B.; Sun, X.; Lin, Y.H.; Arlinghaus, R.B. Combination of jak2 and hsp90 inhibitors: An effective therapeutic option in drug-resistant chronic myelogenous leukemia. *Genes Cancer* **2016**, *7*, 201. [PubMed]

145. Wagle, M.; Eiring, A.M.; Wongchenko, M.; Lu, S.; Guan, Y.; Wang, Y.; Lackner, M.; Amler, L.; Hampton, G.; Deininger, M.W. A role for FOXO1 in BCR–ABL1-dependent tyrosine kinase inhibitor resistance in chronic myeloid leukemia. *Leukemia* **2016**, *30*, 1493. [CrossRef] [PubMed]

146. Scott, M.T.; Korfi, K.; Saffrey, P.; Hopcroft, L.E.M.; Kinstrie, R.; Pellicano, F.; Guenther, C.; Gallipoli, P.; Cruz, M.; Dunn, K. Epigenetic reprogramming sensitizes CML stem cells to combined EZH2 and tyrosine kinase inhibition. *Cancer Discov.* **2016**, *6*, 1248–1257. [CrossRef] [PubMed]

147. Zhou, H.; Mak, P.Y.; Mu, H.; Mak, D.H.; Zeng, Z.; Cortes, J.; Liu, Q.; Andreeff, M.; Carter, B.Z. Combined inhibition of β-catenin and Bcr–Abl synergistically targets tyrosine kinase inhibitor-resistant blast crisis chronic myeloid leukemia blasts and progenitors in vitro and in vivo. *Leukemia* **2017**, *31*, 2065. [CrossRef]

148. Eiring, A.M.; Jonathan, A.; Clinton, C.M.; Ethan, D.; Thomas, H.; Michael, W.D. Loss of G0S2 in Kinase-Independent TKI Resistance and Blastic Transformation of CML. *Am Soc. Hematol.* **2017**, *4173.

149. Zhu, Y.; Lu, L.; Qiao, C.; Shan, Y.; Li, H.; Qian, S.; Hong, M.; Zhao, H.; Li, J.; Yang, Z. Targeting PFKFB3 sensitizes chronic myelogenous leukemia cells to tyrosine kinase inhibitor. *Oncogene* **2018**, *37*, 2837. [CrossRef]
150. Bhatia, R.; Holtz, M.; Niu, N.; Gray, R.; Snyder, D.S.; Sawyers, C.L.; Arber, D.A.; Slovak, M.L.; Forman, S.J. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood* 2003, 101, 4701–4707. [CrossRef]

151. Chu, S.; McDonald, T.; Lin, A.; Chakraborty, S.; Huang, Q.; Snyder, D.S.; Bhatia, R. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. *Blood* 2011, 118, 5565–5572. [CrossRef]

152. Chomel, J.-C.; Bonnet, M.-L.; Sorel, N.; Bertrand, A.; Meunier, M.-C.; Fichelson, S.; Melkus, M.; Bennaceur-Griscelli, A.; Guilhot, F.; Turhan, A.G. Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. *Blood* 2011, 118, 3657–3660. [CrossRef]

153. Chomel, J.C.; Bonnet, M.L.; Sorel, N.; Sloma, I.; Bennaceur-Griscelli, A.; Rea, D.; Legros, L.; Marfaing-Koka, A.; Bourhis, J.-H.; Ame, S. Leukemic stem cell persistence in chronic myeloid leukemia patients in deep molecular response induced by tyrosine kinase inhibitors and the impact of therapy discontinuation. *Oncotarget* 2016, 7, 35293. [CrossRef] [PubMed]

154. Bocchia, M.; Sicuranza, A.; Abruzzese, E.; Iurlo, A.; Sirtori, S.; Gozzini, A.; Galimberti, S.; Aprile, L.; Martino, B.; Pregno, P. Residual peripheral blood CD26+ leukemic stem cells in chronic myeloid leukemia patients during TKI therapy and during treatment-free remission. *Front. Oncol.* 2018, 30, 194–201. [CrossRef] [PubMed]

155. Graham, S.M.; Jørgensen, H.G.; Allan, E.; Pearson, C.; Alcorn, M.J.; Richmond, L.; Holyoake, T.L. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood* 2002, 99, 319–325. [CrossRef] [PubMed]

156. Jørgensen, H.G.; Allan, E.K.; Jordanides, N.E.; Mountford, J.C.; Holyoake, T.L. Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. *Blood* 2007, 109, 4016–4019. [CrossRef]

157. Corbin, A.S.; Agarwal, A.; Loriaux, M.; Cortes, J.; Deininger, M.W.; Druker, B.J. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. *J. Clin. Invest.* 2011, 121, 396–409. [CrossRef]

158. Hamilton, A.; Helgason, G.V.; Schemionek, M.; Zhang, B.; Myssina, S.; Allan, E.K.; Nicolini, F.E.; Müller-Tidow, C.; Bhatia, R.; Brunton, V.G. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. *Blood* 2012, 119, 1501–1510. [CrossRef]

159. Neviani, P.; Harb, J.G.; Oaks, J.J.; Santhanam, R.; Walker, C.J.; Ellis, J.J.; Ferrenchak, G.; Dorrance, A.M.; Paisie, C.A.; Eiring, A.M. PP2A-activating drugs selectively eradicate TKI-resistant chronic myeloid leukemic stem cells. *J. Clin. Invest.* 2013, 123, 4144–4157. [CrossRef]

160. Holyoake, T.L.; Vetrie, D. The chronic myeloid leukemia stem cell: Stemming the tide of persistence. *Blood* 2017, 129, 1595–1606. [CrossRef]

161. Modi, H.; McDonald, T.; Chu, S.; Yee, J.-K.; Forman, S.J.; Bhatia, R. Role of BCR/ABL gene-expression levels in determining the phenotype and imatinib sensitivity of transformed human hematopoietic cells. *Blood* 2007, 109, 5411–5421. [CrossRef]

162. Kuma, A.; Brendel, C.; Hochhaus, A.; Neubauer, A.; Burchert, A. Low BCR-ABL expression levels in hematopoietic precursor cells enable persistence of chronic myeloid leukemia under imatinib. *Blood* 2012, 119, 530–539. [CrossRef]

163. Naka, K.; Hoshii, T.; Muraguchi, T.; Tadokoro, Y.; Oshio, T.; Kondo, Y.; Nakao, S.; Motoyama, N.; Hirao, A. TGF-β–FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature* 2010, 463, 467. [CrossRef] [PubMed]

164. Pellicano, F.; Scott, M.T.; Helgason, G.V.; Hopcroft, L.E.M.; Allan, E.K.; Aspinall-O’Dea, M.; Copland, M.; Pierce, A.; Hultly, B.J.P.; Whetton, A.D. The antiproliferative activity of kinase inhibitors in chronic myeloid leukemia cells is mediated by FOXO transcription factors. *Stem Cells* 2014, 32, 2324–2337. [CrossRef] [PubMed]

165. Dierss, C.; Beigi, R.; Guo, G.-R.; Zirlik, K.; Stegert, M.R.; Manley, P.; Trussell, C.; Schmitt-Graeff, A.; Landwehr, K.; Veelken, H. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. *Cancer Cell* 2008, 14, 238–249. [CrossRef] [PubMed]
166. Zhao, C.; Chen, A.; Jamieson, C.H.; Fereshteh, M.; Abrahamsson, A.; Blum, J.; Kwon, H.Y.; Kim, J.; Chute, J.P.; Rizzieri, D. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature 2009, 458, 776. [CrossRef]

167. Reya, T.; Duncan, A.W.; Ailles, L.; Domen, J.; Scherer, D.C.; Willert, K.; Hintz, L.; Nuusse, R.; Weissman, I.L. A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature 2003, 423, 409. [CrossRef]

168. Zhao, C.; Blum, J.; Chen, A.; Kwon, H.Y.; Jung, S.H.; Cook, J.M.; Lagoo, A.; Reya, T. Loss of β-catenin impairs the renewal of normal and CML stem cells in vivo. Cancer Cell 2007, 12, 528–541. [CrossRef]

169. Krause, D.S.; Lazarides, K.; von Andrian, U.H.; Van Etten, R.A. Requirement for CD44 in homing and engraftment of BCR-ABL1-expressing leukemic stem cells. Nat. Med. 2006, 12, 1175. [CrossRef]

170. Krause, D.S.; Lazarides, K.; Lewis, J.B.; von Andrian, U.H.; Van Etten, R.A. Selectins and their ligands are required for homing and engraftment of BCR-ABL1+ leukemic stem cells in the bone marrow niche. Blood 2014, 123, 1361–1371. [CrossRef]

171. Yamamoto-Sugitani, M.; Kuroda, J.; Ashihara, E.; Nagoshi, H.; Kobayashi, T.; Matsumoto, Y.; Sasaki, N.; Shimura, Y.; Kiyota, M.; Nakayama, R. Galectin-3 (Gal-3) induced by leukemia microenvironment promotes drug resistance and bone marrow lodgment in chronic myelogenous leukemia. Proc. Natl. Acad. Sci. USA 2011, 108, 17468–17473. [CrossRef]

172. Zhang, B.; Ho, Y.W.; Huang, Q.; Maeda, T.; Lin, A.; Lee, S.; Hair, A.; Holyoake, T.L.; Huettnner, C.; Bhatia, R. Altered microenvironmental regulation of leukemic and normal stem cells in chronic myelogenous leukemia. Cancer Cell 2012, 21, 577–592. [CrossRef]

173. Mumprecht, S.; Schürch, C.; Schwaller, J.; Solenthaler, M.; Ochsenbein, A.F. Programmed death 1 signaling on chronic myeloid leukemia-specific T cells results in T-cell exhaustion and disease progression. Blood 2009, 114, 1528–1536. [CrossRef] [PubMed]

174. Riether, C.; Schürch, C.M.; Ochsenbein, A.F. Regulation of hematopoietic and leukemic stem cells by the immune system. Cell Death Differ. 2015, 22, 187. [CrossRef] [PubMed]

175. Tarafdar, A.; Hopcroft, L.E.M.; Gallipoli, P.; Pellicano, F.; Cassels, J.; Hair, A.; Korfi, K.; Jørgensen, H.G.; Vetrie, D.; Holyoake, T.L. CML cells actively evade host immune surveillance through cytokine-mediated downregulation of MHC-II expression. Blood 2017, 129, 199–208. [CrossRef] [PubMed]

176. Marum, J.E.; Wang, P.P.; Stangl, D.; Yeung, D.T.; Mueller, M.C.; Dietz, C.T.; Walker, I.; Nataren, N.; Donaldson, Z.; Parker, W.T. Novel fusion genes at CML diagnosis reveal a complex pattern of genomic rearrangements and sequence inversions associated with the Philadelphia chromosome in patients with early blast crisis. Blood 2016, 128, 1219. [CrossRef]

177. Marum, J.E.; Yeung, D.T.; Purins, L.; Reynolds, J.; Parker, W.T.; Stangl, D.; Wang, P.P.; Price, D.J.; Tuke, J.; Schreiber, A.W. ASXL1 and BIM germ line variants predict response and identify CML patients with the greatest risk of imatinib failure. Blood Adv. 2017, 1, 1369–1381. [CrossRef] [PubMed]

178. Lucas, C.M.; Harris, R.J.; Giannoudis, A.; Copland, M.; Slupsky, J.R.; Clark, R.E. Cancerous inhibitor of PP2A (CIP2A) at diagnosis of chronic myeloid leukemia is a critical determinant of disease progression. Blood 2011, 117, 6660–6668. [CrossRef] [PubMed]

179. Rousselot, P.; Prost, S.; Guéllot, J.; Roy, L.; Etienne, G.; Legros, L.; Charbonnier, A.; Coiteux, V.; Cony-Makhoul, P.; Huguet, F. Pioglitazone together with imatinib in chronic myeloid leukemia: A proof of concept study. Cancer 2017, 123, 1791–1799. [CrossRef]