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CHRONIC MYELOID LEUKEMIAS (G SAGLIO, SECTION EDITOR)

The Hidden Pathogenesis of CML: Is *BCR-ABL1* the First Event?

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Abstract

Purpose of Review Identification of the *BCR-ABL1* fusion oncogene in patients diagnosed with chronic myeloid leukemia (CML) led to the development of targeted therapy responsible for the dramatic survival benefits observed in the past two decades. However, despite these revolutionary findings, there remains marked disparity in patient outcomes. Why do some patients present de novo while others evolve to the more aggressive stages of CML? Why can select patients successfully discontinue therapy as part of a treatment-free remission attempt whereas others fail to meet specific molecular milestones?

Recent Findings *BCR-ABL1* kinase mutations are only identified in approximately 50% of patients with poor responses and disease progression, suggesting the presence of alternative resistance mechanisms. Numerous institutions have identified the presence of additional genomic events in addition to *BCR-ABL1* with the increasing availability of next-generation sequencing.

Summary We explore the potential pathways and events that may cooperate with *BCR-ABL1* to answer these questions but also challenge the fundamental tenet that *BCR-ABL1* is always the sole event initiating CML.

Keywords Next-generation sequencing · Mutations · Disease progression · Resistance

Introduction

For decades, it has been postulated that cancer development is a multi-step evolution [1] with Knudson formalizing the hypothesis that cancer is at minimum a "two-hit" process based on his work in pediatric retinoblastoma [2]. However, CML has defied this long-standing principle with only the Philadelphia (Ph) chromosome consistently identified in patients. It has long been the prototype of a disease with a clear genetically based diagnosis due to the unassailable presence of the oncogene, *BCR-ABL1*, formed by the translocation and fusion of *ABL1* from chromosome 9 to the *BCR* gene on chromosome 22 [3]. The development of the *BCR-ABL1* fusion gene has always been considered to be the inciting event in the pathogenesis of CML with its acquisition in a pluripotent stem cell conferring a marked survival advantage to its progeny via hyper-proliferative and anti-apoptotic mechanisms, resulting in effacement of normal hematopoiesis [3]. The identification of this single mutation is also the only molecular requirement for the establishment of the diagnosis of CML [4, 5], unlike other haematological malignancies such as acute myeloid leukemia [6] or myelodysplastic syndrome which have a far more heterogeneous molecular profile. Adding to the burden of evidence regarding the importance of *BCR-ABL1* in the pathogenesis of CML is the marked efficacy of the currently available targeted molecular therapies, the tyrosine kinase inhibitors (TKIs)—imatinib, nilotinib, dasatinib, bosutinib and ponatinib, which can induce significant cytogenetic and molecular remissions resulting in dramatic survival benefits [7–10] for CML patients compared to historical controls. Conversely, despite that only a single mutational event need be identified for patients to manifest

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disease responses and outcomes in CML are heterogeneous. While many patients are able to attain and maintain deep molecular responses, a proportion of patients either present or progress to the more aggressive accelerated phase (AP) or blast crisis (BC) CML, despite appropriate TKI therapy [11–13, 14•]. Other patients are able to successfully discontinue TKI under appropriate medical supervision as part of a treatment-free remission attempt [15–17], clearly demonstrating the diverse clinical landscape associated with CML. The *BCR-ABL1* oncogene is clearly sufficient to initiate chronic phase CML, but resistance and evolution to the more aggressive stages requires accumulation of additional events.

BCR-ABL1 Must Occur in the Pluripotent Stem Cell to Cause Disease

Transfection of murine stem cells with the *BCR-ABL1* fusion gene has resulted in the rapid development of a myeloproliferative neoplasm very similar to CML, leading to almost definitive confirmation that *BCR-ABL1* is the mandatory event required to trigger leukemic evolution [18, 19]. However, Fialkow's establishment of multiple B-lymphoid cell lines from a CML-affected patient with co-existing heterozygous expression of glucose-6-phosphate dehydrogenase revealed that only a fraction of resultant daughter cells expressed *BCR-ABL1* [20]. These experiments and observations indicate that the *BCR-ABL1* alone is sufficient to provide a survival advantage for leukemic cells, but an additional event is likely required for transmission to the descendants of the mutated leukemic stem cell (LSC) [20]. *BCR-ABL1* has also been detected in healthy individuals with neither clinical nor laboratory evidence of CML [21–23]. With limited follow-up, these patients do not develop CML, likely as these events are generally detected in terminally differentiated leukocytes as opposed to the LSC [23]. The absence of *BCR-ABL1* in the pluripotent stem cell explains the lack of proliferative potential, corroborating that this genetic event must develop in the LSC for CML to develop.

Disease Progression Requires Additional Events

BCR-ABL1-Dependent Resistance

TKI failure still occurs with progression to AP/BC occurring in 4–7% despite potent *BCR-ABL1* inhibition [11–13, 24]. Intrinsically, *BCR-ABL1* promotes genetic instability, more so with the co-occurrence of deletions of the derivative chromosome 9 [25], providing the perfect environment for the further gain of additional mutations [14•, 26, 27••]. Kinase domain (KD) mutations, currently the best-understood resistance mechanism, are an adverse prognostic factor, conferring an increased risk of transformation to AP/BC CML while predicting for an inferior progression-free survival (PFS) and overall survival (OS) [28•]. The risk of KD mutation development with upfront second-generation TKI therapy is lower compared with imatinib therapy although the prognostic implication is uncertain [12, 13]. The most common *BCR-ABL1* mutation, T315I, confers resistance against all first- and second-generation TKIs and results in inferior OS and PFS, even in comparison with the other KD mutations [28•]. Harbouring multiple KD mutations is also associated with a poorer outcome [29]. IC_{50} (50% inhibitory concentration) values are published for the most frequently detected KD mutations, enabling appropriate sequential therapy selection [30]. Inadvertent selection of a TKI with reported resistance against the identified mutation is usually associated with therapy failure [31]. However, KD mutations are not always identified in TKI-resistant patients, suggesting the presence of alternative resistance pathways. Furthermore, despite appropriately selected therapy based on identified KD mutations, not all patients respond.

At diagnosis, additional chromosomal abnormalities are observed in ~ 5% of chronic phase (CP) patients but more frequently in AP/BC. Historically, major route abnormalities (trisomy 8, isochromosome 17q, a second Philadelphia chromosome, trisomy 19) at diagnosis predicted for poorer survival and molecular outcomes [32•, 33•] although a recent publication questions this [34•]. Increased *BCR-ABL1* expression (via a second Philadelphia chromosome, *BCR-ABL1* gene amplification or increased oncogene transcription), although uncommon, has also been associated with TKI resistance [35]. Efflux and influx transporters have been implicated in resistance mechanisms in many malignancies, and in vitro, overexpression of ABCB1 and ABCG2 is associated with a reduction in intracellular TKI concentrations, potentially contributing to clinical TKI resistance [36]. Influx transporters such as OCT-1 mediate imatinib entry into cells, and patients with low OCT-1 activity have been shown to have inferior molecular and survival outcomes, increasing the risk of mutation development and disease progression [37].

Potential BCR-ABL1-Independent Resistance Mechanisms

While the mechanisms underlying disease progression are poorly understood, for some patients, it is likely dependent on the cooperation of *BCR-ABL1* with other dysregulated genes and signalling pathways (including STAT3, PI3K, SRC) implicated in malignancy [38]. The inherent genetic instability associated with unrestrained *BCR-ABL1* activity has been demonstrated to trigger the development of point mutations, insertions and deletions [14•, 27••, 38]. The largest published investigation to date into the genomic profile of CML patients revealed that at the time of diagnosis, 54% of

patients who eventually progressed to AP/BC had additional detectable cancer gene variants, which also predicted for earlier onset of BC [39]. The most frequently mutated cancer-associated genes were *ASXL1*, *IKZF1* and *RUNX1* [39••]. Patients with optimal outcomes, defined as eventual major molecular response, were less likely to acquire additional genomic events, occurring in 16% of the tested cohort [39••].

The mutational landscape observed in patients evolving to more aggressive disease is heterogeneous, with no individual signature profile observed. As expected, almost 60% of patients had identified KD mutations at the time of progression [39••]. At the time of BC, all patients sequenced had at least 1 variant detected in known cancer genes which were frequently mutated at diagnosis in other CP patients [39••]. Achievement of basic cytogenetic responses may also be prevented by the acquisition of these variants at diagnosis as 75% of poor outcome patients with failure to achieve at least a major cytogenetic response (6 out of 8 patients) had additional mutations in cancer genes, whereas this was only observed in 17% (1 out of 6 patients) achieving a complete cytogenetic response [39••].

BC can be myeloid, lymphoid or undifferentiated/mixed phenotype, supporting the hypothesis that *BCR-ABL1* develops in the pluripotent stem cell [40•]. There are some detectable differences in mutation profile depending on whether patients were either in lymphoid or myeloid blast crisis [39••]. KD mutations involving *BCR-ABL1* were more often observed in patients with lymphoid as opposed to myeloid BC [39••, 41•]. Mutations within the tumor suppressor gene, *TP53*, have been implicated in the progression to myeloid BC in almost 25% of patients [42–44], although a clear correlation with *TP53* has not been consistently demonstrable [39••, 45]. However, stabilization of *TP53* in BC has been shown to restore apoptotic regulation, terminating primitive granulocytic proliferation [46]. Gene fusions, where one of the gene partners is a cancer-associated gene, and *ASXL1* mutations also accompany myeloid BC [39••, 44, 47, 48]. *CDKN2A*, a gene frequently involved in the pathogenesis of acute lymphoblastic leukemia (ALL), regulates *TP53* and *RB1* activity [49]. Deletions involving the *CDKN2A* locus have been demonstrated in patients with BC, predominantly lymphoid [50]. Co-deletions in *IKZF1* (IKAROS), another gene implicated in the development of ALL [51], are also frequently observed in patients progressing to lymphoid BC [39••, 48, 52].

One of the most frequently mutated genes in CML is *ASXL1* [39••], which is also implicated as an adverse prognostic marker in other haematological malignancies including acute myeloid leukemia and myelodysplastic syndromes [6, 53–55]. At the time of diagnosis, evidence of *ASXL1* mutations have been identified in approximately 10% of CML patients [27••, 39••, 45]. However, not all patients had an adverse outcome with only 56–66% progressing to blast crisis [27••, 39••]. The median time to progression to BC was also prolonged compared with patients without *ASXL1* mutations [39••]. At the time of BC, the *ASXL1* mutations that were identified at diagnosis were frequently not detected, although other cancer gene variants were [39••]. For patients progressing to BC with *ASXL1* mutations identified only at the time of progression, these variants generally co-existed in conjunction with other genomic events [39••, 48]. Surprisingly, some patients with *ASXL1* variants identified at the time of diagnosis actually had optimal outcomes with the eventual achievement of major molecular response [39••, 56]. Subsequent testing on remission samples demonstrated disappearance of mutated *ASXL1* in some cases, suggesting the variant was present in a leukemic clone that was eliminated or reduced with TKI therapy [27, 39••]. Additional studies are required to establish the clinical relevance of mutated *ASXL1* at diagnosis. Acquisition following initial diagnosis is more likely to align with an adverse outcome and due to the co-occurrence of additional events, perhaps indicating underlying genetic instability of the patient [39••, 48].

A putative mechanism for the differentiation block accompanying myeloid blast crisis is diminished expression of *CEBPα*, a key transcription factor regulating granulocytic differentiation [57, 58]. Lower levels of *CEBPα* have been identified in patients demonstrating imatinib-resistance, especially in patients progressing to AP or BC CML [59]. Reduced expression of *CEBPα* has been found in KCL-22, a BC CML cell line, while restoration of *CEBPα* expression in this cell-line results in terminal granulocytic differentiation [60]. Physiological regulation of *CEBPα* in stem cells is performed by a + 37 kilobyte (kb) enhancer, activity of which can be perturbed by the fusion oncoproteins *RUNX1-RUNX1T1* and *CBFB-MYH11* [57]. Identification of these fusions in myeloid BC or patients failing therapy may illuminate a *BCR-ABL1*-independent mechanism of progression [39••, 61]. Hence, in conjunction with other studies evaluating the genomic landscape of CML [14•, 48, 62, 63], the presence of additional mutations is perhaps a hallmark of the risk of evolution to AP/BC.

Evidence of Preleukemic Variants

With increasing ability to identify variants due to technological and bioinformatic advances, the identification of clonal mutations in the absence of clinical disease is a recent topic of interest. These variants are frequently detected in healthy older individuals and are termed clonal hematopoiesis of indeterminate potential (CHIP). The presence of CHIP predicts for a slight increase in risk of development of myeloid neoplasms, but frequently, these patients never manifest any evidence of malignancy [1]. The most frequently implicated CHIP mutations are *DNMT3A*, *TET2* and *ASXL1*, which are often identified in myeloid malignancies [1]. CHIP mutations are not disease-specific and are often detected in patients with existing myeloid malignancies, obscuring the significance of

the identified variants. Persistence of CHIP mutations despite successful curative therapy suggests clearance of the dominant disease-initiating clone while the less pathogenic sub-clones persevere [1]. Due to the potential of these driver mutations to persist at high levels despite appropriate therapy, they are excluded from minimal residual disease analysis in acute myeloid leukemia, obscuring the prognostic implications of these mutations [64]. While the CHIP mutations in myeloid malignancies generally connote an adverse outcome, perhaps their first contribution to malignancy is to prime sub-clones for the acquisition of additional disease-causing events [1].

Persistence of leukemic clones despite appropriate *BCR-ABL1* inhibition has been identified in CML [14•]. Kim *et al.* found persistence of *TET2* mutations on diagnostic and follow-up samples of responsive patients with minimal variance in allele burden, attributing the presence of *TET2* to a Ph-negative preleukemic clone [27••, 45]. Likewise, Schmidt *et al.* demonstrated *DNMT3A* persistence in both diagnostic and follow-up remission samples, again present from a preleukemic clone [65]. Conversely, *DNMT3A* and *TET2* have been identified in remission samples alone, implying evolution within Ph-negative clones [39••, 65]. Other patients had mutations, frequently the CHIP-associated variants, identified at diagnosis that were not detected in remission samples, likely involving the Ph-positive clone [27, 65]. The presence of the preleukemic variants may be a manifestation of aging, but we postulate that in select patients, the presence of *ASXL1*, *DNMT3A*, *TET2* and other CHIP mutations could possibly indicate a favourable milieu for the development of further mutations, including *BCR-ABL1*.

Conclusion

The *BCR-ABL1* fusion gene is sufficient to manifest CP CML. However, disease progression and resistance likely occur due to the accumulation of additional events including kinase domain mutations, additional chromosomal abnormalities and variants in known cancer genes. Inadequate disease control, either through intrinsic resistance mechanisms or non-compliance, promotes genetic instability required for these additional events to develop. By highlighting these alternative pathways, more appropriate targeted therapy can follow, perhaps enhancing the 'cure' rate of CML with more patients able to discontinue TKI therapy instead of anticipating decades of treatment with associated toxicities. The detection of mutated cancer-associated genes at diagnosis indicates that these mutations either occurred soon after the acquisition of the *BCR-ABL1* fusion or in some cases, pre-dated the acquisition of the fusion. With increasing use of next-generating sequencing techniques, the clinical relevance of mutated cancer genes detectable at the time of CML diagnosis should be resolved over the coming years. Important questions remain to be answered, not least of which is whether additional mutations at diagnosis impact the chance of achieving treatment-free remission.

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Compliance with Ethical Standards

Conflict of Interest N.S. received honoraria from Novartis and Bristol-Myers Squibb and travel and accommodation expenses from Novartis, Gilead, Amgen and Janssen. S.B. is a member of the advisory boards of Qiagen, Novartis and Bristol-Myers Squibb and received honoraria from Qiagen, Novartis, Bristol-Myers Squibb and Cepheid.

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- • Of importance
- •• Of major importance

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