SPECIAL REPORT

STAT3 as a mediator of BCR-ABL1-independent resistance in chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a hematopoietic cell disorder, with an annual incidence of 1–1.5 per 100 000 in the United States. The *BCR-ABL1* oncogene is necessary and sufficient to cause the leukemic phenotype. CML is typically diagnosed in the chronic phase, where the myeloid cell compartment is expanded, but cellular differentiation maintained. Without effective therapy, there is inevitable transformation to the blastic phase, which resembles an acute leukemia of myeloid or lymphoid phenotype. Current CML therapy is based on tyrosine kinase inhibitors (TKIs), which block progression from the chronic to the blastic phase in the majority of patients. The first-generation TKI, imatinib mesylate (IM), is generally well tolerated, but has a failure rate of about 25% due to resistance or intolerance. Second-generation TKIs (dasatinib; nilotinib) provide effective salvage options, but some patients will eventually progress to blastic phase and have a poor prognosis. Additionally, even in patients with profound responses (complete molecular response), recurrence is common upon discontinuation of TKI therapy, a situation referred to as persistence. TKI resistance can result from two fundamentally different mechanisms: BCR-ABL1-dependent resistance, in which kinase domain mutations restore kinase activity by disrupting TKI binding,^{1,2} and BCR-ABL1-independent resistance, which can be divided into (i) extrinsic resistance, in which contact between CML cells and bone marrow-derived factors protects CML cells from TKIs,^{3,4} and (ii) cell-autonomous (intrinsic) resistance, in which CML cells activate alternative signaling pathways in the absence of bone marrow-derived factors.^{5,6} Importantly, BCR-ABL1-independent resistance occurs despite continued suppression of BCR-ABL1 activity. It is thought that persistence of residual leukemia in patients responding to TKIs is due to the innate BCR-ABL1 independence of CML stem cells.⁷ Consistent with this, we have found that primitive CML progenitor cells, including quiescent Lin[−] CML cells, survive despite TKI inhibition of BCR-ABL1.⁸ Our recent studies have implicated STAT3 in BCR-ABL1-independent resistance. In line with previous reports, we have found upregulation of pSTAT3^{Y705} in TKI-naïve CML progenitor cells grown in HS-5 bone marrow stromal cell-conditioned medium in the presence of IM, and that pSTAT3^{Y705} promotes CML cell survival, implicating STAT3 as a critical mediator of extrinsic BCR-ABL1-independent resistance. Interestingly, we have also found pSTAT3^{Y705} activation in CML CD34⁺ cells from patients with intrinsic TKI resistance (clinical resistance in the absence of BCR-ABL1 mutations). This suggests

that extrinsic and intrinsic BCR-ABL1-independent resistance mechanisms converge on pSTAT3^{Y705}, a notion that was validated with STAT3-specific small hairpin RNA and dominant-negative mutants. Our observations prompted us to evaluate the effects of small-molecule STAT3 inhibitors that block STAT3 dimerization through binding to the SH2 domain. Successive STAT3 inhibitor library screens identified BP-5-087 as a highly potent and selective STAT3 inhibitor. Computational modeling and physicochemical assays confirmed binding of BP-5-087 with STAT3. In biological assays using TKI-resistant progenitors, we found that BP-5-087 combined with imatinib reduces survival and colony formation by CML progenitor and stem cells from patients with BCR-ABL1-independent resistance, consistent with a synthetically lethal pharmacologic interaction. Taken together, our data indicate that activation of pSTAT3^{Y705} is central to BCR-ABL1 kinase-independent TKI resistance, and that genetic, functional or pharmacologic inhibition of STAT3 reduces TKI-resistant clonogenic potential *in vitro*. BP-5-087 is a highly potent and selective STAT3 inhibitor. Combinations of BP-5-087 with imatinib greatly reduce the survival of primary TKI-resistant stem and progenitor cells, and as such, may be considered a promising combinatorial therapy to overcome BCR-ABL1-independent TKI resistance and eliminate residual leukemia.

CONFLICT OF INTEREST

MWD served as a paid advisory board member and consultant for Bristol-Myers Squibb, ARIAD Pharmaceuticals Inc., Novartis, Incyte Corporation and Pfizer. The remaining authors declare no conflict of interest.

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