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EDITORIAL COMMENT

ROCK and Rolling Towards Predicting BCR-ABL Kinase Inhibitor-Induced Vascular Toxicity*

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Chronic myeloid leukemia (CML) is the prototype for successful molecularly targeted cancer therapy.[1] CML is caused by the Philadelphia chromosomal translocation that produces a BCR-ABL fusion with constitutive ABL kinase activity that drives myeloid cell proliferation. The development of imatinib, the first generation BCR-ABL tyrosine kinase inhibitor (TKI), has turned CML from a cancer with high mortality despite chemotherapy and bone marrow transplantation into a chronic disease treated with pills. The emergence of ABL kinase mutations that render leukemic cells resistant to imatinib has driven the development of second- and third-generation CML TKIs. These later generation drugs significantly improved the rate of molecular remission while being less specific for ABL kinase. The rationale for specifically targeting the genetic defect driving a particular cancer is to mitigate the harmful side effects of traditional cytotoxic chemotherapy. As such, a meta-analysis of clinical trials revealed that later generation BCR-ABL TKIs increase the risk for serious cardiovascular adverse events (CVAEs), thereby mitigating the cancer survival benefit of these newer agents.[2] Specifically, nilotinib, ponatinib, and dasatinib treatment portends a 3- to 4-fold increased risk for CVAEs, including myocardial infarction, stroke, and acute limb ischemia, compared with imatinib. A flurry of research has followed to uncover mechanisms driving CVAEs in order to mitigate risk. In this issue of *JACC: CardioOncology*, Yu et al[3] identify Rho-associated coiled-coil containing kinase (ROCK) as a potential mediator of BCR-ABL TKI-induced endothelial dysfunction and a possible biomarker to predict CVAE risk.

Yu et al[3] first examined ROCK activity in peripheral blood mononuclear cells from patients with leukemia treated with BCR-ABL TKIs. This clinical study, including 53 Philadelphia chromosome–positive patients with leukemia and 15 control subjects without leukemia, revealed that: 1) untreated patients with leukemia ($n = 8$) have higher leukocyte ROCK activity than control subjects; 2) patients with leukemia taking TKIs associated with CVAEs (nilotinib, ponatinib, and dasatinib; $n = 35$) have higher ROCK activity than those on imatinib ($n = 11$); 3) patients with leukemia with ROCK activity above the third quartile have an odds ratio >5 for CVAEs; and 4) in 5 of 6 patients with samples available before and after treatment with ponatinib or dasatinib, ROCK activity increased after treatment. The rest of the study used human aortic endothelial cells (ECs) to model the impact of BCR-ABL TKIs in vitro, showing that: 1) nilotinib, dasatinib, and ponatinib (but not imatinib) increase ROCK activity in ECs in a dose-dependent manner; 2) dasatinib and ponatinib impair EC function (permeability, nitric oxide production) in a ROCK-dependent manner; and 3) imatinib and nilotinib enhanced phosphorylation of p190RhoGAP, a known ABL kinase substrate and inhibitor of ROCK.

By adding to the growing body of knowledge regarding the impact of TKIs on ECs, this study has potential implications for prevention of TKI-induced CVAEs. Current strategies to prevent BCR-ABL TKI-induced CVAE are extrapolated from prevention

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guidelines in the noncancer population and include consideration of statins or aspirin. Clinical trials of cardiovascular disease prevention strategies specifically in the CML population are needed. Although studies exploring direct prothrombotic effects of BCR-ABL TKIs on platelets have yielded variable results, substantial data implicate EC damage in the pathogenesis of BCR-ABL TKI-induced CVAEs (reviewed in Haguet et al[4]), but the signaling mechanisms driving EC dysfunction remain unclear. The present study extends a prior observation implicating ROCK in dasatinib-induced endothelial dysfunction in vitro[5] by comparing dasatinib with other BCR-ABL TKIs and by testing ROCK activity in samples from patients with leukemia. Future studies are needed to confirm the role of ROCK in vivo by examining whether blocking ROCK activity protects ECs and mitigates the adverse impact of later generation BCR-ABL TKIs in atherothrombotic disease models.

The possibility that peripheral leukocyte ROCK activity may be a biomarker of CVAE risk also has important clinical implications. Extended patient survival due to the success of BCR-ABL TKIs has dramatically increased the prevalence of CML, a cancer that typically affects people in their 60s with baseline prevalent cardiovascular risk factors that predispose to ischemic events.[6] This high baseline risk is substantially exacerbated by treatment with later generation BCR-ABL TKIs, with up to 25% of ponatinib-treated patients developing CVAE over 5 years.[7] The timing of CVAEs is late, generally occurring 6 months to several years after starting treatment, and the events are acute and severe, including myocardial infarction, stroke, and critical limb ischemia. Hence, these side effects have a substantial adverse impact on a growing number of cancer survivors. Bosutinib and asciminib are 2 additional approved TKIs that were not tested in this study. Bosutinib, like imatinib, is not associated with increased risk for CVAE. Asciminib was recently approved for patients with the BCR-ABL T315I mutation, for which ponatinib was previously the only effective therapy, but long-term safety data are not yet available.[6] Thus, treatment options for Philadelphia chromosome–positive leukemias are growing, and the choice by oncologists could be modulated by better cardiovascular risk prediction. There are no proven biomarkers that specifically indicate risk in BCR-ABL TKI-treated patients with leukemia, so traditional cardiovascular risk scores are currently used to guide therapy. Risk scores derived in the general population are likely to be poorly calibrated in the CML population and may underestimate cardiovascular risk. Future validation that ROCK activity improves prediction over current risk scores could change CML management to allow modification of TKI therapy prior to development of CVAEs.

Limitations of this study should be acknowledged and provide opportunities for future research. A major limitation of the clinical study is the small sample size and substantial variability in patient treatments. Identification of higher ROCK activity in patients treated with second-generation TKIs is based on a comparison of patients treated with a mix of 3 later generation TKIs with only 11 patients on imatinib. With only 7 patients with blood samples available before and after treatment, causation cannot be distinguished from correlation. Indeed, untreated patients with CML already have higher ROCK activity compared with control patients, suggesting a drug-independent effect, although these data are also limited by the small number of imperfectly matched control subjects. In addition, whether traditional cardiovascular risk factors associate with ROCK activity needs to be examined and adjusted for, requiring a larger sample size. Thus, additional and much larger studies are certainly needed prior to considering ROCK activity as an actionable biomarker of CVAE risk in patients with leukemia.

The in vitro studies also have important limitations. Although the data show that the 3 drugs associated with CVAEs all increase ROCK activity, only dasatinib and ponatinib impaired EC function in a ROCK-dependent manner. As nilotinib also associates with CVAEs, one might conclude that ROCK-mediated EC dysfunction does not correlate with the clinical observation. Another potential explanation, however, could be related to drug dosing. In this study, ECs were treated with the same concentration of each TKI (100 nM to 1 μM) despite substantially different drug potencies in vivo. Indeed, the maximal effective concentrations in clinical trials for imatinib (4 μM) and nilotinib (3 μM) are more than an order of magnitude higher than dasatinib (0.2 μM) or ponatinib (0.1 nM) and higher than any concentration tested in this in vitro study. Thus, it remains possible that imatinib or nilotinib might affect EC function if tested at concentrations representative of those achieved in patients. An alternative explanation is that the BCR-ABL TKIs promote CVAEs by distinct mechanisms. For example, nilotinib increases fasting glucose and lipids and hence may promote atherosclerotic events via exacerbation of traditional risk factors,[6] whereas dasatinib and ponatinib appear to have a greater negative impact on EC function even in studies using more clinically relevant drug concentrations.[8] As such, whether ROCK activity is relevant for nilotinib-induced EC dysfunction and

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vascular risk requires further study. In vivo validation is an important next step to test whether ROCK inhibition may protect from BCR-ABL TKI-induced endothelial dysfunction using preclinical atherothrombosis models before considering randomized trials testing ROCK-targeted vascular protective agents in patients with leukemia.

Despite these limitations, this study supports the possibility that peripheral blood mononuclear cell ROCK activity may be a biomarker of CVAE risk in patients with leukemia treated with second- and third-generation BCR-ABL TKIs and that ROCK inhibition is a potential target to protect the endothelium from the adverse impact of these drugs. If confirmed in vivo and in larger clinical studies, these findings have substantial implications to change the management and improve overall outcomes in the rapidly growing CML population.

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