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Response to Comment on Incidence and Risk Factors of Bleeding-Related Adverse Events in Patients with Chronic Lymphocytic Leukemia Treated with Ibrutinib

We thank Drs. Tam and Kamel for their thoughtful comments on our recent publication on the incidence and risk factors of bleeding-related adverse events in patients with chronic lymphocytic leukemia (CLL) treated with the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib.[1] We welcome the opportunity to answer their questions and address their concerns.

At a median follow-up of 24 months we recorded grade ≤2 bleeding-related adverse events in 55% of 85 patients. No grade ≥3 events occurred. We reported two types of analyses in these patients. First, we prospectively assessed platelet function and coagulation factors at baseline and after 4 weeks of therapy and found that parameters associated with a significantly increased risk of bleeding-related events were present at baseline, including prolonged epinephrine closure time, and lower levels of vWF activity and FVIII. Secondly, we compared platelet aggregation response to collagen and ADP in 30 patients treated with ibrutinib for >6 months to responses in 12 healthy volunteers, 13 patients with treatment-naïve CLL, and 3 patients with XLA (who carry loss-of-function mutations in BTK).

Cognizant of a prior report that CLL lymphocytes themselves inhibit platelet aggregation,[2] we chose to use whole blood for platelet aggregation testing rather than platelet-rich plasma, as others have done, in order to capture the effect of CLL cells on platelet aggregation. While laboratory testing may never fully reflect in vivo situations, whole blood studies arguably more closely reflect the condition of blood circulating in vivo than do studies using platelet rich plasma. Our key findings were that compared to normal controls, response to both agonists was decreased in all patients with CLL, whether on ibrutinib or not. Compared to untreated CLL patients, response to collagen showed a mild further decrement on ibrutinib, while response to ADP improved. Drs. Tam and Kamel rightly point out that the platelet count could influence the analysis of platelet function by aggregation and we appreciate the opportunity to provide the requested additional data and clarifications.

Figure 1 shows the distribution of platelet counts in the groups tested: healthy volunteers (median 205 k/μL; IQR 192–229); untreated CLL control subjects (median 161 k/μL; IQR 121-225); and patients on ibrutinib (median 146 k/μL, IQR (118-184). Thrombocytopenia <100 k/μL was present in 4 out of 30 patients on ibrutinib (platelet counts of 72 k/μL, 86 k/μL, 88 k/μL and 97 k/μL, respectively). Drs. Tam and Kamel write that several groups have observed baseline defects in platelet aggregation in CLL that occurs "non-specifically across multiple agonists", and conclude that this effect was "mainly related to thrombocytopenia" as accurate platelet aggregometry using platelet-rich plasma requires platelet counts greater than 100-150 k/μL.[3] We agree that thrombocytopenia would result in a global decrease in aggregation with all agonists; accordingly, the fact that ADP and collagen aggregation were affected in a divergent fashion argues against simple thrombocytopenia (or its reversal by treatment) as the cause for our findings. Additionally, there was no statistically significant difference in platelet counts between healthy controls and untreated CLL patients in our analysis, suggesting that lower platelet counts do not account for the observed baseline aggregation defect in CLL. Furthermore, given that platelet counts did not differ between patients on ibrutinib and CLL controls, we do not believe that treatment related recovery of platelet counts influenced the improvement in ADP response. The significantly lower platelet counts in ibrutinib treated CLL patients (compared to normal subjects) undermines the case for attributing slightly inferior collagen aggregation responses to a drug toxicity, as lower platelet counts confound this inference. Additionally, adjusting for the observed difference in platelet counts would serve only to amplify the relative contribution of ibrutinib to improved ADP-mediated aggregation. Thus, on the whole, we do not believe platelet counts explain the marked differences in collagen and ADP responses which we reported.

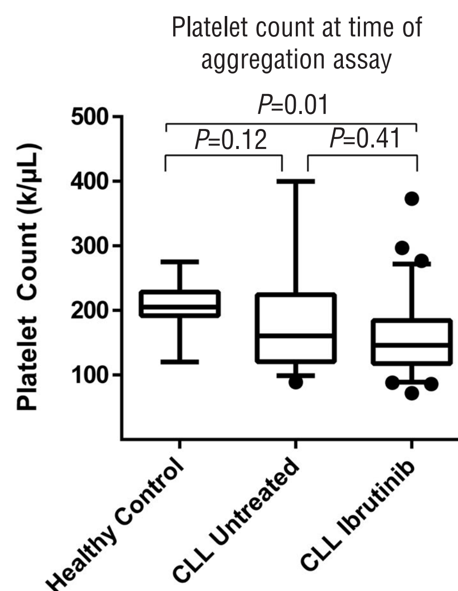

Figure 1. The distribution of platelet counts in the groups tested.

Drs. Tam and Kamel are concerned our results may lead to a perception of "blame the disease [CLL], not the drug". Clearly, bruising and low-grade bleeding adverse events were more common in patients randomized to ibrutinib compared to the control arm,[4] and the drug's package insert recommends weighing the risks and benefits of interrupting treatment with ibrutinib for invasive procedures. We agree that ibrutinib contributes to platelet dysfunction, and do not argue otherwise. However, prior work by others demonstrated platelet function defects in CLL patients studied,[5] and Pulte et al. showed that the addition of CLL lymphocytes to platelet-rich plasma diminishes aggregation responses to ADP.[2] In our analysis of whole blood, compared to normal controls platelet aggregation was significantly decreased in untreated CLL patients who had a 43% reduction in response to low dose collagen and 57% reduction in response to high dose ADP. Granule release was similarly impaired, by > 80% in response to collagen and 79% in response to ADP. In summary, our findings and those of others suggest there is some contribution by CLL cells to platelet dysfunction that is not attributable to ibrutinib. Notably, our observation that the risk of bleeding-related adverse events appears to decrease after the first 6 months on ibrutinib is consistent with a contribution of disease factors to overall bleeding risk.

Finally, recent studies indicate that the aggregation defect in collagen-evoked signaling in suspension assays may play

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less of a role *in vivo* than the initial studies would suggest. For example, by utilizing several methods, including single-cell imaging measuring Ca++ concentrations in individual platelets, Bye et al. found that the collagen signaling deficiency caused by ibrutinib is milder during adhesion to immobilized collagen compared to in suspension.[6] Furthermore, using whole blood they found that initial adhesion to collagen under arterial shear stress was not significantly inhibited by ibrutinib, but stable thrombus formation was strongly inhibited.

Combined, all these data suggest that ibrutinib's effect on platelet function involves several signaling pathways, and a better understanding of the interplay between these factors will contribute to the safe use of ibrutinib, in particular when combinations with anti-platelet agents or anticoagulants have to be considered.

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