LETTER TO THE EDITOR

Pirtobrutinib results in reversible platelet dysfunction compared to ibrutinib and acalabrutinib

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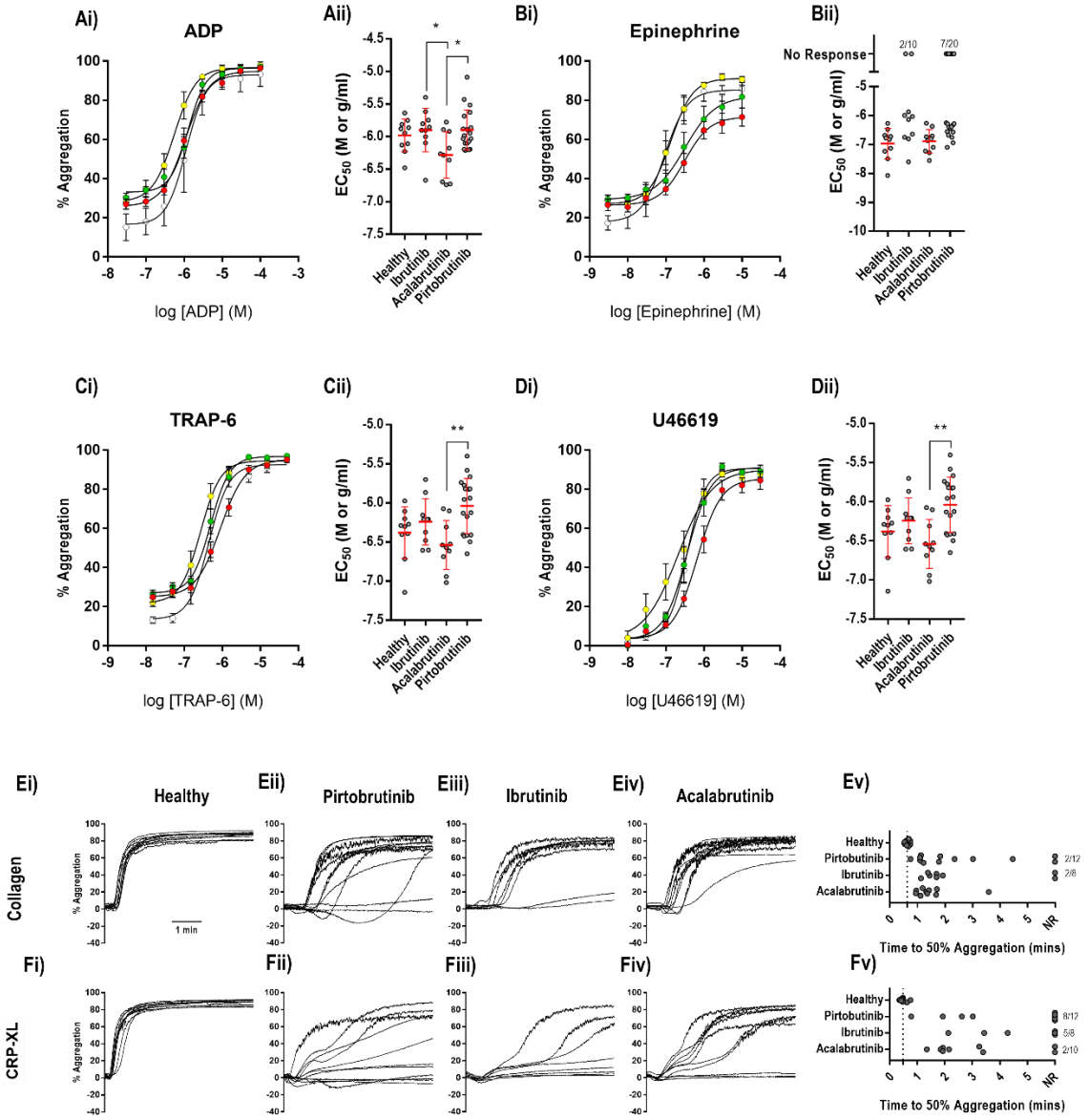
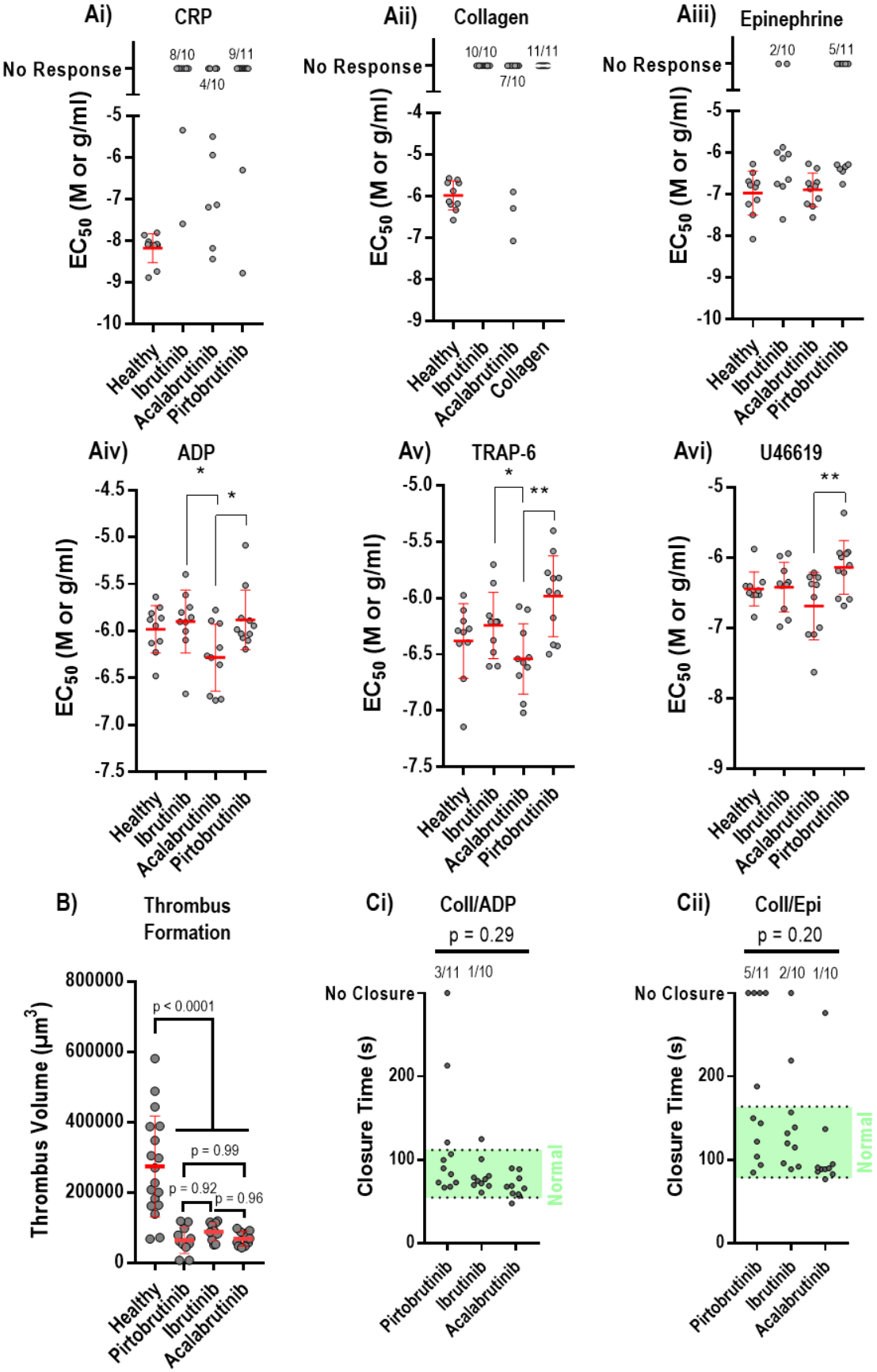
Supplementary Figure 1



Supplementary Figure 1. Pirtobrutinib, ibrutinib and acalabrutinib therapy causes platelet dysfunction downstream of GPVI. Aggregation of PRP from healthy donors or patients receiving BTKi therapy measured in 96-well plates following stimulation with concentration ranges of A) ADP, B) epinephrine, C) TRAP-6 and D) U46619**.** Plots of i) concentration-response curves for each agonist in which points represent the mean response to each concentration ± s.e.m. and ii) scatter plots of EC_{50} values, bars represent the mean ± S.D. Failure to induce concentration-dependent aggregation was designated 'No Response'. The proportion of non-responders is noted at the top of relevant scatter plots. Statistical comparisons were performed by two-way ANOVA with Tukey multiple comparisons test. * p

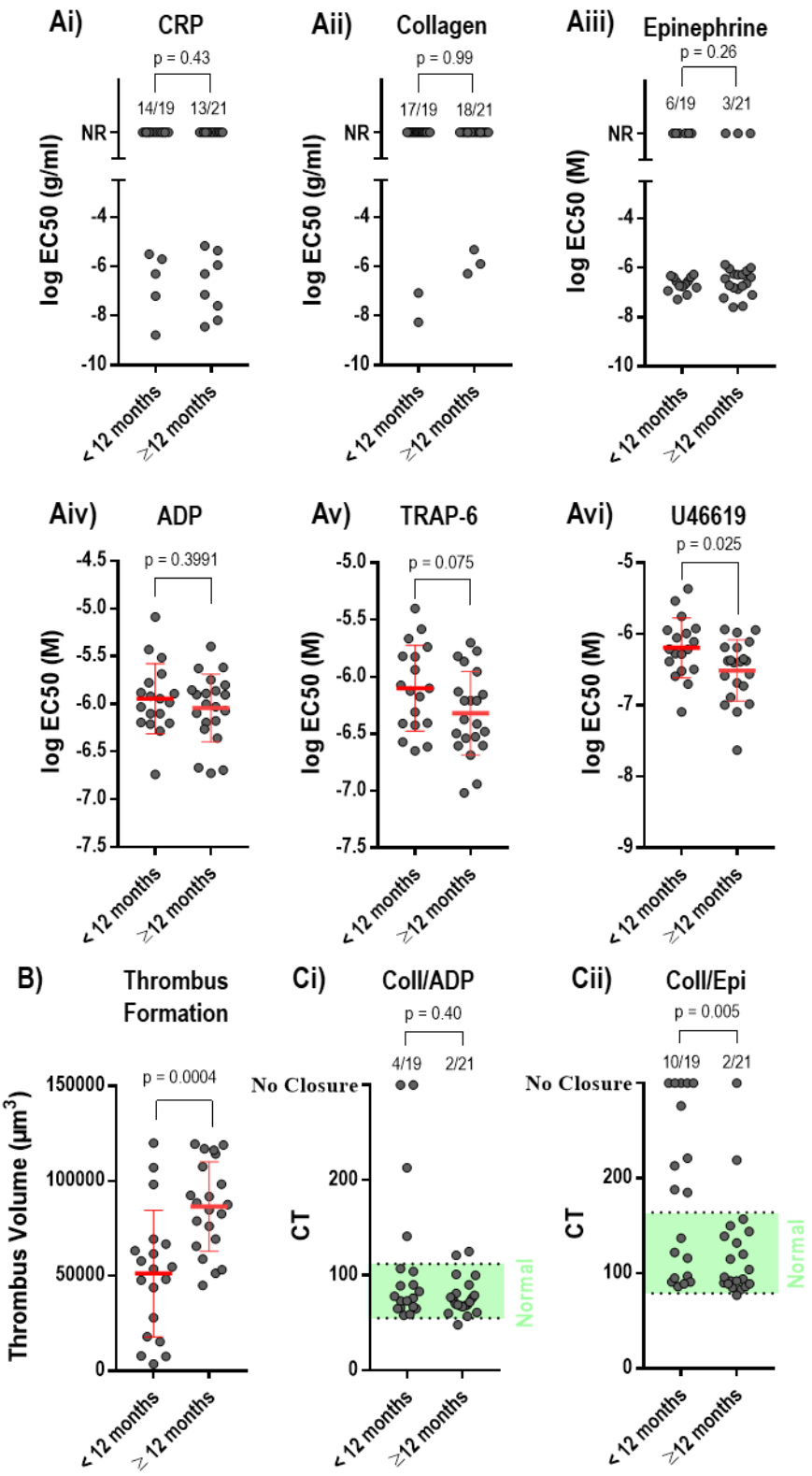
< 0.05, ** p < 0.01. Aggregation measured by Born aggregometry stimulated by E) 10µg/ml Collagen or F) 3µg/ml CRP-XL of PRP samples taken from i) healthy donors or patients receiving ii) pirtobrutinib, iii) ibrutinib or iv) acalabrutinib. Scatter plots of v) time taken to achieve 50% aggregation. The dotted line represents the mean time to 50% aggregation of healthy donors stimulated with each agonist. Failure to achieve 50% aggregation within 5 minutes was designated 'not reached' (NR). The proportion of responses that did not reach 50% aggregation is noted to the right of the graphs.

Supplementary Figure 2



Supplementary Figure 2. Platelet function parameters for patients with CLL only. A) Scatter plots of the EC_{50} of PBA responses of patients with CLL receiving BTKi to i) CRP-XL, ii) collagen, iii) ADP, iv) epinephrine, v) TRAP-6 and vi) U46619. Bars represent the mean EC50 ± S.D. The p-values were calculated by t-test to compare mean EC_{50}. B) A scatter plot of thrombus volumes formed in collagen-coated microfluidic chambers. Bars represent the mean volume ± S.D. and the p-value was calculated by 1-way ANOVA. Scatter plots of PFA-200 closure times measured using Ci) collagen/ADP and Cii) collagen/ADP cartridges grouped by duration of BTKi therapy including the normal CT range (green) and proportion of abnormal CT (top of graph). The p-values were calculated using Fisher's Exact Test to compare the proportion of prolonged CTs in the groups.

Supplementary Figure 3



Supplementary Figure 3. Duration of BTKi therapy is a stronger determinant of whole blood haemostasis parameters than pathway-specific platelet function measured by aggregometry. A) Scatter plots of the EC_{50} of PBA responses of patients receiving BTKi to i) CRP-XL, ii) collagen, iii) ADP, iv) epinephrine, v) TRAP-6 and vi) U46619 by duration of BTKi therapy. Bars represent the mean EC50 ± S.D. The p-values were calculated by t-test to compare mean EC_{50} or by chi-square to compare proportions of non-responders (NR) as appropriate. B) A scatter plot of thrombus volumes formed in collagen-coated microfluidic chambers compared by duration of BTKi therapy. Bars represent the mean volume ± S.D. and the p-value was calculated by t-test. Scatter plots of PFA-200 closure times measured using Ci) collagen/ADP and Cii) collagen/ADP cartridges grouped by duration of BTKi therapy including the normal CT range (green) and proportion of abnormal CT (top of graph). The p-values were calculated using Chi-square analysis to compare the proportion of prolonged CTs in the groups.