In response to the comment by Hechler *et al.*: Amotosalen/UVA pathogen inactivation technology reduces platelet activatability, induces apoptosis and accelerates clearance.

We would like to thank B. Hechler and colleagues for the interest shown in our paper on the effects of the Amotosalen/UVA on platelet function, and we are pleased to take the opportunity to reply to their comments.

They note correctly that the results of our study are divergent from their previously published observation, but state that "..Stivala et al. quote our study in a way which may lead to convergent results, which is obviously not the case." In the discussion of our paper, we refer to the publication by Hechler et al.[1] in three separate instances:

1. In the second paragraph of the Discussion section, where we speculate that the reduced platelet adhesion to collagen under flow conditions observed in our study may be due to a reduced amount of surface GPV as reported by Hechler et al., since GPV has been implicated to participate in the platelet response to collagen (Moog et al.[2]);

2. In the third paragraph of the same section, where we clearly state that "In contrast to previous studies - with reference to Hechler et al. and two others- we did not detect an increased activation of the fibrinogen receptor GpIIbIIIa" and we continue by proposing that "this could be partly explained by the different protocol used for platelet collection, which was shown to affect platelet activation.[3,4]" Hechler et al. also agrees with us that "…differences in the overall initial collection procedure and/or storage conditions may play a role and explain such differences between centers."

3. Finally, in the second to last paragraph of the Discussion, we specifically cite the study by Hechler in "…although one study reported no change in platelet aggregation when washed platelets were used", again clearly stating the different results between our study and the one from Hechler et al. Therefore, we believe that careful reading of our manuscript does not lead to the assumption that our results are in agreement with those of Hechler et al.

Regarding the influence of the storage medium, Hechler et al. propose that it may have "inhibitory yet reversible effects on platelet responsiveness." Undoubtedly the storage medium influences platelet response as reported by several studies,[5,6] yet it might indeed have longer-lasting effects which are not reversible, especially after longer storage time (i.e., 3-4 days). In our study, platelets were resuspended in the same storage medium for all treatment groups (untreated and IBS-treated with or without pre-treatment with inhibitors) so the difference between untreated and Amotosalen treated platelets cannot solely be explained by a negative impact of the storage medium nor by the pH, which, even though significantly lower for the Amotosalen/UVA samples (as reported in other studies too[7,8]), was nevertheless in an acceptable range observed by others and above 7. Besides, for the in vivo analysis of platelet survival in mice, human platelets were washed and resuspended in sterile PBS before i.v. injection, therefore, any possible effect of the medium itself on the outcome (platelet survival) can be excluded.

Regarding the in vivo model itself, several studies[9-11] have shown a correlation between platelet survival and platelet damage upon injection of human platelets in immunodeficient mice and have therefore confirmed the usefulness of this model and its reproducibility. We also agree that some studies did not report an increased usage of platelet components with Amotosalen/UVA, but others did in fact observe a decreased count increment (CI) and corrected count increment (CCI),[12-14] which could be explained primarily by an increased platelet clearance. Finally, we would like to emphasize again (as we state at the end of our paper) that the primary goal of our study was to better understand the effect of Amotosalen/UVA on platelet function at the molecular level in order to develop newer and better pathogen inactivation technologies.

We want to thank Hechler et al. for their constructive input and we hope that we were able to clarify it.

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