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Editorial

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# Failure of MMP-9 Antagonists in IBD: Demonstrating the Importance of Molecular Biology and Well-Controlled Early Phase Studies

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Tissue remodelling plays an important role in inflammatory bowel disease [IBD].[1] Matrix metalloproteinases [MMPs] are key enzymes during this chronic process as they are responsible for cleaving extracellular matrix [ECM] components and thus contribute to the destruction of the intestinal barrier.[2] MMPs are secreted as latent pro-enzymes and require cleavage of the pro-peptide to obtain activity. MMP-9 has drawn most attention because it has been shown to be involved in multiple disease pathways. However, the question remains whether MMP-9 actually plays a causal role in these disease pathways, or if its upregulation is rather a consequence of the overall inflammation [mainly mediated by neutrophils, a major source of MMP-9]. Indeed, MMP-9 was previously identified as an important marker of inflammation in both ulcerative colitis [UC] and Crohn's disease [CD].[3–5] Although previous studies claimed that genetic deletion or inhibition of MMP-9 improved murine colitis,[6–8] the causality of MMP-9 could not be validated in a more recent study with multiple well-controlled preclinical murine models.[9] Therefore, the therapeutic potential of anti-MMP-9 antibodies in human IBD could not be clearly distilled from animal models.

In this issue of the *Journal of Crohn's and Colitis*, Sandborn *et al.*[10] and Schreiber *et al.*[11] address the efficacy of andecaliximab [GS-5745], a recombinant chimeric IgG4 monoclonal antibody directed against both latent pro-MMP-9 and activated MMP-9, in patients with moderate to severe UC and CD, respectively. In a previous phase 1 dose-escalation study in patients with UC,[12] 5 weeks of both intravenous [IV] and subcutaneous [SC] administration of andecaliximab was well tolerated and—in an exploratory analysis—a higher efficacy with better clinical, endoscopic and histological responses compared to placebo was suggested. In the phase 2/3 study in UC patients described by Sandborn *et al.*,[10] 165 patients were randomized [1:1:1] to subcutaneously receive either placebo, 150 mg andecaliximab every 2 weeks [Q2W] or 150 mg andecaliximab weekly [QW]. The primary endpoint was endoscopy/bleeding/stool [EBS]-defined clinical remission at week 8 with a Mayo endoscopic sub-score of 0 or 1, a rectal bleeding sub-score of 0, and ≥1 point decrease from baseline in stool frequency achieving a sub-score of 0 or 1. However, there was no significant difference in the proportion of patients achieving EBS clinical remission at week 8 between placebo and Q2W or QW andecaliximab [7.3, 7.4 and 1.8%, respectively]. The Mayo Clinic Score response, endoscopic response, mucosal healing and adverse events rates were also not significantly different. In a parallel phase 2 study in CD patients performed by Schreiber *et al.*,[11] 187 patients were randomized 1:2:2:2 to subcutaneously receive placebo, andecaliximab 150 mg Q2W, andecaliximab 150 mg QW or andecaliximab 300 mg QW for 8 weeks. The co-primary endpoints were clinical response (liquid or very soft stool frequency and abdominal pain composite [based on PRO2] score ≤8 at week 8) and endoscopic response (≥50% reduction from baseline of Simple Endoscopic Score for Crohn Disease (SES-CD) after 8 weeks). No differences in the proportion of patients treated with placebo, andecaliximab 150 mg Q2W, andecaliximab 150 mg QW or andecaliximab 300 mg QW were observed with regard to achievement of clinical response [14.3, 17.0, 13.2 and 11.3%, respectively] or endoscopic response [10.7, 11.3, 13.2 and 7.5%, respectively]. In addition, no differences in adverse event rates were reported.

We believe both studies faced multiple limitations suggesting early termination of the clinical development of andecaliximab. Exploratory efficacy analyses of the preceding phase 1 study included clinical response defined as reduction in the complete Mayo Clinic score of ≥3 points and a decrease in at least 30% from the baseline score with ≥1 point decrease in the rectal bleeding sub-score or a rectal bleeding sub-score ≤1, and clinical remission defined as complete Mayo Clinic score ≤2 with no sub-score >1 at day 36.[12] Clinical response occurred in 43% of the patients receiving andecaliximab [pooled multiple dose cohorts] compared with 13% receiving placebo, whereas clinical remission occurred in 14% treated with andecaliximab and 0% who received placebo. Importantly, these endpoints were different from those used in the phase 2/3 studies. Moreover, looking at the patient characteristics, UC patients included in the phase 1 study had a median faecal calprotectin level of 544.1 mg/kg [vs 1478 mg/kg in phase 2/3]. In the phase 1 study, 74 patients were randomized to receive single or multiple doses every 2 weeks by IV infusions [0.3, 1.0, 2.5 or 5.0 mg/kg andecaliximab or placebo] or weekly SC injections [150 mg andecaliximab or placebo].

Ultimately, only ten patients received 150 mg andecaliximab subcutaneously. Together with the differences in patient characteristics, this small cohort size may explain the lack of reproducibility in the phase 2/3 study due to inaccurate reflection of the general UC population. Indeed, the efficacy endpoints in the phase 1 study were only exploratory, and therefore no firm conclusions on efficacy could be made.

Second, the authors reported no differences in adverse event rates between patients treated with andecaliximab or placebo. This was an important observation because MMP inhibition has a history with severe adverse events [SAEs], such as musculoskeletal syndrome. However, as for most phase 2/3 trials the study was not designed and therefore underpowered to evaluate safety issues. If we look more closely at the reported SAE and treatment emergent adverse event [TEAE] rates, there does seem to be a numerical difference with regard to andecaliximab-treated patients compared to placebo, especially for those treated at higher frequency or with higher doses. More specifically, in the UC study by Sandborn *et al.*, two patients treated with 150 mg QW andecaliximab experienced SAEs, compared to one patient in the placebo group. Importantly, three patients in the QW group [vs one in the Q2W group and one in the placebo group] experienced TEAEs, resulting in premature study discontinuation. In the CD study by Schreiber *et al.*, six patients on 150 mg andecaliximab QW had serious TEAEs as well as eight patients on 300 mg andecaliximab QW compared to one patient on 150 mg andecaliximab Q2W and three patients on placebo. Three patients on 150 mg andecaliximab QW and four patients on andecaliximab 300 mg QW had to discontinue treatment compared to one patient on placebo. It will be important to understand if these TEAEs were specifically related to MMP-9 inhibition, given that MMP-9 does not only play a role in tissue destruction, but also in regulation of the inflammatory response and cell signalling.[13]

A third concern relates to the structural properties and *in vivo* neutralizing effect of the antibody. Andecaliximab, the humanized version of AB0041, was generated through immunization of BALB/c mice with recombinant human MMP-9 and targets both the pro-domain and the catalytic domain in a non-competitive way. In the original study by Marshall *et al.*[7] this was portrayed as a therapeutic advantage. More elaborate biochemical analyses were subsequently performed by Appleby *et al.*, showing that andecaliximab exploits its inhibitory potential in two ways: as an allosteric inhibitor of MMP-9 activity and by preventing activation of the secreted pro-enzyme.[14] Importantly, the authors stated that andecaliximab binds to active MMP-9 with ~150–400-fold weaker affinity compared with pro-MMP-9. Although selective for MMP-9, andecaliximab therefore does not completely inhibit MMP-9 activity and substrate binding, but it may affect substrate orientation and subsequent cleavage. Sandborn *et al.* mentioned that they did not measure MMP-9 coverage in stool or colonic tissue because no validated techniques exist.[10] However, it will be important to generate these data to better understand the neutralizing effect of andecaliximab. Moreover, other techniques [e.g. gelatin zymography[15], a specialized technique to distinguish pro-, activated, complexed, monomer or trimer forms of MMP-9] can be performed to study MMP-9 levels in stools or tissue.

Based on the fact that andecaliximab did not show efficacy in UC and CD, Gilead decided to terminate its further development in IBD. However, there might still be a chance for andecaliximab in a specific subset of patients, namely those with fistulizing CD. In these patients, the intestinal tissue is excessively broken down, creating fistulae, indicating a severe imbalance between MMPs and their inhibitors (tissue inhibitors of MMPs [TIMPs]). Indeed, previous studies have shown that MMP-9 is increasingly expressed surrounding fistulae tracts.[16] Interestingly, Schreiber *et al.* described that 18% of the CD patients included in the phase 2 study had fistulizing disease prior to andecaliximab.[9] It will be important to understand if an improvement of the fistulae was observed after 8 weeks of andecaliximab, and if so, using which dosing regimen[s]. Nevertheless, given the outcomes of the phase 2/3 trials, it is clear that studying the effect of andecaliximab in a murine model of fistulizing disease [e.g. SAMP1/YitFc mice] must proceed its potential use in patients.

In conclusion, phase 2/3 studies with andecaliximab did not show efficacy in UC and CD patients, despite promising earlier phase 1 safety and exploratory efficacy data. This may partially be explained by differences in study endpoints, patient characteristics and the small number of patients actually treated subcutaneously with andecaliximab in the phase 1 study. Importantly, recent preclinical murine models on the role of MMP-9 also generated conflicting data. Of note, andecaliximab might cause an increase in SAEs at higher/more frequent doses. The non-competitive character of the antibody and lack of *in vivo* neutralizing data warrant further research. Despite these limitations and given the excessive imbalance between MMPs and TIMPs in patients with fistulizing disease, MMP-9 inhibition in this specific subgroup of patients might still be worth pursuing, provided the availability of preclinical evidence.

## Conflict of Interest

MdB has no conflict of interest. MF is a Senior Clinical Investigator of the Research Foundation Flanders [FWO], Belgium, and received financial support for research from Takeda and Janssen; lecture fees from Ferring, Boehringer-Ingelheim, Chiesi, MSD, Tillotts, Janssen Biologics, Abbvie, Takeda, Mitsubishi Tanabe, Zeria; and consultancy fees from Abbvie, Boehringer-Ingelheim, Ferring, MSD and Janssen Biologics.

## Author Contributions

MdB and MF drafted and revised the text. Both authors approved the final version of the manuscript.

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