Additional File 3 – Supplementary Discussion

We found that the patients for which we observed these associations were frequently treated with vancomycin, ciprofloxacin, and especially ceftazidime at time points early post-HSCT. This is in contrast to our previous study that revealed high *Lachnospiraceae* and *Ruminococcaceae* abundances and rapid B and NK cell reconstitution in the absence of vancomycin and ciprofloxacin treatment [1]. Vancomycin and ciprofloxacin are among the broad-spectrum antibiotics that have previously been attributed a detrimental effect on the commensal microbiota, especially on *Clostridiales* [1–3]. The effect of ceftazidime however is controversially discussed. On the one hand, commensal sparing has previously been observed for cefepime, which belongs to the same antibiotic class as ceftazidime (cephalosporins) [4]. On the other hand, ceftazidime treatment could not rescue bacterial alpha diversity compared with e.g. vancomycin or ciprofloxacin treatment in a previous report [2]. In the present study, high abundances of clostridial microbiota members despite vancomycin and ciprofloxacin treatment might point to a potential beneficial effect of the additional treatment with ceftazidime. Moreover, future studies could assess if reconstitution in different NK cell subsets might differ with regards to time point and dependency on microbial composition and antimicrobial treatment.

We found that a specific *Parabacteroides distasonis* ASV (family *Tannerellaceae*) predicted moderate to severe aGvHD. In this context, it is interesting that our multivariate analyses indicated that a set of other *Parabacteroides* spp. ASVs was associated with high eosinophil counts from month +3 onwards. *Parabacteroides* members are providing the SCFA propionate which has previously been found to bind to the G 500 protein-coupled receptor GPR43 on eosinophils, although the exact mechanisms of GPR43-mediated immune modulation are yet to be investigated [5,6]. Interestingly, increased eosinophil numbers prior to and during aGvHD have been described before [7]. Therefore, an explanation for our prediction of moderate to severe aGvHD from high pre-HSCT *Parabacteroides* spp. abundances could involve a potential propionate-mediated activation of eosinophils, which might contribute to aGvHD. In contrast, propionate can also stimulate T_{reg} cells, which in turn prevent T_H17-induced inflammation involved in aGvHD [8,9]. We did not observe any correlation between *Parabacteroides* members and T cell counts in our study, however T cell subsets were measured after aGvHD and thus might be altered according to the inflammatory condition itself or its treatment.
In addition, a specific *Lachnospiraceae* ASV in the gut predicted subsequent moderate to severe aGVHD when highly abundant prior to HSCT. This family has previously been reported to be reduced prior to aGvHD (but post transplantation) [10,11]. However, here we examined pre-HSCT abundances to make predictions, i.e. we did not aim at elucidating *Lachnospiraceae* abundances concurrent to aGvHD. Particular members of this bacterial family are crucial producers of the SCFA butyrate, which has been suggested to prevent aGvHD directly by improving epithelial integrity in GvHD-target tissue, and/or indirectly by inducing T_{reg} cells that reduce inflammation [9,12]. Of note, we did not observe any strong associations between bacterial abundances and inflammation (represented by CRP levels) at any body site in this study. It is of interest for future studies to reveal the exact mechanisms by which certain *Lachnospiraceae*, and specifically the predictive ASV we identified, might promote aGvHD, or whether their increase might be compensatory.

In addition to ASVs in the gut, we also identified oral and nasal cavity ASVs with pre-transplant abundances predicting subsequent aGvHD. For instance, in the oral cavity, an ASV affiliated with *Prevotella melaninogenica* strongly predicted aGvHD. This species is known to cause oral mucosal infections, and to be elevated in abundance in oral cancer [13,14]. Oral mucositis in allogeneic HSCT has been linked to elevated aGvHD risk [15,16]. It may be speculated that this could account for the observed association between aGvHD and high levels of this *Prevotella* ASV, although we did not investigate manifestations of mucositis in the present study. This highlights the need for direct evaluation of oral mucositis in this context.

Other microbial predictors of aGvHD in the oral and nasal cavity included members of the order *Actinomycetales* (*Actinomyces* spp., *Pseudopropionibacterium propionicum*, *Rothia* sp.). The order *Actinomycetales* comprises numerous commensals colonizing the oral and nasal cavities in healthy individuals, but can cause opportunistic infections in e.g. allo-HSCT patients [17]. It has been demonstrated that aGvHD increased the susceptibility to infections in allogeneic HSCT patients, but a predisposition to aGvHD due to a preceding infection has also been suggested for *Clostridium difficile* [18]. Whether actinomycosis might play a role in facilitating aGvHD development remains to be investigated. Interestingly, an increase of oral *Actinobacteria* in the gut at the time of neutrophil recovery has been found to be correlated with subsequent severe aGvHD [11]. One potential explanation involves the ability of *Actinomyces* spp. to drive biofilm formations, e.g. in periodontitis and during colorectal cancer [19].

Interestingly, among all ASVs significantly predicting aGvHD at any body site, we found only one ASV, namely a nasal *Rothia* sp. ASV, with high pre-HSCT abundances predicting that patients would be spared from subsequent aGvHD. *Rothia mucilaginosa* was among the species that, when present in the gut post-transplant, correlated with moderate to severe aGvHD in a previous study [11]. However, it has to date not been assessed in which way different *Rothia* species in the nasal cavity might be associated with aGvHD. This discrepancy again emphasizes the importance of high taxonomic resolution in this context, and the examination of the microbiota at different body sites.

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