BACKGROUND: The bacterial microflora aggravates graft-versus-host-disease (GvHD) after allogeneic stem cell transplantation, but the underlying mechanisms of manifestations of intestinal GvHD (iGvHD) in the gut remain poorly understood. AIM: To analyse the gut flora composition and the impact of bacterial sensing via Toll-like receptors (TLRs) in iGvHD.

METHODS: By mimicking clinical low-intensity conditioning regimens used in humans, a novel irradiation independent, treosulfan and cyclophosphamide-based murine allogeneic transplantation model was established. A global survey of the intestinal microflora by cultural and molecular methods was performed, the intestinal immunopathology in TLR-deficient recipient mice with iGvHD investigated and finally, the impact of anti-TLR9 treatment on iGvHD development assessed.

RESULTS: The inflammatory responses in iGvHD were accompanied by gut flora shifts towards enterobacteria, enterococci and Bacteroides/Prevotella spp. Analysis of iGvHD in MyD88(-/-), TRIF(-/-), TLR2/4(-/-), and TLR9(-/-) recipient mice showed that bacterial sensing via TLRs was essential for iGvHD development. Acute iGvHD was characterised by increasing numbers of apoptotic cells,
proliferating cells, T cells and neutrophils within the colon. These responses were significantly reduced in MyD88(-/-), TLR2/4(-/-), TRIF(-/-) and TLR9(-/-) mice, as compared with wild-type controls. However, TRIF(-/-) and TLR2/4(-/-) mice were not protected from mortality, whereas TLR9(-/-) mice displayed increased survival rates. The important role of TLR9-mediated immunopathology was independently confirmed by significantly reduced macroscopic disease symptoms and colonic apoptosis as well as by reduced T-cell and neutrophil numbers within the colon after treatment with a synthetic inhibitory oligonucleotide. CONCLUSIONS: These results emphasise the critical role of gut microbiota, innate immunity and TLR9 in iGvHD and highlight anti-TLR9 strategies as novel therapeutic options.