**P1312 FORODESINE AMPLIFIES HOST INNATE IMMUNE RESPONSE THROUGH TOLL-LIKE RECEPTOR 7 ACTIVATION WHILE PREVENTING EXPERIMENTAL GRAFT-VERSUS-HOST DISEASE**

**Topic:** 21. Stem cell transplantation - Experimental

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**Background:** Forodesine, a novel inhibitor of purine nucleoside phosphorylase (PNP), causes nucleotide pool imbalances by accumulating intracellular deoxyguanosine, thereby resulting in selective apoptosis of T-cells. Recent evidence indicates that specific types of nuclear acids including guanosine and its derivatives act as natural ligands for toll-like receptor 7 (TLR7). So far, it is unclear whether and how forodesine can modulate host innate immunity, including antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs), which express TLR7 abundantly.

**Aims:**

The aim of this study is to investigate the role of forodesine in host APC function as well as donor T-cell activation both in vivo graft-versus-host disease (GVHD) models and in vitro cell assays.

**Methods:** NOG mice were subjected to 250 cGy total body irradiation followed by intravenous injection of human isolated T-cells. Mice were treated with forodesine (20 mg/kg) or vehicle daily after transplantation.

**Results:** Despite the mice without treatment developing severe GVHD after transplantation, guanosine and deoxyguanosine were hardly detected in the plasma, suggesting that purine nucleosides released from damaged tissues were rapidly degraded by PNP. In fact, inhibition of PNP activity by forodesine markedly increased plasma guanosine levels, whereas deoxyguanosine was still undetectable. This was also the case in GVHD target organs, including liver and lungs. At the same level of guanosine in the liver, forodesine promoted TNF-a production from in vitro alveolar macrophage cultures. Preincubation with a TLR7 antagonist, ODN20958, attenuated the TNF-a production from macrophages stimulated by guanosine and forodesine. Nuclease treatment did not reduce macrophage TNF-a production, suggesting that single-strand RNA, a well-known TLR7 ligand, is not involved in this response. Similar results were observed in Flt3-ligand-induced plasmacytoid DCs. Not only TNF-a but also IL-12 production was induced by the combination of guanosine and forodesine, and was comparable to that in pDCs pulsed with R848, a potent agonist of TLR7/8. These observations suggest that a sustained high level of guanosine caused by forodesine treatment lead to continuous activation of TLR7 signaling, resulting in cytokine production from APCs. Meanwhile, as reported previously, forodesine inhibited the proliferation of human T-cells activated with CD3 and CD28 antibodies in the presence of deoxyguanosine. Of note, however, this combination did not impair but rather stimulated IFN-g production from activated T-cells cocultured with pDCs. This suggests that T-cell priming capacity mediated by the interaction with pDCs is conserved through TLR7 activation. Finally, we investigated the in vivo effects of forodesine on survival, body weight change, and disease severity of GVHD mice. Forodesine treatment significantly prolonged survival of the mice compared with control mice, with a median survival of 17.5 days vs 14 days, respectively (p = 0.029). The mean body weight loss percentage at day 9 was 11.5% vs 19.0% in the forodesine group and control, respectively (p = 0.003). Furthermore, the clinical GVHD scores were also lower in the forodesine group vs control (4.9 vs 0.6 points, p = 0.001).

**Image:**

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Summary/Conclusion: This study demonstrates that forodesine amplifies host innate immune response through TLR7 signaling, while ameliorating T-cell GVH reaction. Further studies are needed to investigate whether forodesine can provide host protection against infections in a transplant setting.