Impact of the NF-kappa B inducing kinase inhibitor SMI1 on graft-versus-host disease

Objectives: Allogeneic stem cell transplantation remains a cornerstone of the treatment of some hematological malignancies, although it is still associated with a significant morbidity and mortality which are mainly related to graft-versus-host disease (GVHD). NK-kB signaling pathways play a determining role in the pathogenesis of the disease. Two prior reports in mouse-to-mouse models of GVHD have demonstrated the implication of the NF-kappa inducing kinase (NIK) in the pathogenesis of GVHD (Sánchez-Valdepeñas *et al.* 2010, Murray *et al.* 2011). Indeed, mice transplanted with NIK-deficient donor T-cells did not develop GVHD. This was attributed to the fact that recently activated T-cells tended to enter in apoptosis in the absence of NIK. Moreover, the absence of NIK blocked the activation of donor T-cells via the OX40 receptor, the latter being required for acute GVHD development, whereas constitutive expression of NIK in regulatory T-cells (Treg) was shown to decrease their *in vitro* and *in vivo* function (Polesso *et al.* 2017).

In order to determine whether NIK inhibition could become a tool against GVHD in humans, we assessed the ability of the NIK inhibitor SMI1 at preventing GVHD in a humanized mouse model combining xenogeneic and allogeneic GVHD.

Methods: NSG-HLA-A2-HDD mice were transplanted with human HLA-A2 negative peripheral blood mononuclear cells (PBMC) on day 0. SMI1 was administered by oral gavage twice daily from day 1 to day 20. Blood samples were drawn on days 14, 20 and 28 and stained for spectral flow cytometry to assess immune reconstitution. Single cell RNA sequencing and TCR repertoire analyses were performed on human CD3+ T-cells isolated from day 20 blood samples. Mice follow-up included GVHD scoring and survival.

Results: Treated mice harbored lower human $CD45^+$ engraftment, lower $CD8^+$ T-cells counts and a longer persistence of Treg. Single cell RNA sequencing evidenced a lower proportion of $CD8^+$ T-cells but a higher proportion of Treg and naive $CD4^+$ T-cells in treated mice. Furthermore, genes involved in the cytotoxic function of T-cells, such as Granulysin, Granzyme B and Perforin 1, were downregulated in treated mice. In addition, treated mice had lower GVHD scores during treatment.

Conclusion: Our preliminary results suggest that the NIK inhibitor SMI1 could attenuate GVHD in a humanized mouse model.