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**Title of Presentation: Evaluation of Thymus as a Novel Source of T Regulatory Cells for Therapy after Transplantation**

**Abstract**

**Introduction:** Transplantation is often subject to the risk of graft rejection or graft-versus-host disease (GVHD). Cell-based therapy with FOXP3+ T regulatory cells (Tregs) to induce tolerance to alloantigens could eliminate these complications. However, expanding enough human Tregs from blood to use in patients is challenging due to limited growth and potential for contamination with effector T cells. Discarded pediatric thymuses from cardiac surgery could provide an alternative source of Tregs which are less likely to be contaminated with effector T cells. However, whether thymic Tregs are as effective as peripheral Tregs at suppressing responses to transplanted antigens is unknown.

**Methods / Results:** Human Tregs were isolated from peripheral blood or pediatric thymus and stimulated with high doses of human IL-2 and artificial antigen presenting cells (APCs) that express human CD58, CD86 and the human CD32 Fc receptor to immobilize soluble anti-CD3 mAbs. Culture for ~14 days resulted in over 500-fold expansion of peripheral and about 40-fold expansion of thymic Tregs. Due to considerably higher starting cell numbers of thymic Tregs similar final counts were achieved and thymic Tregs retained a significantly higher proportion of FOXP3+ cells compared to peripheral Tregs. Tregs from both sources were equally effective at suppressing proliferation of effector T cells in vitro. To investigate the suppressive function in a humanized-mouse model of GVHD, immunodeficient NSG mice were injected with 10x106 PBMCs with or without equal numbers of expanded thymic Tregs. After ~2 weeks, mice receiving no Tregs showed severe signs of GVHD whereas injection of Tregs significantly delayed onset and prevented rapid and severe progression of the disease. The proportions of the human CD45+, CD4+ and CD8+ cell populations in the blood were monitored by flow cytometry and supported the clinical GVHD scores.

**Conclusion:** We have optimized the expansion conditions for peripheral and thymic Tregs and demonstrated that thymic Tregs are able to significantly delay the onset of GVHD. Further direct comparison of Tregs from peripheral blood and thymus will reveal whether thymuses are a suitable source for continued development of Treg cell-based therapy.