could have therapeutic potential in the setting of cGvHD as a selective inhibitor of activated, alloreactive T cells.

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Ptcy Prevents Xenogeneic Gvhd without Abrogating Gvl Effects

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Background: Post-transplant administration of high-dose cyclophosphamide (PTCy) is one of the most efficient way to prevent graft-versus-host disease (GVHD) after allogeneic hematopoietic cell transplantation (allo-HCT). However, the impact of PTCy on immune-mediated graft-versus-leukemia (GvL) effects has remained debated. Here, we investigated the impact of PTCy administration on xenogeneic GVHD and allogeneic GvL effects in two humanized mouse models.

Impact of PTCy on xGVHD: A single i.p. injection of cyclophosphamide (100 mg/kg) 3 days after infusion of 2×107 human PBMC in NSG mice significantly delayed xGVHD (64 vs 43 days, $P < 0.0001$). However, xGVHD was not abrogated and the majority of treated mice eventually succumbed from xGVHD. On day 21 after PBMC infusion, (human) blood T-cell counts were significantly lower in PTCy than in control mice ($P = 0.0028$) while the proportions of $KI67^+$ $CD4^+$ and $CD4^+$ T cells were similar. Interestingly, at that time point the frequency of Treg in the blood was significantly higher in PTCy than in control mice (12.9 vs 4.3 %, $P = 0.03$). In further analyses, we compared human cells recovered from mouse blood and organs on day 6 after PBMC infusion (60 hours after PTCy in PTCy-treated mice). In comparison to untreated mice, PTCy mice had a dramatically lower proportion of KI67+ T cells as well as a much higher proportion of CD4+ apoptotic T cells. Treg numbers in the peripheral blood were also significantly lower in PTCy than in control mice. Looking at the bone marrow and other xGVHD target organs, PTCy-treated mice had a lower proportion of proliferative (i.e. KI67+) CD4+ T cells as well as of proliferative Tregs.

Impact of PTCy on GvL effects: The impact of PTCy on GvL effects was assessed in NSG-HLA-A2/HHD mice (a strain of NSG mice that express in addition to mouse MHC, the human HLA-A0201). All mice were i.v. injected with the human (HLA-A0201) AML cell line THP-1 transfected with the luciferase gene on day 0, while a second injection of THP-1 cells was administered on day 5. Four groups of mice were compared, a group of mice given only THP-1 cells, a second group given THP-1 cells and PTCy on day 3, a third group given THP-1 cells and human PBMC from a non HLA-A2 donor, and a last group of mice given THP-1 cells, PTCy and human PBMC from the same non HLA-A2 donor. On day 34, none of the THP-1+ PBMC mice had detectable tumors while THP-1 + PTCy + PBMC mice had detectable tumors but to a lesser extent that THP-1 only mice or THP-1+PTCy mice. The best survival was observed in THP-1+PTCy+PBMC mice while THP-1+PBMC mice died from xGVHD whereas THP-1+PTCy as well as THP-1 mice died from leukemia.

Conclusions: These results suggest that PTCy prevents xGVHD by depleting proliferative T cells and favoring the recovery of Treg. Further, GvL effects (although decreased) were not abrogated by PTCy.

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Preventing Post-Transplant Diabetes Mellitus Via Orchestration of the IL-33/ST2 Axis in Pancreatic Islets

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Post-transplant diabetes mellitus (PTDM) occurs after allogeneic hematopoietic cell transplantation (HCT) in over 50% of HCT recipients and will increase 3-fold their risk of death. Further, levels of soluble Stimulation-2 (ST2) are predictive of PTDM in adults and children. However, molecular pathology of PTDM remains poorly understood, and therapeutic strategies for PTDM are limited. PTDM is initiated by hyperinsulinemia and hyperglycemia, and the alarmin IL-33 has been found to contribute to the secretion of insulin in islets by activating innate lymphoid cells type 2 (ILC2s) via Stimulation2 (ST2), the IL-33 receptor (*Dalmas E. et al, Immunity 2017*). This ST2/IL-33 regulatory axis is also critical for regulating inflammation and the balance between cytopathic T effector cells (Teffs) and regulatory cells ($CD4^+FOXP3^+$ Tregs and ILC2s). Therefore, we explored the role of ST2/IL-33 in pancreatic islets post-HCT.

First, neither fasting blood glucose nor glucose tolerance (time and area under the curve) was significantly different between syngeneic and allogeneic wild-type (WT) or $ST2^{-/-}$ recipients of minor mismatch HCT (B6→C3H.SW) (Fig. 1A, 1B). Total infiltrating $CD90.2^+$ T cells in the pancreas were increased in the allogeneic groups but not different between WT or $ST2^{-/-}$ recipients (Fig. 1C). However, pancreatic $IFN\gamma^+$ T cells were significantly decreased while total Tregs in $ST2^{-/-}$ recipient were increased (Fig. 1D, 1E). Second, using ST2 blockade with a neutralizing antibody from day -1 to day 9, 100 μg every over day for 6 doses), we showed significant improvement of survival in the treated group as compared to the isotype group (Fig. 2A), consistent with an increase in plasma IL-33 (Fig. 2B) as well as decreased plasma TNFα and IFNγ (Fig. 2C, 2D). Lastly, increased β-cell proliferation before immune cell invasion prevents progression of type 1 diabetes (*Ercument. et al, Nature Metabolism 2019*), and we found increase T cell infiltration. Thus, we specifically sought to analyze the ratio of β-cells/α-cells and both Teffs and regulatory cells in the pancreatic islets in anti-ST2 antibody treated vs. isotype groups. We found that the average ratio of β-cells/α-cells was decreased in the treated group in comparison to the isotype group (Fig. 3A). In addition, both frequencies of Tregs and ILC2s significantly increased while frequency of $CD8^+$ Teffs decreased in the treated group as compared to the isotype group (Fig. 3B, 3C, 3D).

We conclude that the effects of PTDM on glucose tolerance in the acute preclinical HCT model are not immediate while the

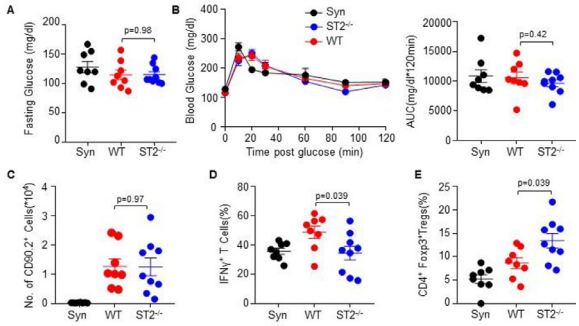

Figure 1. No difference in blood glucose between syngeneic and minor mismatch allogeneic WT or $ST24^{-/-}$ recipients.