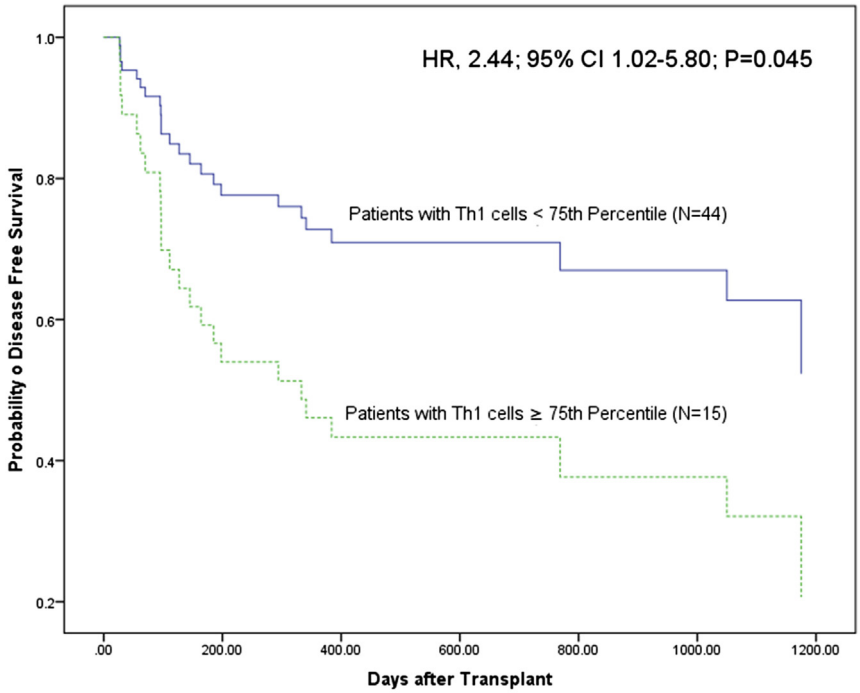

engraftment can identify patients who may benefit from pre-emptive therapies to promote GVL and prevent malignancy relapse.

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Higher Mycophenolic Acid (MPA) Trough Levels Result in Lower Day 100 Severe Acute Graft-Versus-Host Disease (aGVHD) without Increased Toxicity in Double-Unit Cord Blood Transplantation (CBT) Recipients

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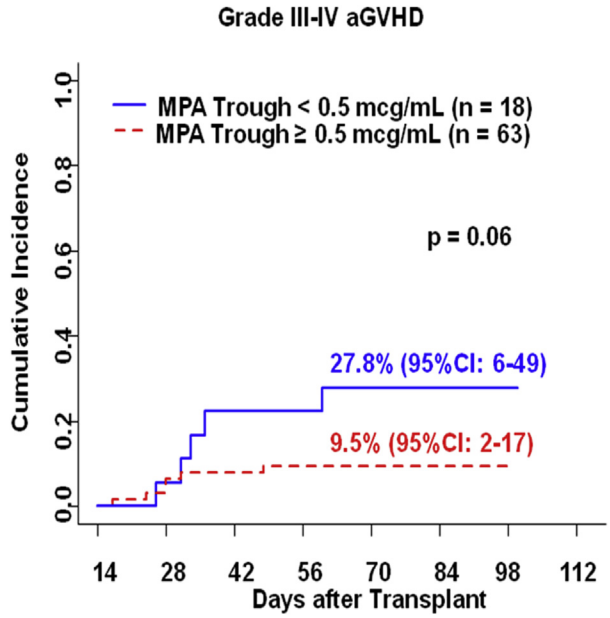
Background: Mycophenolate mofetil (MMF) is frequently combined with cyclosporine-A (CSA) as immunosuppression in CBT. Drug monitoring of MPA, the active MMF metabolite, is advisable based on variable MMF pharmacokinetics and the association of low unbound MPA AUCs with increased aGVHD. This is highly relevant in CBT as aGVHD is a leading cause of mortality. However, a limited pharmacokinetic parameter such as MPA troughs is ideal. Additionally, the toxicity associated with MPA troughs is not established and is of concern in CBT due to the theoretical risk of myelosuppression from high levels.
Methods: We evaluated the association between serial (weeks 1-6) total MPA trough levels and outcomes in double-unit CBT recipients transplanted 8/2009-11/2012. Intravenous CSA/MMF commenced on day -3. Trough levels were dichotomized into <2 mcg/mL and ≥ 2 mcg/mL for the toxicity analysis (engraftment, gastro-intestinal toxicity as measured by TPN duration and CMV infection) at each time point. For the efficacy (aGVHD prevention) analysis, mean week 1 and 2 trough levels were split at <0.5 vs ≥ 0.5 mcg/mL.
Results: Eighty-three patients (median age 44 years, range 1-71) had weekly MPA levels for 6 weeks after CBT. Sixty-nine (83%) received myeloablative (MA) conditioning and 45 (54%) were CMV seropositive. Median trough levels ranged 0.6-1.3 mcg/mL. Trough levels increased over time (p=0.03). Myeloablative (MA) had lower MPA troughs than non-myeloablative recipients (p=0.02). Younger age (0-15 years old) was associated with lower trough levels (p=0.002). By time-dependent Cox regression analysis, there was no association between troughs and toxicity, and MA recipients



with high troughs ≥2 mcg/mL had enhanced platelet recovery (p=0.005). In a competing risk 2-week landmark analysis, there were no differences grade II-IV aGVHD incidences (61% vs 57%, p=0.52) according to troughs. However, patients with a low MPA trough early post-CBT had nearly triple the incidence of grade III-IV aGVHD (27.8% vs 9.5%, p=0.06, Figure).

Conclusions: Higher total MPA troughs are safe and may protect against severe aGVHD. The platelet benefit could be explained by the lower severe aGVHD incidence. Prospective investigation of MPA troughs, and ultimately intervention based on drug monitoring in CBT recipients is warranted.

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Inhibition of Cdk2 Inactivates EZH2 and Induces Epigenetic Regulation of Fopx3 Leading to the Generation of CD8+ Treg and Protection from GvHD

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In spite of intense efforts, control of graft versus host disease (GvHD) remains incomplete and novel therapeutic approaches are required. Cdk2 has a central role in cell cycle re-entry of mature T lymphocytes and inhibition of Cdk2 is mandatory for induction of T cell anergy in vitro and tolerance in vivo. To determine the effects of Cdk2 inhibition on GvHD, we used the B6D2F1 mouse model of allogeneic BMT and two different Cdk2 inhibitors (Cdk2i), CYC202 and CYC205. Lethally irradiated B6D2F1(K^d) recipients were infused with bone marrow from C57BL/6(K^b) donors with (BMT) or without splenocytes and were subsequently treated with each Cdk2i for three weeks. Treatment was administered daily during week 1, every other day on week 2, and twice a week on week 3 followed by assessment of GvHD during a 70-day period. BMT recipients treated with Cdk2i displayed a transient weight loss and subsequently regained weight to levels comparable to controls. Treated BMT recipients also displayed delayed GvHD mortality (p=0.0054). Treg have a central role in mediating protection from GvHD. To examine whether Cdk2i induced Treg, we used GFP^- T cells from Foxp3.GFP-KI mice as a source of T cells. Assessment of peripheral blood lymphocytes, splenocytes, lymph nodes and intestinal lymphoid cells (ILC) in treated and control BMT recipients revealed no differences in $CD4^+GFP^+$ Treg. In contrast, $CD8^+GFP^+$ Treg were increased in the treated group, predominantly in ILC, which displayed a 5-fold increase of $CD8^+$ Treg (p=0.05). To investigate the mechanisms via which Cdk2i had a selective effect on $CD8^+$ Treg, we isolated $CD4^+GFP^-$ and $CD8^+GFP^-$ T cells from Foxp3.GFP-KI mice and subjected them to in vitro Treg polarization. Cdk2i had almost no effect on $CD4^+GFP^+$ cells but induced a 2-4 fold increase of $CD8^+GFP^+$ cells. Culture of $CD8^+GFP^-$ cells with stable concentrations of Cdk2i and decreasing concentrations of TGF-β revealed that Cdk2i induced $CD8^+$ Treg differentiation in the presence of TGF-β concentrations that failed to induce $CD8^+$ Treg cells when used alone. Expression of FOX family genes is regulated by transcriptional and epigenetic mechanisms. A critical epigenetic regulator of FOX transcription factors in cancer cells is the Polycomb group (PcG) protein, enhancer of zeste homologue 2 (EZH2), which promotes histone H3 lysine 27 trimethylation (H3K27me3) and induces epigenetic gene silencing. Cdk1 and Cdk2 phosphorylate EZH2 at Thr350 in an evolutionarily conserved motif. Phosphorylation of Thr350 is important for EZH2 recruitment and maintenance of H3K27me3 levels at EZH2-target loci. Upon polarizing $CD8^+$ T cell culture, EZH2 displayed robust phosphorylation on Thr350, which was blocked by Cdk2i. This event temporally coincided with a 44-fold increase in Foxp3 mRNA expression compared to control T cells. These results reveal an unexpected mechanism via which Cdk2 inhibitors induce $CD8^+$ Treg and protection from GvHD.

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IL-22 Administration Protects Intestinal Stem Cells from Gvhd

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Factors regulating damage and regeneration of the intestinal epithelium after allogeneic BMT are poorly understood. We have previously shown that IL-22 produced by recipient-derived innate lymphoid cells (ILCs) provides a critical signal for epithelial recovery following experimental BMT. However, intestinal IL-22 levels are reduced in GVHD due to the elimination of radioresistant host ILCs. We therefore sought to determine if IL-22 administration post-BMT could negate the effect of ILC elimination and reduce GVHD pathology. We utilized a clinically modeled LP into C57BL/6 (B6) minor antigen mismatched model with T cell-depleted marrow and purified T cells transplanted into lethally irradiated mice.

We found that daily administration of rIL-22 (4ug IP starting day +7) led to decreased GVHD pathology in recipient small and large intestine three weeks post-BMT (p<0.001). Further assessment of the intestinal pathology indicated that recipients of rIL-22 had decreased intestinal crypt apoptosis in both small and large intestine (p<0.01) with no difference in intestinal and splenic lymphocytes or inflammatory cytokine levels.

To assess the effects of IL-22 administration on the intestinal stem cell (ISC) compartment, we performed LP into B6 allo-HCT using Lgr5-LacZ ISC reporter mice. Recipients treated with rIL-22 demonstrated increased numbers of Lgr5+ ISC three weeks post-HCT during active GVHD with no immunosuppression (p<0.05). Furthermore, we found increased ISC Ki-67 expression in Lgr5-GFP reporter mice with GVHD after IL-22 treatment, indicating increased ISC proliferation in response to IL-22.

In addition to Lgr5+ cells, it has been reported that BMI-1+ crypt cells may possess ISC activity after crypt damage. Crypt cells from BMI-1-GFP reporter mice were indeed found to be IL-22R+ at baseline (7-10% IL-22R surface expression). However, BMI-1 mRNA expression in small intestine of mice with GVHD was not affected by IL-22 administration, suggesting that the effect of IL-22 administration in vivo was not due to stimulation of BMI-1+ cells. Additionally, there was no difference in Wnt3 or EGF expression, arguing that improved ISC survival after IL-22 administration was not due to improvement in ISC niche function. In contrast, IL-22 treatment demonstrated increased Reg3γ (p<0.001) and Reg3β (p<0.01) expression, suggesting a potential antimicrobial benefit of IL-22 administration.

In summary, we found that IL-22 administration could reduce intestinal pathology and improve ISC recovery in GVHD. This appeared to be due to direct stimulation of Lgr5+ ISCs, and not due to improved support of the ISC niche. These