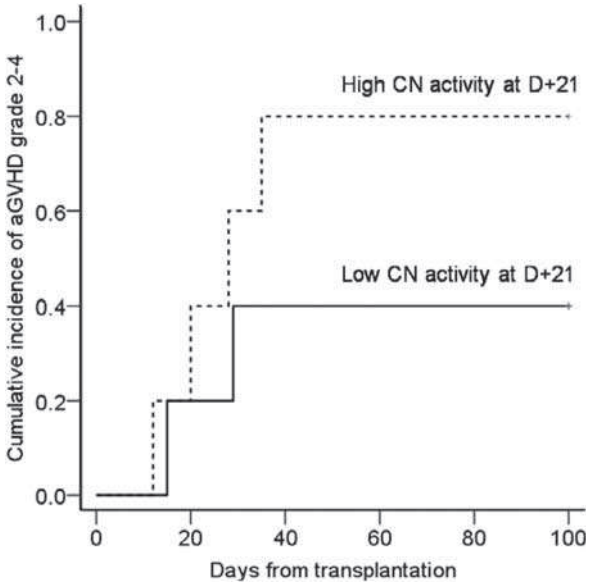
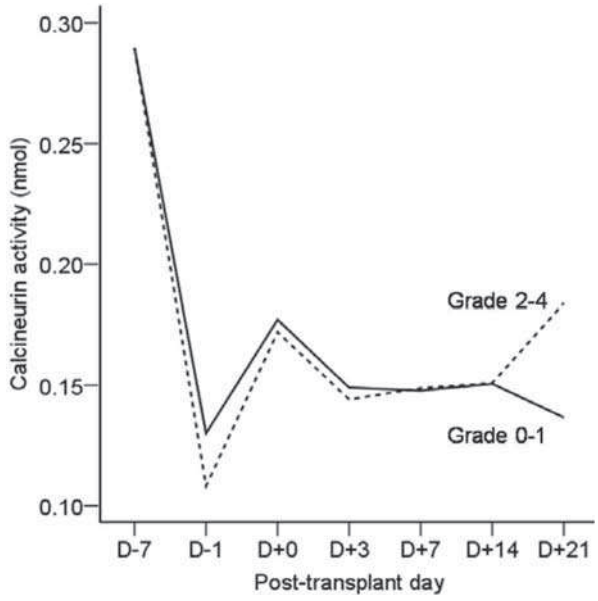
Wilcoxon). Combining mAb together did not increase cell binding inhibition. The contribution of CD44 could not be assessed because the addition of HA to the assay did not generate reproducible results. None of the mAbs, nor HA (from 1 ug to 1 mg/ml) affected T cell proliferation in the absence of MSC, or impaired MSC-induced T cell inhibition, thus indicating that integrin-induced cell-cell interaction was not the effector of the inhibition. Moreover experiments performed with transwell systems showed that T cell transmigration under MSC was not associated with inhibition as long as the compartment where the cells landed after transmigration was voluminous.
In conclusion, the binding of activated T cells to MSC is only partially mediated by beta-2 intergrins and seems not associated with MSC regulatory function. However cell-cell interactions may favor the action of soluble inhibitory effectors synthesized by MSC.

P602
Pilot study on the measurement of calcineurin phosphatase activity on day 21 in allogeneic stem cell recipients
S.K. Sohn (1), J.H. Moon (1), Y.J. Lee (1), S.J. Lee (1), Y.R. Do (2), Y.K. Kim (1), J.S. Suh (1), M.S. Lim (1), Y.R. Yoon (1)
(1)Kyungpook National University Hospital (Daegu, KR); (2)Keimyung University Dongsan Hospital (Daegu, KR)

Background: Tacrolimus (TAC) suppress T-cell activation by inhibiting calcineurin (CN). CN activity was assessed in the allogeneic recipients who were treated with TAC for graft-versus-host disease (GVHD) prophylaxis to investigate whether CN activity was increased in patients with severe acute GVHD.
Methods: CN activity was analyzed in 10 consecutive patients who underwent allogeneic stem cell transplantation (SCT). TAC was administered at a dose of 0.03 mg/kg intravenously from day-1 to +21. TAC levels and CN activity was assessed on day-1 before TAC administration and days 0, +3, +7, +14, and +21. Target TAC concentration (15-20 ng/ml) was maintained during the current study.
Results: The cumulative incidence of acute GVHD (74.1% *vs.* 60.3%, p=0.888) and severe chronic GVHD (22.5% *vs.* 33.3%, p=0.539) were not different between high and low TAC trough levels. CN activity on day-1 was 0.12±0.09 nmol and had decreased from baseline level (0.29±0.15 nmol, p<0.001). There was no correlation between CN activity and TAC concentrations (r2=0.024). CN activity was steady-state during post-transplant day+0 to +14 regardless of acute GVHD, CN activity on day+21 for those with grade 2-4 acute GVHD showed higher CN activity (0.18±0.04 nmol) compared to those without grade 2-4 acute GVHD (0.14±0.05 nmol, p=0.462). Cumulative incidence of acute GVHD (40% *vs.* 80%, p=0.248) and chronic GVHD (20% *vs.* 70%, p=0.464) between low and high CN activity group were not significantly different.
Conclusion: Maintaining target TAC trough level did not correlate with the development of GVHD in allogeneic recipients. Although GVHD was higher for the high CN activity on day+21, this pilot study failed to demonstrate significant difference due to small sample size. However, the patients manifesting GVHD with high CN activity on post-transplant D+21 may need to be treated with other kinds of immunosuppressive agent regardless of drug level.

[P602]





P603
Adaptation of a murine chronic GvH model to study graft-versus-myeloma effect after allogeneic transplantation
M. Binsfeld (1), L. Belle (1), M. Hannon (1), P. Hofgaard (2), S. Dubois (1), E. Otjacques (1), Y. Beguin (1), B. Bogen (2), F. Baron (1), J. Caers (1)
(1)University of Liège (Liège, BE); (2)University of Oslo (Oslo, NO)

To elucidate the mechanisms behind graft versus tumor effect (GVT) and graft versus host disease (GVH), our laboratory adapted a murine model of allogeneic bone marrow (BM) transplantation using B10.D2 donor mice and Balb/cJ recipient mice that were inoculated with MOPC-315.BM myeloma cells. Balb/cJ recipient mice were intravenously (IV) injected with $2{,}5x10^5$ luciferase transfected MOPC-315.BM cells. At day 30 after inoculation, 6 mice received an autologous transplantation

(Balb/cJ cells) and 8 mice received an allogeneic transplantation (B10.D2 cells) by IV injection of $10x10^6$ BM cells and $70x10^6$ spleen cells. Prior to transplantation, both groups of mice were irradiated with 6 Gy. Tumor development was followed by measuring their bio-luminescence using VIVOVISION IVIS 200 (Xenogen). Immune responses were followed by taking blood samples before transplantation (day -2), and at days 7 and 19 after transplantation, analysing lymphocyte counts and NK, NKT and T-cell subpopulations. When mice showed signs of paraplegia or signs of GVH disease, they were sacrified and analysed for immune activation and regulation in different organs (blood, spleen, lymph nodes, thymus and bone marrow). *In vivo* imaging showed disappearance of the luciferase signal in 7 of the 8 allografted mice. All the mice that received an autologous transplantation developed myeloma disease. The recovery of myeloma diseased mice by this allogeneic transplantation could be attributed to an immune graft versus myeloma effect. Further analysis of the cellular subpopulations at sacrifice (Allografted *vs* Autografted mice) showed a decrease in regulatory T cells [% of CD4: Blood 5,5±2,6 *vs* 18,4±4,4 ($p=1,6x10^{-5}$); Spleen 20,6±8 *vs* 24,7±4,7 (p=0,29); BM 10,9±6.5 *vs* 48,1±6,4 ($p=1,79x10^{-7}$)] and activation (CD69) of both CD4 T lymphocytes [% of CD4: Blood 45,4±14,5 *vs* 14,6±8,1 (p=0,0006); Spleen 44,9±8,9 *vs* 23,1±5,5 (p=0,0002); BM 60,4±14,4 *vs* 46,2±5,6 (p=0,04)] and CD8 T lymphocytes [% of CD8: Blood 51,6±14,8 *vs* 12,3±6,7 ($p=6,2x10^{-5}$); Spleen 46±7,9 *vs* 11,2±2,7 ($p=2,5x10^{-7}$); BM 69,6±9,6 *vs* 37,6±10,9 ($p=8,1x10^{-5}$)]. The same trends were observed in simply allografted control mice.
This model will be used for studying the mechanisms behind graft versus tumour effect (antigen mismatches, activation of T cell subpopulations) and the effects of immune suppressors (e.g. rapamycin) on the graft versus tumour effect.

P604
Intrinsic survival capacity of cord blood T-cells is heterogeneous and can be promoted by repeated low doses of recombinant interleukin-7
E. de Berranger, L. Pascal, J. Trauet, B. Choufi, S. Thiant, J.-P. Dessaint, I. Yakoub-Agha, M. Labalette
CHRU Lille (Lille, FR)

Background: Umbilical cord blood (CB) transplantation is known to be associated with delayed and defective immune reconstitution, in part because recent thymic emigrants (RTEs), have limited intrinsic survival. Interleukin-7 (IL-7) has been reported to increase the initial recovery of the graft-derived T-cells compartment. The aim of this study was to investigate CB-derived T-cell survival and to define a minimal effective dose of recombinant human IL-7 (rIL-7), considering that in addition to its anti-apoptotic effect, IL-7 may enhance immune reactions that occur in the host, including acute GVHD.
Methods: Fifteen CB were obtained after normal-term delivery, using the same procedure as for CB banking. T cells were isolated within 12 hours by negative magnetic bead sorting and cultured for 2 weeks either directly or after being frozen. Unstimulated T cell cultures were conducted in parallel in medium (RPMI supplemented with 10% normal AB serum) alone or supplemented with a range of rIL-7concentrations added either only once at day 0 (100 to 1000 pg/mL) or every day (10 to 100 pg/mL). Cell viability was assessed by flow cytometric scatter analysis and staining with 3,3'-dihexyloxacarbocyanine iodide [DiOC6(3)] and propidium iodide.
Results: In basic culture conditions, the majority of T cells had died over 2 weeks. There was heterogeneity in cell survival, with 2% to 50% viables T cells (median 15%) at day 6 of culture. Interestingly, the same intrinsic characteristics of survival were observed in T cells that underwent beforehand a freezing-thawing procedure. In cultures supplemented with rIL-7, we confirmed the efficacy of rIL-7 in maintaining CB T cell survival. A minimal dose of 25 pg/mL added daily was sufficient to induce the prolonged survival of CB T cells, with more than 95% of viable cells at day 6. Noteworthy, these concentrations allowed prolonged survival of CD T cells without inducing any cell proliferation, as shown by the absence of CFSE dilution. Similar results were observed comparing CB cells that had been frozen or not.
Conclusion: Repeated low-doses of rIL-7 are required to preserve the survival capacity of CB T cells without inducing their proliferation. These results could represent a framework for clinical studies aiming at improving T cell reconstitution following allogeneic CB transplantation. Repeated administration of low-doses of rIL-7 might be especially useful in patients with low serum levels of IL-7 after conditioning.

P605
Intestinal toxicity after busulfan and cyclophosphamide treatment in piglets
P.E.L. Pontoppidan (1), R. Shen (1), C. Heilmann (2), K. Müller (2), P. Saingild (1)
(1)University of Copenhagen (Frederiksberg, DK); (2)Rigshospitalet (Copenhagen, DK)

Objectives: Chemotherapy treatment related toxicities are common among children undergoing Haemopoietic Stem Cell Transplantation (HSCT). Gastrointestinal complications, such as oral and intestinal mucositis, are related to increased risk of severe infections and increased mortality. The objective of this study was to investigate intestinal structural, functional and inflammatory markers after treatment with busulfan (Bu) and cyclophosphamide (Cy) in a piglet model of chemotherapy-induced mucositis.
Methods: Piglets were allocated to receive either 4 d of Bu plus 2 days of Cy (1.6 and 60 mg/kg/d, respectively, Bu-Cy group, n=9) or saline (controls, n=8) intravenously. After 6 days chemotherapy or saline, pigs were kept for another 5 days until when intestinal samples were collected for histomorphometry (villus height and crypt depth), activity of six brush border enzymes (disaccharidases, peptidases) and tissue levels of cytokines (IL-8, IL-1beta and TNF-alfa) in the proximal and distal intestine.
Results: Bu-Cy pigs had 40% shorter villi in the proximal intestine, relative to controls ($p<0.01$) while no difference was seen in the distal intestine. Crypt depths were smaller in both proximal and distal intestine (18-24%, $p<0.05$). Likewise, the activity of the brush border enzymes in the proximal intestine (sucrase, ApA, ApN and DPPIV) were 30-50% lower in Bu-Cy pigs compared with controls pigs ($p<0.01$ for all) while in the distal intestine only sucrase was lower in Bu-Cy pigs ($p<0.05$). No differences were found for maltase and lactase. IL-8 were 40% higher ($p<0.05$) in the proximal intestine while TNF-alfa levels were 50% higher in the distal intestine of Bu-Cy pigs ($p<0.05$). No difference was found for IL-1-beta.
Conclusion: The Bu-Cy treatment resulted in structural damage and reduced functions, especially in the proximal part of the small intestine within 5 days after the 6 days treatment protocol. A faster enterocyte turnover in the proximal versus the distal intestine may explain that chemotherapy induces more marked effects proximally. The associated increases in tissue IL-8 and TNF-alfa levels reflect local bacteria-stimulated inflammatory reactions. These may accelerate damage and prime the tissue to later development of graft versus host diseas following infusion of a transplantat. Pharmacological and dietary factors should be investigated as means to reduce the immediate intestinal effects of Bu-Cy treatment.

P606
Fludarabine-cytabarine-melphalan combination – a novel high-dose chemotherapy conditioning regime for autologous peripheral blood stem cell transplant in lymphoma
Y.K. Guan, T.C. Ong, K.M. Chang, M.Z. Zakaria, J.S. Rajasuriar, T.C. Tan
Hospital Ampang (Ampang, MY)

Background: Fludarabine-Cytabarine-Melphalan combination (FLAM) is a novel combination chemotherapy which theoretically has the advantage of being able to provide Central