trunk, arms, hands, face and oropharynx; there was no evidence of liver dysfunction. No mutations suggestive of hereditary hemorrhagic telangiectasias were identified. An IgG-kappa monoclonal gammopathy was detected at a concentration of approximately 0.7 g/dl without lytic bone lesions and the bone marrow biopsy revealed 7% plasma cells, consistent with monoclonal gammopathy of undetermined significance (MGUS). The bilateral perinephric fluid collections developed slow and progressive over 5 years from completely normal appearing kidneys. A liver biopsy was normal without any evidence of elevated pressures through the hepatic or portal systems. Therapeutic phlebotomy was initiated but ultimately discontinued due to development of shortness of breath following phlebotomy. Microscopic intrapulmonary shunting was identified and slowly progressed with worsening hypoxia and shortness of breath, finally requiring continual supplemental oxygen. There was no evidence of pulmonary hypertension on echocardiogram or right heart catheterization. The pathofysiology of the TEMPI syndrome is unclear.

## Abstracts posters stem cell biology and transplantation P.05 – P.17

### P.05. Imatinib and nilotinib do not prevent adhesion and migration of human CD34+ cells in vitro and in immunodeficient NSG mice

*L. Belle[1], F. Bruck[2], J. Foguenne[2], A. Gothot[2], Y. Beguin[2], F. Baron[2], A. Briquet[2]*

*[1]CHU Liège, Belgium, [2]University of Liège, Belgium*

**Background**
The BCR-ABL tyrosine kinase inhibitor (TKI) imatinib has previously been shown to also inhibit the tyrosine kinase c-kit, the stem cell factor receptor. Nilotinib is 30 times more potent than imatinib to inhibit BCR-ABL in vitro. But very few information is available on its inhibitory effects on c-kit and thus on $CD34^+$ cell adhesion and migration since this receptor is implicated in these biological processes.

**Aims**
To compare, in vitro and in vivo, the inhibitory effects of imatinib and nilotinib on adhesion, migration and engraftment capacity of human cord blood $CD34^+$ cells.

**Results**
Analysis of VLA-4, VLA-5 and CXCR-4 cell surface expression by flow cytometry after 48 hours of culture have shown that both VLA-4 and VLA-5 expression (n=3) were significantly decreased in presence of imatinib or nilotinib at physiological concentrations (1 and 5 μM) while CXCR-4 expression was not affected (n=3). However, nor imatinib nor nilotinib decreased the adhesion of $CD34^+$ cells to retronectin (n=4). Further, migration through a SDF-1 gradient was not affected by a 48-hour cell culture in presence of TKIs (n=3). Finally, we compared the impact of imatinib and nilotinib on engraftment in a xenotransplantation model. Twenty-five NSG mice sublethally irradiated and inoculated intravenously with $6.10^5$ human $CD34^+$ cells, were treated orally with a placebo, imatinib 150 mg/kg/day or nilotinib 75 mg/kg/day for 42 days. Bone marrow chimerism was analyzed by flow cytometry. No significant differences were seen between mice treated with imatinib (47.7 % ± 5.3; n=8; p=0.4130) or placebo (52.5 % ± 2.7; n=9), while engraftment of human CD34+ cells was slightly decreased (40.6 % ± 4.4; n=8; p=0.0314) in mice treated with nilotinib.

**Conclusion**
TKIs do not prevent adhesion and migration of human cord blood $CD34^+$ cells both in vitro and in NSG mice even if chimerism was slightly lower in mice given nilotinib.

### P.06. Evidence for expansion of host-derived CMV-specific CD8+ T cells after allogeneic transplantation with nonmyeloablative conditioning

*C. Menten-Dedoyart, E. Castermans, M. Hannon, S. Ormenese, Y. Beguin, F. Baron*

*ULg, Liège, Belgium*

It has been suggested that significant numbers of host-derived CMV-specific T cells could persist in patients given grafts following nonmyeloablative conditioning. In the current study, we challenged this hypothesis by assessing chimerism levels among CMV-specific CD8+ T cells (labelled by specific pMHC multimers) around day 40, 100 and 180 after allo-HCT in a cohort of 24 patients given allogeneic grafts after nonmyeloablative conditioning. Four of 17 CMV-seropositive recipients given grafts from CMV-seronegative donors had higher (>25%) proportion of cells of recipient origin among CMV-specific CD8+ T cells (ranging from 32.4 to 100%) than among other CD8+ T cells. Interestingly, the 2 patients with CMV-specific CD8+ T cells of 100% recipient origin on day 100 had relatively high counts of CMV-specific CD8+ T cells on that day (13.1 and 14.7 cells/μL), demonstrating that high number of CMV-specific CD8+ T cells of recipient origin could persist after allo-HCT in a proportion of nonmyeloablative recipients.

### P.07. Adaptation of a murine chronic GVH model to study graft versus myeloma effect after allogeneic transplantation

*M. Binsfeld, L. Belle, M. Hannon, S. Dubois, E. Otjacques, Y. Beguin, F. Baron, J. Caers*

*University of Liège, Belgium*

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, before and after transplantation 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, whereas all 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 kinetics showed a decrease in regulatory T cells and activation of both CD4 and CD8 T lymphocytes in the allografted 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.

### P.08. Bone marrow-derived mesenchymal stromal cells failed to prevent experimental xenogeneic graft-versus-host disease

*F.B. Bruck[1], L. De Leval[1], L.B. Belle[1], C. Lechanteur[2], M. Hannon[1], A. Briquet[1], S. Dubois[1], E. Castermans[1], S. Humblet-Baron[1], S. Rahmouni[1], Y. Beguin[1], F. Baron[1]*

*[1]University of Liege, Belgium, [2]Laboratory of Cell and Genetic Therapy, Liege, Belgium*

**Background**
Graft-versus-host disease (GVHD) is a life-threatening complication

of allogeneic hematopoietic cell transplantation caused by donor T cells reacting against host tissues. Previous studies have suggested that mesenchymal stromal cells (MSC) could exert potent immunosuppressive effects.

**Aim**
The aim of the study was to assess the ability of MSC to prevent xenogeneic GVHD in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) and in NOD/SCID/IL-2Rγ (null) (NSG) mice transplanted with human peripheral blood mononuclear cells (PBMC).

**Methods**
MSC were expanded from human bone marrows and injected intraperitonealy (i.p.) into immunodeficient mice.

**Results**
Injection of 200x10$^6$ human PBMC i.p. into sub-lethally (3.0 Gy) irradiated NOD/SCID mice also given anti-asialo GM1 antibodies i.p. 1 day prior and 8 days after transplantation induced a lethal xenogeneic GVHD in all tested mice. Co-injection of 2x10$^6$ MSC i.p. on day 0 failed to prevent lethal xenogeneic GVHD induced by injection of human PBMC. Similarly, i.p. injection of 30x10$^6$ human PBMC into sub-lethally (2.5 Gy) irradiated NSG mice induced a lethal xenogeneic GVHD in all tested mice. Injection of 3x10$^6$ MSC i.p. on days 0, 7, 14 and 21 failed to prevent lethal xenogeneic GVHD induced by injection of human PBMC.

**Conclusion**
Injection of MSC failed to prevent xenogeneic GVHD in these two humanised mice models.

## P.09. Bone marrow seems to be the best source of mesenchymal stem cells to repair injured liver

*A. Briquet, F. Comblain, A. Halleux, S. Dubois, A. Gothot, C. Lechanteur, Y. Beguin*

*Ulg, Liège, Belgium*

Several genetic hepatic metabolic diseases alter physical and neurological development as well as life expectancy of affected children. The only potential curative option to date for these patients is a liver transplant. Given the shortage of organs, development of cellular sources other than human liver is urgent. The main objective of this project is to demonstrate the feasibility of treating liver metabolic diseases by MSC transplantation.
Human MSC from umbilical cord (UC-MSC), bone marrow (BM-MSC) or liver (L-MSC) were transplanted into NSG mice after CCl4-induced liver injury. In order to support MSC homing towards injured liver, we induced expression of the CXCR4 receptor on their surface.
NSG mice received 3 CCl4 3% IP injections per week during 4 weeks. 48h after the last injection, mice received 500,000 MSC by intravenous tail injection. We injected UC-MSC, BM-MSC or L-MSC (CXCR4- or CXCR4+). We examined MSC homing by real-time PCR and MSC function by quantitative image analysis of sirius red staining and blood enzyme analysis.
Data confirm that CCl4 treatment induced hepatic fibrosis. PCR showed that human MSC, after injection in mice, were found partly in their liver. In addition, BM-MSC seemed to be the most promising cells. Indeed, they stabilized the rates of plasma albumin and alanine amino-transferase of mice. Moreover, these cells decreased hepatic fibrosis. CXCR4 expression did not improve the homing nor function of MSC. BM-MSC CXCR4+ or UC-MSC or L-MSC (expressing CXCR4 or not) seemed less effective. These results need to be confirmed in a larger number of animals.

## P.10. Optimisation of cryopreservation conditions for mesenchymal stem cells used for bone repair

*A. Briquet, A. Halleux, C. Lechanteur, Y. Beguin*

*Ulg, Liège, Belgium*

The utilization of tissue engineering in the repair of bone defects has shown great promise recently. In combination with appropriate biomaterials and growth factors, bone marrow-derived mesenchymal stem cells (MSC) have been proven to significantly enhance bone repair in large animal fracture models. MSC banking is feasible but the optimal technique of cryopreservation must be developed. First, we tested different cryoprotectants (CPA) (DMSO and/or trehalose and/or sucrose) and different concentrations. Then, we studied speed of freezing process as well as technique of elimination of CPA after thawing process.2x106 cells were transferred to a cryovial containing each CPA solution. Cryovials were immediately frozen at -80°C during 24h then transferred into a liquid nitrogen cylinder at -196°C. After 1 week, the vials were removed from liquid nitrogen, placed in a 37°C water bath. Then, cellular suspension was washed twice with cold culture medium. Viability was analysed with a Trypan blue dye exclusion assay. Cell proliferation of cryopreserved MSC was determined after 7 days of culture.No significant differences in viability percentage were detected among cryopreservation solutions with 5% and 10% DMSO independent of addition of trehalose or sucrose. When cells were cryopreserved with 2,5% DMSO, fewer than 30% of MSC were viable. Proliferation didn't change significantly after thawing process in 15 media tested. However, proliferation tends to be more important when MSC are frozen in 5% DMSO + trehalose. When MSC were freezed with a freezing container (-1°C/min) and when DMSO were washed drop by drop, viability percentage reached more than 90%. In conclusion, it would be possible to replace standard CPA (10% DMSO) by a solution with 5% DMSO + 60mM trehalose. It is preferable to freeze the cells in a progressive way and to slowly wash the cells.

## P.11. Day 100 PET scan positivity predicts for worse survival in lymphoma patients given allogeneic peripheral blood stem cells after non-myeloablative conditioning

*A. Jaspers[1], P. Fosse[2], N. Withofs[2], M. Lejeune[2], E. Willems[2], K. Hafraoui[2], R. Hustinx[2], F. Baron[2], Y. Beguin[2]*

*Ulg, Liège, Belgium, [2]CHU de Liège, Belgium*

**Background**
PET scan is increasingly used in the follow-up of lymphoma patients given allogeneic hematopoietic cell transplantation (Allo-HCT). However, whereas several studies addressed the question of the impact of PET positivity after autologous transplantation on transplantation outcomes, very few have been performed after allo-HCT. This is the aim of the current retrospective study.

**Methods**
We analyzed data from 50 lymphoma patients who underwent an allo-HCT after non-myeloablative conditioning. The diagnoses were Hodgkin's lymphoma (n=8) and non-Hodgkin's lymphoma (n=42). PET scans were scheduled on days 100, 180 and 365 and then yearly for a total of five years.

**Results**
Day 100 PET scans were not performed in 5 patients. Among the remaining 45 patients, 20 (44.4%) presented hypermetabolic lesions, including 9 patients (20%) who had hypermetabolic lesions evocative of lymphoma. One-year OS (Figure 1) was higher in patients whose PET scan was negative or positive for infectious/inflammatory reasons than for those with typical lymphoma lesions (85% vs 44%, p=0.0013).
During further follow-up, twenty patients (44.4%) never presented hypermetabolic lesions after transplantation and 25 (55.6%) had at least one abnormal PET scan. Among the 25 patients, 11 (24.5%) had probable/proven neoplasia : 3 residual disease, 5 relapses, 1 progression, 1 lung cancer and 1 lung PTLD.
The other 14 patients (31.1%) presented suspicious lesions, but none of these proved to be a relapse. Six biopsies were performed, including 2 lymph node (1 normal and 1 lymphoid hyperplasia), 2 lung (1 normal and 1 aspergillosis) and 2 GI (1 normal and 1 GVHD) biopsies. For 6 patients, imaging studies were normal or demonstrated infectious or inflammatory disorders. The last 2 patients were thought to relapse, but refused biopsies, then their lesions regressed spontaneously.