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X. Leleu (Lille, France)

12.30 Conclusion, awards & lunch

ABSTRACTS SPECIAL HIGHLIGHTS LECTURES AND SATELLITE SYMPOSIA

Treatment of relapsed DLBCL

C. Gisselbrecht, Hôpital Saint Louis, Paris, France

Introduction

Aggressive lymphoma patients who relapse or fail to achieve a CR have a poor outcome with a life expectancy of 6 months. Since < 10 %t of these patients obtain long-term disease-free survival with a salvage regimen alone, it has long been established before rituximab era, that salvage chemotherapy should, whenever possible be followed in a chemosensitive patient by consolidation with HDT and then ASCT.

All patients are now treated with front line rituximab (R) and chemotherapy. The analysis of randomized studies and registry data from patients treated with R CHOP confirmed that a major improvement in the treatment of diffuse large B cell lymphoma is observed in the general population.

Fewer relapses are seen among patients with 0-2 IPI factors (10-20%) however 30-50% of relapses are still seen for patients with more than 2 IPI factors.

In the absence of transplantation the outcome of relapsing patients is still poor. In the long-term analysis of data from the LNH 98-5 trial, comparing CHOP and R CHOP in patients over the age of 60 years[1] survival after progression, whatever the type or progressive disease, is poor for most of the patients. Median OS after progression was 0.6 month and 0.7 month for CHOP and R-CHOP respectively. However, some patients responded to salvage therapy and had a long survival after progression: 10 year survival was 10.5% and 8.6% for CHOP and R-CHOP respectively. In younger or fit elderly patient based on the PARMA study [2], HDT/ASCT has become the standard of care in younger patients with chemosensitive relapsed or primary refractory aggressive lymphoma. Induction therapy before HDT/ASCT consists in salvage regimens and several important issues to obtain the best CR are still in question: first the type of salvage regimen to choose; second, the efficacy of rituximab used in an era when R-CHOP is accepted as standard care in frontline therapy; third the risk factors as second line age-adjusted IPI (s-aaIPI) or relapse less than 12 months from diagnosis. When patient is not candidate to HDT/ASCT, other therapeutic options such as new biological therapies may be considered. Improvements in outcome may potentially be achieved through a greater understanding of the genetic abnormalities specifically associated with poorer-prognosis, and of factors that lead to unresponsiveness to chemotherapy.

Selecting a salvage regimen

Various old and new drugs are treatment options for DLBCL in the salvage setting. The effectiveness of these agents has been evaluated mainly in non-randomized studies and the difficulty of obtaining a cure or a prolonged disease-free period with conventional salvage chemotherapy may explain the large number of phase II studies that have been conducted in this setting. Consequently, salvage regimens outcomes are generally expressed as response rates and the possibility of collecting stem cells for ASCT. Survival data very often represent a mixture of transplanted patients and those not eligible for transplantation. No clear superiority of one regimen over the other has been demonstrated in the absence of randomized study.

Advances in salvage therapy are needed for two reasons: first, to overcome resistance to chemotherapy, enabling more patients to achieve a CR, thus allowing suitable candidates to proceed to transplantation and, second, to optimize transplantation procedures.

The addition of rituximab to CHOP chemotherapy has significantly improved the CR rate, event-free and overall survival rates compared to CHOP alone, as first-line treatment of aggressive NHL, without increasing toxicity. The clear demonstration of the addition of rituximab to platinum-based salvage regimens was provided in the study conducted by the HOVON group; 239 patients with relapsed or refractory DLBCL received a salvage regimen consisting of DHAP-VIM-DHAP ± rituximab followed by ASCT. Analysis of 225 patients showed that after two courses of chemotherapy, PR/CR was obtained in 54% of the patients in the DHAP arm and 75% in the R-DHAP arm (p≤0.01). Post-transplantation PR/CR was obtained in 50% and 73% of the patients, respectively (p=0.003) However, at the time of the study less than 5% of the patients have been previously exposed to Rituximab [3]. What is the optimal chemotherapy regimen to combine with rituximab as salvage therapy for DLBCL? The CORAL intergroup trial compared the association rituximab, Ifosfamide, etoposide, carboplatinum, R-ICE and rituximab dexamethasone aracytine and cisplatinum R-DHAP. DLBCL CD 20+ in first relapse or patients' refractory after first line therapy were randomized between R-DHAP and R-ICE. Responding patients received BEAM and ASCT and were randomized between observation or maintenance with rituximab for 1 year. Intent to treat analysis was made on the first 396 pts randomized in 11 countries (R ICE:202; R DHAP:194) [4]. The median age was 55 years. In 225 patients a relapse >12months was observed after initial complete remission. In 166 cases patients did not achieve initial complete remission (refractory) or had an early relapses < 12 months. 244 patients were treated with combination chemotherapy with prior exposure to rituximab. At the time of inclusion in the study there were 240 patients with Stage 3-4; 198 patients with elevated LDH. At relapse 226 patients had a secondary IPI 0-1 and 149 patients sIPI 2-3. Patients with prior exposure to rituximab had more refractory disease and adverse prognostic factors. The overall response rate was 63%, with 38% complete remission. There was no difference in response rate between R-ICE 63.5% (CI: 56-70%) and R-DHAP 62.8% (CI: 55-69%), and in mobilization adjusted response rate 52% vs 54%. Factors significantly affecting response (p<0.0001) were: refractory/relapse < 12 months with a response rate of 46 % vs 88 %, secondary IPI >1: 52% vs 71% and prior exposure to rituximab: 51% vs 83%. There were fewer serious adverse events in the RICE regimen when compared to R DHAP. From this first randomized study on relapses which was recently updated, there was obviously no difference in response rate and the ability to mobilize stem cell between the two major regimens used around the world in DLBCL.

Rituximab as post-transplantation maintenance/consolidation

Despite transplantation, the rate of progression was in the CORAL study at 3 years 39%. Progress should be made to prevent relapses. Maintenance rituximab post-ASCT has been evaluated as a means to reduce minimal residual disease. Two institutions have independently reported improvements in DFS and OS rates with use of rituximab post-ASCT. In the first study [5], rituximab was introduced at the dose of 1000mg/m² before collection of peripheral blood stem cell and after transplant on day 1 and 8. The DFS rate after a median follow-up of 20 months was 67%, compared with 43% in a historical control group (p=0.004). The 2-year OS rate was 80%, compared with 53% in historical controls who underwent ASCT without rituximab (p=0.002). In the second study [6] rituximab was given once-weekly at weeks 4–8 after salvage ASCT (and repeated if needed over 4 weeks at month 6) to 21 patients with relapsed or refractory large-cell lymphoma. After a median follow-up of 30 months, the EFS rate was 81% and the OS rate was 85%. It should be noted that there was an increased risk of prolonged neutropenia complicated with infection and hypogammaglobulinemia. In the CORAL study, after a second randomization post transplant with or without rituximab after HDT/ASCT, rituximab was given at the dose of 375 mg/m² every two months for one year. For the maintenance, ASCT was performed in 255 pts and 242 randomized:122pts rituximab (R), 120 pts observation(O). Distribution between R/O arms were respectively: median age 54 /53 yr, Male 76/83; female 46/37; secondary IPI 0-1: 84/81; sIPI 2-3: 36/36. 89/76 relapses >12months, 33/41 refractory/early relapses. With a median follow up of 44 months there were 111 events. Four years EFS was 52.8 % (CI 46-59) with 63% (CI 56-69) OS. There was no difference in event free survival, progression free survival and overall survival between rituximab and observation arm. In multivariate analysis, secondary IPI2-3 significantly affected EFS, PFS, OS (p=0.0004). Gender female (83pts) had a better 4 yr EFS 63% than male (159pts) 37% (p=0.01). This difference was seen only for female in the rituximab arm (p=0.004). In multivariate analysis, gender was an independent prognostic factor in the rituximab arm[7].

New approaches

Is there a place for RIC allograft?

A number of studies showed that it was only advisable to proceed to

an autograft if the patient had responded to initial salvage therapy with response being defined by clinical and CT criteria. With CT/PET scanning now widely available, it appears that autografts are of major benefit only in those patients with no metabolically active disease[8], and further attempts with standard dose therapy should be made to achieve such a state before proceeding to an autograft. In the CORAL study, for the patients who had a CT/PET before transplant there was also an advantage in PFS to PET negative patients with a 3 year PFS at 62%, however not all the patients with PET positive experienced a relapse with a 3 year PFS at 35% (p<0.0001). The patients failing to achieve a metabolic CR after initial salvage therapy clearly represent a poor prognostic group, and there is enthusiasm for considering these patients for reduced intensity allografts. One study has suggested that the allograft procedure overcomes the poor prognosis associated with a persistently positive PET scan but this requires confirmation [9].

Currently the major role of reduced intensity allogeneic transplantation (RIT) is in those patients who have failed an autograft or in whom an autograft is not possible, and the results from some centres are encouraging. However, less favourable results have been reported from some other centres, and stringency of patient selection is likely to be a major reason for such discrepancies.

Unlike ASCT, allogeneic SCT (alloSCT) generates an allogeneic graft-versus-lymphoma effect that reduces the likelihood of disease relapse following transplantation. The advent of reduced-intensity conditioning (RIC) regimens has renewed interest in alloSCT, which reduces non-relapse mortality while maintaining a graft-versus-lymphoma effect, and therefore allows the treatment of elderly patients and/or patients with co-morbidities. Although RIC alloSCT has only been used for a few DLBCL patients, the results suggest that it may be beneficial. In previously published studies of RIC alloSCT, the rates of relapse at 2 or 3 years ranged from 33 to 79 % [10]. The use of RIC allo in 48 consecutive patients with DLBCL (18 transformed from follicular lymphoma), 69% of whom had failed a previous autograft was reported [11]. The overall survival at 4 years was 47%. Recently, the French Society of Marrow Transplantation and Cellular Therapy reported on 68 patients [12]. They had received a median of 2 regimens of therapy prior to RIC alloSCT, and 54 (79%) had already undergone ASCT. Prior to transplantation, 32 patients (47%) were in complete remission (CR). With a median follow-up of 49 months, estimated 2-year OS, PFS and the cumulative incidence of relapse were 49, 44, and 41% respectively. The 1-year cumulative incidence of non-relapse mortality was 23%. Given the poor prognosis of this subset of patients when treated by conventional therapy, these results suggest that RIC alloSCT is an attractive therapeutic option for patients with high-risk DLBCL. An exciting finding of this study is that a history of anti-CD20 therapy prior to allogeneic transplant did not significantly affect the incidences of disease progression or relapse (2–yr PFS: 40 vs 48%, p=0.59). The results of all these studies can be considered encouraging and confirmed by EBMT registry data[13].

How to use new agents in combination

We now better understand that the treatment of DLBCL must take into account individual factors related to biological characteristics of tumours and patients. Although DLBCL is a well-defined entity and has been characterized since the first classification of NHL, the complexity and heterogeneity of the disease has just been demonstrated over the past 10 years: according to the most recent WHO classification, it includes no less than 15 different sub entities [14]. Prognostic discrimination can also be achieved through gene expression profiling (GEP) [15], sub-dividing DLBCL lymphoma into the germinal center (GC) type, the activated B-cell (ABC) type and primary mediastinal B-cell lymphoma. The prognostic stratification between GC and ABC subtypes remains valid in patients receiving chemoimmunotherapy with a 3-year OS of 84 and 56%, respectively. However, GEP is technically demanding, and robust kits have not entered the routine use, either for broad-based diagnosis or for DLBCL sub categorization.

Although there are technical limitations, immunophenotyping is an essential diagnostic method that can identify DLBCL and further classify DLBCL into the GC type (CD10 + or CD10-, BCL6+ MUM1-) and the non-GC (ABC) type (CD10- BCL6- or CD10 – BCL6+ MUM1+) [16]. In the CORAL study, the analysis of a subset of 235 patients with GCB DLBCL (116) according to Hans's algorithm had a better PFS than patients with non-GCB DLBCL (119) (p=0.09). A more comprehensive and global view of molecular heterogeneity of the tumor and of the host response will help us to design more accurate and rational approaches to successfully treat these patients by targeted therapies[17].

It would be preferable to use one drug to treat patients with DLBCL, regardless of their subsets. However, it has not yet been clearly elucidated whether there are common factors among the subsets to target or whether we have to treat patients using different strategies. The rate of relapse and failure was dramatically reduced with the combination of rituximab and chemotherapy, mostly in the GCB group. Salvage chemotherapy is less effective in patients with previous exposure to rituximab . Therefore, new therapies should focus on patients with the high-risk IPI or the ABC subtype. There is an unmet need for this population, and new drugs could be evaluated in patient more quickly. However, despite the large amount of data collected in the last 10 years by wide-genomic analyses, only a few identified targets have progressed to phase II trials for DLBCL[18], [19]. Of the many new agents developed for lymphoma, that are not monoclonal antibodies, some have been shown to be promising for future therapy of DLBCL and that are now under clinical investigations. These new drugs include immunomodulators and mTOR as well as kinase, proteasome, and histone deacetylase inhibitors, most of which have been tested in indolent lymphomas or mantle cell lymphoma, showing significant single-agent activity.

Multiple new agents targeting various pathways have shown some clinical activity in lymphoma. When the limit of standard chemoimmunotherapy treatment is reached, we need to incorporate these agents in the armamentarium, taking into account the additive toxicity. Understanding the relationship of tumor biology to outcome is important for the identification of molecular targets and for improvement of therapy. Recent advances in GEP confirmed that patients with the ABC subtype are less likely to respond well to CHOP-based regimens than those with GCB subtype. Hypothesis proposed by Wilson et al for a different result of infusional R-EPOCH with a better efficacy in GC B-cell like DLBCL than ABC like DLBCL was due to a prolonged exposure of agents. In contrast, the poor outcome of ABC like DLBCL may relate to the constitutive activation of the nuclear factor-k B pathway [20].

In the CORAL study, the subgroup of patients with GCB profile by immunohistochemistry had a better outcome under RDHAP regimen, but difference was observed for the ABC subtype. These findings underlined the need to study the effect of new drugs according to DLBCL subtypes[17].

In addition, the prognostic significance of MYC$^+$ DLBCL in a selected population of patients included in the CORAL study was evaluated. Twenty-four (16%) out of 150 patients analysed exhibited a MYC rearrangement, associated in 87.5% (n=21) of the cases with a BCL2/18q21 rearrangement (n=16), with a BCL6/3q27 rearrangement (n=4), and with both BCL2/18q21 and BCL6/3q27 rearrangements (n=4). Patients with MYC$^+$ DLBCL presented with a more advanced disease, and have a significant inferior prognosis than patients with MYC$^-$ DLBCLs. Their outcome seems not influenced by the proposed salvage therapy [21].

However, at the present stage, most of the studies with new drugs were conducted in a limited number of refractory patients. The response rate in DLBCL was in the order of 30%, with few complete remissions and a short duration. Most of these agents appeared to be less toxic than conventional chemotherapy or showed a different spectrum of toxicity. However, hematotoxicity with thrombocytopenia remained one of the most common toxicities. Many of these agents are cytostatic rather than cytotoxic. Their benefit might be only detectable when they are combined with standard regimens.

The easiest and most logical approach is to combine these agents with rituximab, given its low toxicity. Inotuzumab ozogamicin is a humanized anti-CD22 antibody conjugated to cytotoxic calicheamicin (INO) is an attractive approach when combined to rituximab. It achieved a response rate >35% in selected patients with poor prognosis relapses[22]. Phase 1 is ongoing with combination to rituximab gemcitabine and oxaliplatine in relapsed DLBCL.

The combination of lenalidomide and rituximab increased the response rate in follicular lymphoma and is worthwhile to be tested

in DLBCL. Rituximab and temsirolimus also appeared to be promising in mantle cell lymphoma. However, assessing the real benefits of these combinations will be proven only in randomized studies. The addition of new agents to well-established chemoimmunotherapy may improve the baseline results and is easier to apply to patients not heavily pre-treated. Bortezomib has been incorporated in several standard regimens: RCHOP, R CVP, R bendamustine. The rationale for treating DLCBL is that the ABC subtype is sensitized to chemotherapy under exposition to bortezomib.
Lenalidomide can also be incorporated to RCHOP or other combination chemotherapy, with the possible goal of achieving a better activity in ABC-DLBCL.
Another attractive approach is the combination of these new agents. Combining agents that inhibit cell growth through different mechanisms should be developed, although this is also an enormous challenge that should be supported by reliable preclinical models in vitro and in vivo.
It must be kept in mind that the approval of two drugs in combination requires outstanding results and cost effectiveness. The number of drugs to be tested is enormous, and all too often, companies are conducting similar studies in similar patient populations, using molecules with similar targets. For diseases such as lymphoma, the incidence is too low for all of the different studies, and there is a risk of increasing the cost and significantly delaying the clinical development of new drugs.

Recent advances in Severe Combined Immune Deficiency

L.D. Notarangelo, Children's Hospital Boston, USA

Severe Combined Immune Deficiency (SCID) includes a heterogeneous group of genetic disorders that affect T cell development and in some cases also B and/or NK cell development. In spite of the immunological and genetic heterogeneity of SCID, the clinical presentation is typically characterized by early-onset severe infections and failure to thrive. Unless immune reconstitution is achieved with treatment, SCID is inevitably fatal within the first years of life.
In the last years, significant advances have been made in the characterization of the molecular basis of SCID, definition of the clinical and immunological phenotype, early recognition, and treatment.
In particular, unexpected phenotypic heterogeneity has been identified in patients carrying mutations in SCID-causing genes, as best exemplified by mutations in the RAG genes, that have been associated with T^- B^- NK^+ SCID, Omenn syndrome, leaky SCID, CID with expansion of gd T cells, delayed-onset immunodeficiency with granuloma, and idiopathic CD4 lymphopenia. To define the basis of this clinical and immunological heteroegenity, we have developed a flow-cytometric assay that makes use of a $Rag1^{-/-}$ tg.bcl2 abelson-transformed pro-B cell line that has been engineered to contain an inverted GFP cassette flanked by recombination signal sequences (RSS) as a single integrant. Upon infection with retroviral vector expressing wild-type or mutant human RAG1, GFP expression is measured as read-out of V(D)J recombination activity. In a series of more than 40 different RAG1 mutants analyzed, a tight correlation has been observed between degree of residual activity and clinical/immunological phenotype. Of note, mutations associated with Omenn syndrome have very low activity (0.5-2% of wild-type), whereas mutations associated with the mildest forms of disease (idiopathic CD4 lymphopenia and granuloma formation) carry significant residual activity (up to 40-60% of wild-type) on one allele. We have also demonstrated that mutations that impair (but do not abolish) RAG activity are associated with abnormalities in the mechanisms that govern central and peripheral T and B cell tolerance, with impaired expression of aire in the thymus and in the periphery, and increased production of autoantibodies. These data provide the first comprehensive genotype-phenotype correlation analysis in RAG deficiency with a novel, semiquantitative assay and provide mechanistic insights into the immune dysregulation of the disease.
Treatment of SCID is mainly based on hematopoietic cell transplantation (HCT and, in selected cases, gene therapy. Results of HCT are particularly good when the transplant is performed early in life, prior to development of severe infections. Early recognition of SCID is therefore key to promote better outcome. Quantification of T cell receptor excision circles (TRECs), a by-product of V(D)J recombination during generation of $TCRab^+$ thymocytes, in Guthrie cards allows identification of SCID at birth. In Massachusetts, a universal newborn screening for SCID is active since 2009, and 4 infants have been successfully identified and transplanted. Extension of this protocol to other States and countries is anticipated to allow precise definition of the prevalence of SCID, and to permit timely definitive cure.
Gene therapy has proven effective in infants with X-linked SCID and ADA deficiency, however 5 out of 20 patients with X-linked SCID treated by gene therapy have developed leukemic proliferation due to insertional mutagenesis. This has prompted development of novel, potentially safer vectors. A multicenter trial has been recently opened that makes use of a self-inactivating gammaretroviral vector in which expression of the gc is driven by an internal cellular promoter (EF-1a). One infant with X-linked SCID has been treated with this vector in Boston, USA. One year after gene therapy, he has achieved immune reconstitution, with no evidence for clonal dominance.

Regulation of Hematopoietic Stem Cells and their Bone-Marrow Niches by the Coagulation System.

T. Lapidot , Weizmann Institute, Rehovot, Israel

Navigation of transplanted stem cells to their target organs is essential for clinical bone marrow (BM) reconstitution. Hematopoietic stem and progenitor cells (HSPCs) dynamically change their features and location, shifting from quiescent and stationary cells anchored in their bone marrow niches via adhesion interactions, to cycling and motile cells which egress to the circulation. These dynamic changes are driven by stress signals, activation of proteolytic enzymes, cleavage of adhesion interactions, and generation of reactive oxygen species (ROS). The development of functional, preclinical, immune-deficient NOD/SCID mice transplantation models has enabled the characterization of normal and leukemic human HSCs and investigation of their biology, including HSC homing, bone marrow repopulation, their egress and mobilization. The chemokine SDF-1 (CXCL12) activates CXCR4+ HSPC adhesion interactions with the bone marrow microenvironment. While SDF-1/CXCR4 interactions are essential for maintaining murine HSC quiescence in the bone marrow, this chemokine has also major active migratory roles in hematopoietic stem and progenitor cell (HSPC) homing, egress and mobilization. Human and murine SDF-1 differ in only one amino acid and are cross reactive. The migration potential of human CD34+ HSPC to a gradient of SDF-1 in vitro correlates with their in vivo BM repopulation in transplanted NOD/SCID mice. Importantly, the same correlation also exists in patients where the SDF-1- migration capacity of mobilized patients CD34+ HSPC correlates with their clinical repopulation potential in autologous transplantations
Preliminary results reveal that injection of the coagulation factor thrombin induces upregulation of its major receptor PAR-1 on murine bone marrow stromal cells and on the BM reservoir of immature and maturing leukocytes. Thrombin activation induces rapid SDF-1 secretion from BM stromal cells, including Nestin+ MSC which form a niche for stem cells, and its release to the circulation. Thrombin treatment also increases CXCR4 expression via p38 MAPK and eNOS signaling, leading to rapid stem and progenitor cell mobilization in vivo and increased SDF-1 induced migration in vitro. A specific inhibitor of PAR-1, blocked SDF-1 induced migration in vitro as well as AMD3100 and G-CSF induced mobilizations in mice. Murine long term repopulating stem cells highly express endothelial protein C receptor (EPCR). We have found that these cells also highly express PAR-1 and that Thrombin, AMD3100 and G-CSF induced mobilization involve EPCR shedding from stem cells as part of the mobilization process. Inhibition of PAR-1, or inhibition of ROS, prevents EPCR shedding and stem cell mobilization.
In summary, we have found that the coagulation factor thrombin via its major receptor PAR-1, regulates stem and progenitor cell mobilization via eNOS signaling, CXCR4 upregulation, increased SDF-1 secretion and release and EPCR shedding. Manipulations

of these players could improve clinical mobilization and transplantation protocols.

From Pluripotent stem cells to neural networks

P. Vanderhaeghen, Université libre de Bruxelles, Belgium

The mechanisms that control the generation of neural cells from pluripotent stem cells remain poorly understood, but the identification and characterization of factors capable of (re) specifying neurons of precise identity has important implications regarding our understanding of neurological diseases and in the context of therapies for neurological disorders.
Embryonic stem (ES) and induced pluripotent stem (iPS) cells constitute a promising tool for the modelling and treatment of human neural diseases.
Here we describe a novel pathway by which ES and iPS cells, whether of mouse or human origin, recapitulate in vitro major milestones of brain development, leading to the sequential generation of a diverse repertoire of neurons that display most salient features of genuine neurons of the cerebral cortex. When grafted into the cerebral cortex of newborn mice, or lesioned cortex of adult mice, these neurons develop patterns of axonal projections corresponding to endogenous cortical projections in vivo.
This pathway of corticogenesis sheds new light on the mechanisms of neuronal specification, and constitutes an innovative tool to model normal and pathological brain development, including in the human species. In the long run, cortical neurons generated in vitro could be used also in the perspective of brain repair, for several diseases striking cortical neurons.

Classification and prognosis of patients with Myelodysplastic syndromes

Ulrich Germing, Heinrich-Heine-Universität, Düsseldorf, Germany

In 1982, the French-American-British working group (FAB) proposed a classification for the diagnosis of Myelodysplastic syndromes including CMML based on peripheral and medullary blast count, ring sideroblasts and absolute monocyte count in blood. It was accepted worldwide and used for almost two decades. Two Refinements of this classification resulted in the current WHO classification, published in the Blue Book of the WHO in 2008. Major changes were based on more detailed information. The following characteristics were taken into account: 1) the degree of dysplasia (uni-versus multilineage Dysplasia), 2) ANC, platelet count, 3) presence of isolated del(5q), 4) absence of bcr/abl and PDGFR a and b. To get this information, chromosomal findings as well as molecular findings have to be available.
The WHO classification meanwhile has been validated retrospectively as well as prospectively and is in current use in many Institutions.
The major shortcomings of the WHO classification are the definitions of the category MDS unclassified and the unilineage dysplastic RCUD, because in both cases it is extremely hard to distinguish the findings from secondary anemia in cases without proven clonality. Refinements of the classification are to be expected in the next years.
The gold standard for prognostication of MDS patients still is the IPSS. As the chromosomal risk categorization of the IPSS was based on only a small number of aberrant cases, a new refinement of the chromosomal risk categories has been developed recently (Schanz et al, JCO in press) and refinements of the other risk factors used in the IPSS are underway, potentially resulting in an IPSS-R. The WHO adapted prognostic score (WPSS) takes into account transfusion need and also is able to identify patients with different risk groups. In addition, this score was shown to be able to be used at any given time during the course of the disease. It is simple to identify high risk patients (high medullary blasts, adverse cytogenetics, high comborbidity status, etc), but it is not simple, but more important to identify patients at low risk. Since new drugs are available, a most accurate assessment of the prognosis is more important as in former times.

Treatment of myelodysplastic syndromes (MDS) in 2011

P. Fenaux, Hôpital Avicenne and Groupe Francophone des Myélodysplasies (GFM) France

For therapeutic purposes, the IPSS, based on % marrow blasts, karyotype and cytopenias, although it is imperfect, remains a good basis in MDS. One can schematically separate high and int 2 risk patients (grouped as « higher risk » MDS), where median survival was until recently only about 15 months, most patients dying from MDS, and low and int 1 IPSS patients (grouped as « lower risk » MDS) where survival is longer, many patients dying from causes other than MDS, related to their age. In the former group, treatments aiming with an impact on the disease course should be administered whenever possible. In the latter group, treatment is often mainly directed at correcting cytopenias, mainly anemia, and their clinical consequences

1) Treatment of higher risk MDS

Allogeneic stem cell transplantation (SCT) remains the only curative treatment of higher risk MDS, but it is restricted to patients aged less than 45 to 50 (for «classical» allogeneic SCT) and 65 to 70 (for «nonmyeloablative» SCT with an HLA indentical donor (familial or unrelated) i.e. to only about 20% of higher risk MDS. For the remaining patients, Anthracycline-Ara-C based chemotherapy yields complete remission rates of about 50 % in high risk MDS, but is also limited to a minority of MDS patients, whose age (generally less than 65) and general condition do not preclude such treatment. In addition, duration of CR is generally short (about 1 year) and most patients eventually relapse. Lower intensity chemotherapy regimens, mainly low dose cytarabine (Ara C: 20 mg/m²/day during 10 to 14 days every month) have been largely used in elderly MDS patients, with CR and PR rates of 20 % and 20 %, respectively. In addition, these chemotherapy regimens (either intensive anthracycline- AraC-based chemotherapy or low dose AraC) yield almost no response in patients with an unfavourable karyotype, i.e. monosomy 7/del 7q or complex karyotype.
Increased and aberrant gene hypermethylation increases during progression from lower risk to higher risk MDS. Based on the hypothesis that this hypermethylation might favor leukemogeneis by silencing tumor suppressor genes, demethylating agents, including azacitidine and 5'deoxy-azacitidine (decitabine) have been introduced in the treatment of MDS with the aim to antagonize this process. In a randomized phase III study (CALGB 9221) comparing azacitidine (SC 75 mg/m² for 1 week every 28 days) with best supportive care (BSC, including transfusion and antibiotics) in patients with all MDS FAB subtypes the overall response rate in patients treated with this drug versus those receiving BSC was 60% versus 5% ($p < 0.0001$), with a median response duration of 14 months. The median time to AML or death was 21 months in patients receiving azacitidine, compared with 12 months in patients receiving BSC ($p = 0.007$), and median survival 20 months versus 14 months ($p = 0.1$). Of the 65 patients who were RBC transfusion-dependent at the beginning of the study, 29 (45%) experienced sustained transfusion-independence The most frequent adverse effect of azacitidine therapy was worsening of pre-existing cytopenias.
Subsequently, a phase III multicenter randomized trial (AZA 001 trial) demonstrated a survival superiority of azacitidine over conventional care regimen (CCR) (that included BSC, low dose AraC or intensive chemotherapy) in higher-risk MDS patients, FAB-defined as RAEB, RAEB-T, or CMML (10–29% marrow blasts) ($p=0.0001$)[16]. Azacitidine showed a median Kaplan-Meier OS of 24.4 months versus 15 months with CCR. This trial was the first clinical study to demonstrate that any drug could obtain a significant OS advantage in higher risk MDS[16]. Azacitidine showed improved survival over BSC and low dose AraC, while the direct comparison between azacitidine and intensive chemotherapy was difficult due to small patient numbers. In addition, the benefit of azacitidine over CCR was seen irrespective of age, FAB, WHO type, % marrow blasts, number of cytopenias and karyotype. Finally, responses with azacitidine were often delayed, not occurring before 6 cycles and the large number of cycles (median 15 in responders), probably contributed to the survival benefit
Other studies performed with Decitabine in higher risk MDS found similar response rates as with azacitidine, However, 2 trials (one

in the US and one in Europe) where Decitabine was randomized versus best supportive care found no significant survival advantage for Decitabine. It is unclear why decitabine did not improve survival in those studies. One reason might be that patients only received on the average 2 and 4 cycles of the drug in those trials. Another reason could be a suboptimal Decitabine schedule in those trials (ie every 8 hours , 3 days every 6 weeks) . Other schedules, including every day IV during 5 days every 4 weeks, may yield better results, although confirmation in randomized studies focusing on survival will be required. The favorable results obtained with hypomethylating agents, especially azacitidine, in higher risk MDS open many perspective with those drugs in MDS and AML. In particular, interesting results have been obtained with hypomethylating agents before or after allogeneic SCT, as treatment of MDS and AML following myeloproliferative disorders ((ET and PV), in lower risk MDS with anemia resistant to EPO, and in AML irrespective of the marrow blast %. In addition, many combinations between hypomethylating agents and other drugs, including HDAC inhibitors, lenalidomide, low dose chemotherapy or other drugs are currently being tested in order to further improve the survival advantage observed with hypomethylating agents used alone. In case of failure of hypomethylating agents, or relapse after a response, prognosis is poor (median 5-6 months in the absence of allogeneic SCT). In those patients, several drugs are currently being tested in clinical trials, including On 01910Na, clofarabine and others.

2) Treatment of lower risk MDS

As said above, although this is schematic, treatment of lower risk MDS mainly aims at improving cytopenias, mainly anemia, the most important cytopenia in most lower risk MDS cases.

a) treatment of anemia

Anemia, especially when requiring regular RBC transfusions, is associated with poorer survival in MDS. This probably reflects in some cases more aggressive disease, but the consequences of acute or chronic anemia (especially cardiovascular complications), and of iron overload due to repeated RBC transfusions also contribute to this poorer outcome. Anemia is also associated with poorer quality of life. Therefore, trying to improve anemia with specific agents is a major aim in the treatment of lower risk MDS

Erythropoietic stimulating agents (ESA), including recombinant EPO and Darbepoetin alpha are generally the first line treatment of anemia, except in patients with del 5q that respond better to Lenalidomide (LEN)

With ESAs, erythroid response rates of 40 to 60% are achieved, with median response durations of about 2 years. Higher response rates are seen in patients with low RBC transfusion rates and serum EPO levels below 200 or 500 U/l.

The response rate is however lower, and response duration shorter in lower risk MDS with del 5q. On the other hand LEN, in those patients, can yield RBC transfusion independence in about 65% of the cases, with a median response duration of 2.2 years. Importantly, LEN also yields cytogenetic responses in 60 to 70% of the patients, complete in about 40% of them. This, and other experimental evidence, strongly supports a specific activity of LEN on the del 5q clone that may eventually have a favorable impact on the risk of progression to AML and on survival, although this will have to be firmly demonstrated

After failure of ESA (in non del 5q patients) and LEN (del 5q patients), second line treatments include hypomethylating agents, often used at lower schedules than in higher risk MDS, immunosuppressive treatment including antithymocyte globulin, with or without cyclosporine (and possibly alentuzumab), mainly in relatively young patients with several cytopenias, no excess of marrow blasts, normal karyotype or +8, and HLA DR 15, and finally LEN in non del 5q patients (where the response rate to LEN is lower and response duration shorter than in del 5q patients).

b) neutropenia and thrombocytopenia

Neutropenia and thrombocytopenia are relatively rarely isolated or even predominant in lower risk MDS. For neutropenia, prolonged use of G-CSF has not demonstrated any benefit in terms of survival or occurrence of infections. Therefore, the main measure generally consists of immediate administration of broad spectrum antibiotics in case of fever. For patients where drugs like hypomethylating agents can worsen preexisting neutropenia during relatively prolonged periods, prophylactic antibiotics and antifungals may be indicated. For thrombocytopenia of lower risk MDS, high dose androgens may have some efficacy. In selected patients, an immune component to thrombocytopenia may exist, and response to treatments used in ITP (including splenectomy!) is sometimes seen. The thrombopoietin receptor agonist Romiplostin significantly improves platelet counts in about 50% of lower risk MDS with thrombocytopenia at high dose (about 10ug/k every week or 2 weeks), with prolonged response in most cases, but with possible transient increase in marrow blasts in some cases, requiring close monitoring.

3) iron overload and chelation therapy in MDS

Most MDS patients receive RBC transfusions during the disease course, leading to possible iron overload in the liver, heart and endocrine glands, with their clinical consequences. The importance of those clinical consequences, however, is disputed. Those discussions may arise in particular from the fact that, for example, iron overload (as evidenced by MRI examination) is evident in the liver after less than 20 RBC concentrates, while it appears (with possible consequences) in the heart generally only after 50 to 70 concentrates. However, many lower risk MDS receive more than that number of RBC concentrates, and may therefore be at risk of cardiac failure. Thus, it is recommended to use iron chelation in MDS with relatively favorable prognosis (ie lower risk MDS, and probably higher risk MDS that respond to treatment) after a certain number of RBC concentrates, although that number remains disputed. Another group of patients where iron chelation appears very important is candidates for allogeneic SCT, where iron overload, even at relatively low levels, is associated with increased transplant related mortality. The oral chelating agent deferasirox is gradually replacing deferoxamine which requires SC or IV administration. Deferiprone is less used, notably because it can induce neutropenia in some patients.

SATELLITE SYMPOSIUM THE BINDING SITE

The use of the Freelite™ assay in various conditions.

O. Decaux, CHU de Rennes, France

Freelite™ assay allows the measurement of serum concentration of immunoglobulin free κ and λ light chains (FLC) and a calculation of FLC κ/λ ratio (which identifies the presence of a serum monoclonal FLC). It provides greater sensitivity than serum protein electrophoresis and immunofixation. International Myeloma Working Group (IMWG) recently published consensus guidelines for the use of the Freelite™ assay in the diagnosis and management of multiple myeloma and related plasma cells disorders. Here, we present these consensus recommendations and discuss their application in routine practice. There are three major potential indications for the Freelite™ assay in plasma cells disorders.

Screening

In the context of screening, the Freelite™ assay in combination with serum protein electrophoresis and immunofixation offers high sensitivity, and negates the need for 24-h urine studies for diagnoses other than light-chain amyloidosis (AL amyloidosis). It enables detection of monoclonal protein in some patients with non-secretory myeloma and AL amyloidosis that were previously undetectable by serum protein electrophoresis or immunofixation.

Monitoring

The Freelite™ assay is a valuable laboratory test for monitoring response to treatment of patients with oligo-secretory multiple myeloma and AL amyloidosis. These patients were previously very difficult to monitor with traditional assays. Freelite™ can be very useful for monitoring of patients with light-chains multiple myeloma. There are no data to support using the Freelite™ assay for monitoring of multiple myeloma with measurable disease by serum or urine protein electrophoresis. Further prospective studies with large cohorts of patients should provide additional arguments for its utility in this setting.

Prognosis
Baseline FLC concentration is of major prognostic value in all plasma cells disorders. An abnormal FLC ratio is associated with shorten survival of patients with multiple myeloma.
An abnormal FLC ratio has been shown to be a risk factor for progression of monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM) to symptomatic multiple myeloma. However, because of the very high incidence of MGUS in the general population and because it is increasing with age, Freelite™ should not be performed in all patients with MGUS and should be reserved to young patients with bad prognostic factors.

Novel M-component based biomarkers in Waldenstrom Macroglobulinemia (WM)

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Waldenstrom's macroglobulinemia (WM) is an indolent B cell lymphoma of the lymphoplasmacytic type accompanied by a serum IgM component. Although quantified IgM is a compelling marker of WM, its resolution on serum protein electrophoresis (SPEP) can make accurate measurement difficult. The same applies to total IgM quantification by nephelometry which inherently includes monoclonal and polyclonal immunoglobulins. IgM levels by either technique do not accurately reflect tumor load or prognosis in WM. There is a need to identify new markers that reflect disease burden and correlate with patients'outcome. New serum M-component based biomarkers were developed for routine practice in recent years, such as the Freelite® test and more recently the Hevylite test®. The serum free light chain assay (sFLC; Freelite® test, the Binding Site, Birmingham, UK) is a nephelometric measurement of kappa and lambda light chains that circulate as light chain monomers or dimers and that are not bound to immunoglobulin heavy chain. The serum Hevylite® immunoassay (the Binding Site) is based on specific polyclonal antibodies, which recognise epitopes spanning the junction of the heavy and light chains of the individual immunoglobulin isotypes. The serum IgG and IgA Hevylite assays have been reported to be more sensitive than serum protein electrophoresis (SPEP) and nephelometry for identifying and quantifying monoclonal immunoglobulins. The serum IgM Hevylite assay measures specifically IgMkappa and IgMlambda, separately, and might provide true quantitative measurement of the IgM M-spike. The diagnostic and prognostic potential of these tests are under investigation in WM.
Studies have shown that Freelite accurately differentiated patients with IgM MGUS compared to WM, was a prognostic marker for time to treatment in WM that helps monitoring disease response or progression, and Involved sFLC level was found significantly elevated in patients requiring treatment. Involved sFLC level also appeared a reliable marker to monitor response and progression in WM, although not greater to IgM measured using SPEP. However, the median time to sFLC response was one month shorter compared to the median time to IgM response. Although current data are preliminary, Hevylite was a marker of response and might help to accurately monitor progression of WM.
New serum M-component based biomarkers are under investigation in WM; Freelite and Hevylite tests look promising. The former is very sensitive and provide earlier appreciation of the response status of the patients, and the latter might become the reference technique to monitor the IgM M-spike protein in years to come.

ABSTRACTS SELECTED ORAL PRESENTATIONS

O.01. Influence of pre-analytical storage conditions on quantitative BCR-ABL results in CML: a multi-centre study

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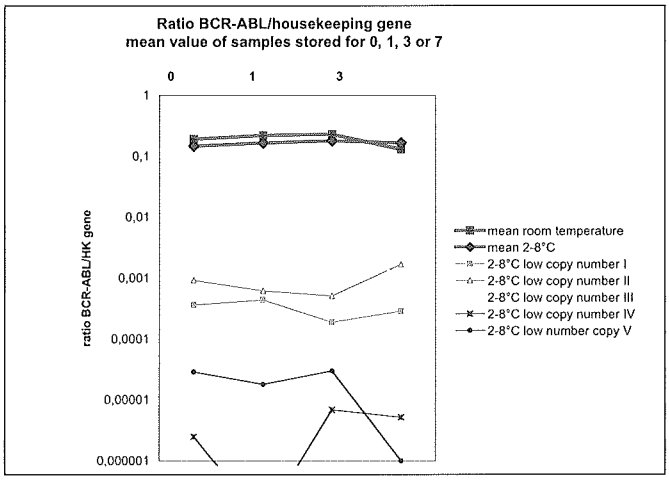
Quality and quantity of RNA extracted from peripheral blood or bone marrow leukocytes play a crucial role for the sensitivity of reverse transcript-PCR assays to monitor the efficacy of therapy (minimal residual disease- MRD) in CML patients characterized by the fusion transcript BCR-ABL. Long transit times of samples are associated with RNA degradation but studies in the literature concern mainly the stabilization with nucleic acid preservative reagents. In this multi-centre study organised by 'MolecularDiagnostics.be' we investigated the influence of pre-analytical storage conditions on quantitative BCR-ABL results in CML patients. Eleven participating laboratories in this multi-centre study divided 20 blood samples suspicious for BCR-ABL (P210) at the day of collection in 4 aliquots, to simulate the situation of sample reception at day 0, 1, 3, and 7. Additionally the samples were divided: one part was stored at room temperature the other part at 2-8°C. After treatment at the different days RNA was extracted and a quantitative BCR-ABL was performed. The ratio of the BCR-ABL/HK gene, the mean value of all samples and the low copy number samples (2-8°C) were calculated (figure 1). The ratio is quite stable when the samples are stored at 2-8°C before treatment. Sample storage at room temperature influences the result after 3 days. The BCR-ABL/HK gene ratio of samples with less than 100 copy numbers of BCR-ABL shows a high variation at the different days of pre-treatment. For a decision of treatment discontinuation on the basis of the 3-log-decline rule at the beginning of therapy, our study indicates that analysing samples stored in the fridge for up to 7 days or at room temperature for up to 3 days can still be informative. However, MRD samples with low copy numbers of BCR-ABL the ratio varies up to 0.5 log at the different days. This could have consequences for reporting molecular responses.



O.02. Ponatinib is active against imatinib resistant mutants of FIP1L1-PDGFRA and KIT, and against FGFR1-derived fusion kinases

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Ponatinib is a third generation BCR-ABL1 inhibitor currently in phase II clinical trials for imatinib resistant CML. In vitro ponatinib activity also encompasses PDGFRA, KIT, and FGFR1, and recently potent activity towards oncogenic fusion or mutant kinases such as FIP1L1-PDGFRA, KIT-N822K and FGFR1OP2-FGFR1 has been documented. Therefore, we investigated the effect of ponatinib on imatinib resistant mutations of FIP1L1-PDGFRA, of KIT, and on an imatinib insensitive FGFR1 fusion. Ponatinib strongly inhibited growth of the FIP1L1-PDGFRA-T674I mutant expressing Ba/F3 cells with an IC50 of 9 nM. It was also active against the FIP1L1-PDGFRA-D842V mutant but with a higher IC50 (154 nM). With western blotting we demonstrate a strong inhibition of the constitutive autophosphorylation of either FIP1L1-PDGFRA-T674I or FIP1L1-PDGFRA-D842V by ponatinib as well as the FIP1L1-PDGFRA downstream targets STAT5 and ERK1/2. Next, we explored the activity of ponatinib against CUX1-FGFR1, a recently described oncogenic FGFR1 fusion kinase, not responding to imatinib. The growth of CUX1-FGFR1-expressing Ba/F3 cells was inhibited by ponatinib with an IC50 of 56 nM. Again, this correlated nicely with decreasing tyrosine phosphorylation of the fusion protein and its downstream targets STAT5 and ERK1/2. Furthermore, we investigated cell-based models of imatinib resistant KIT mutant driven malignancies. Ba/F3 cells were used expressing KIT-D816V, KIT-Y823D, KIT-W557_K558del+T670I and KIT-W557_K558del+D820A. Proliferation experiments supplemented with western blotting demonstrated a potent inhibitory effect of ponatinib towards the tested KIT double mutants. Also KIT-Y823D responded well to ponatinib (IC50: 62 nM), whereas no effect was seen on KIT-D816V. In summary, our data indicate that ponatinib is active in vitro, at clinically achievable IC50, against CUX1-FGFR1, FIP1L1-PDGFRA-T674I, FIP1L1-PDGFRA-D842V, and against specific KIT mutants. Its potential in the therapeutic management of EMS, primary or secondary imatinib resistant GIST, or imatinib resistant FIP1L1-PDGFRA positive disease, needs further evaluation.

O.03. Single-center analysis of biopsy-confirmed post-transplant lymphoproliferative disorder: final analysis

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Purpose
This study aims to better define incidence, clinico-pathological characteristics, risk factors, management and outcome of posttransplant lymphoproliferative disorder (PTLD) in a large cohort of solid organ and hematopoietic stem cell transplant patients.

Patients and methods
Transplant recipients diagnosed with biopsy-confirmed PTLD at the University Hospitals Leuven in the period 1989-2010 were identified. Patient-, transplantation- and disease-related characteristics, prognostic factors and outcome were collected and analyzed.

Results
One hundred-forty biopsy-proven PTLD cases were included. Overall incidence in the transplant population was 2.12% (140/6.607) with heart transplant recipients carrying the highest risk. Most PTLDs were monomorphic (83.6%) with diffuse large B cell lymphoma being the most frequent subtype. The majority of cases (70.7%) occurred > 1

year post-Tx, whereas 66% were Epstein Barr virus positive. Following first line therapy overall response rate was 68.5%, whereas 3-year relapse-free and overall survival were 59% and 49%, respectively. At last follow-up 44% of the patients were alive. Multivariable analysis was able to identify several classical lymphoma-specific poor prognostic factors for the different outcome measures, including higher age at diagnosis, advanced stage, hypoalbuminemia and elevated lactate dehydrogenase. The value of the International Prognostic Index (IPI) was confirmed in our analysis.

Conclusions
PTLD is characterized by a predominance of monomorphic subtypes, with an overall incidence of 2.12%. Except for reduction of immunosuppressive therapy, treatment was very heterogeneous. Despite better knowledge of pathogenesis, prognosis remains poor. Hypoalbuminemia is a very strong poor prognostic factor. Classical prognostic factors for poor outcome, including IPI, also apply to PTLD.

Figure 1. Outcome by histological subtype and Epst.

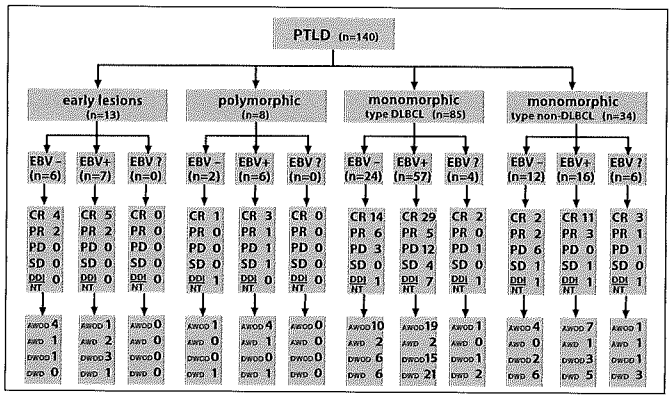
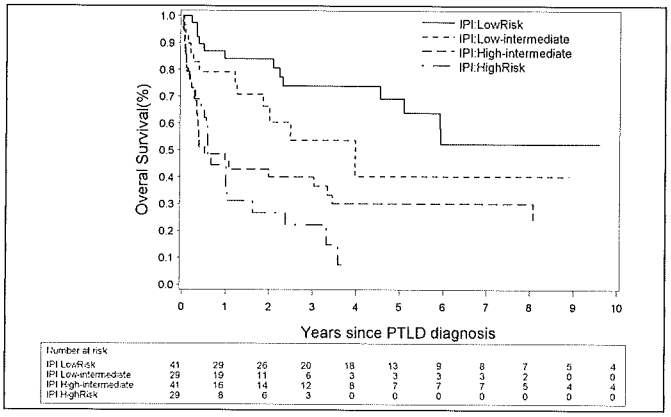


Figure 2. Overall survival for the different IPI.



O.04. The accuracy of PET in detection of Posttransplant Lymphoproliferative Disorder

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Introduction
We investigated the accuracy of Positron emission tomography (PET)-scan in 170 patients with suspected posttransplant lymphoproliferative disorder (PTLD).

Material and methods
All solid organ and hematopoietic stem cell transplant recipients who had a fluorodeoxyglucose (FDG) PET-scan between 2003 and 2010 in our center for the indication PTLD, were retrospectively reviewed and results were compared with biopsy, whenever possible. Hundred-seventy PET-scans in hundred-fifty patients were eligible for evaluation. In 45 cases, the patient had already a bioptic proof of PTLD before PET-scanning and PET was merely performed for staging of the disease. In the remaining 125 PET-scans, PET was performed to differentiate between PTLD and other diseases. FDG-uptake was semiquantitatively expressed by calculation of the maximal and mean standardized uptake value (SUV) in the most intense lesion, or, in the absence of attenuation corrected PET-scans by comparison with liver and mediastinal uptake.

Results
In 119 (70%) patients a biopsy was available. We found a sensitivity of PET of 89%, specificity of 89%, positive predictive value (PPV) of 91% and negative predictive value (NPV) of 87% for the detection of PTLD. In a subanalysis of the 125 scans performed for differential diagnosis PTLD versus other diseases, sensitivity, specificity, PPV and NPV were 90%, 89%, 85% and 93% respectively. FDG-uptake in PTLD was generally high with a median mean and maximal SUV of 9.0 [range 2.0-18.6] and 17.4 [range 2.6-26.4]. PTLD had often an atypical presentation on PET and differentiation with other (mainly inflammatory) diseases is not always evident.

Conclusions
From these data, we can conclude that PET is highly sensitive for the detection of lesions of PTLD, and that PET has an excellent ability to differentiate PTLD from other diseases.

O.05. Positive selection of CD8 T cells in vitro is not dependent on MHC or CD1 expression

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Introduction
Notch-signaling plays a crucial role in T cell development. This finding has lead to the establishment of an in vitro culture system which supports differentiation of murine or human hematopoietic stem cells to mature T cells. HSC from different sources are cultured on a bone marrow stromal cell line, transduced with the Notch ligand delta-like 1 (OP9-DL1) and obtain a mature phenotype. However, we have shown previously that mature T cells, generated in this system harbour characteristics of innate T cells. Moreover, induction of classical MHC molecules on OP9 stromal cells does not seem to increase in vitro maturation efficiency. These findings suggest that positive selection in an OP9-DL1 coculture system could be induced by interactions of the TCR with non-classical TCR-ligands, MHC-like molecules such as the CD1-isoforms.

Methods
HSC, isolated from postnatal thymus, were cultured on an OP9-DL1 stromal cell monolayer in the presence of cytokines (Flt-3L, SCF, IL-7). To explore the nature of the selecting TCR-ligand driving T cell maturation, these cultures were performed in the absence and presence of blocking antibodies against MHC classI, MHC classII, beta2-microglobulin and CD1a. The generation of T cell precursor populations and mature T cells was monitored and quantified.

Results and conclusion
A substantial proportion of CD27+CD1- mature T cells was generated in the cocultures performed. Blockade of classical MHC interactions by MHC class I, beta2-microglobulin or MHC class II did not show a significant change in maturation efficiency. In addition, no significant change in maturation was seen when blocking the non-classical ligand CD1a or other beta2-microglobulin-associated TCR-ligands. This suggests that both classical and non-classical MHC molecules are not involved in positive selection of the majority of T cells maturing in OP9-DL1 cocultures. Perhaps TCR-independent mechanisms could be inducing maturation in a fraction of developing T cell precursors in vitro.

O.06. Rapamycin prevents experimental scleroderma tous chronic graft-versus-host disease in mice

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Background
The most widely used mice model of chronic graft-versus-host disease (cGvHD) is an MHC-matched bone marrow transplantation model of sclerodermatous cGvHD. A limitation of that model is that mortality is relatively low, making difficult to study the impact of potentially therapeutic compounds.

Aims
To develop a more severe model of cGVHD and to assess the impact of rapamycin administration in that model.

Results
Lethally irradiated Balb/C mice were injected with 10x10^6 bone marrow cells and 70x10^6 splenocytes from B10.D2 donor mice. Twenty-one days later, all mice developed cGvHD. For the severe model, donor B10.D2 mice were injected with 0.5x10^6 splenocytes from Balb/C twenty-one days before transplantation. All mice from the severe model (n=8) died a median of 32 days while 3 of 7 mice in the classical model survived beyond day 52. Mean survival was decreased in the severe model compared to the classical model (p=0.0185). Recipient mice in the severe group experienced higher weight loss, hair loss and skin fibrosis. Numbers of T lymphocytes (p=0.0032) and CD4+ T cells (p=0.0018) per microliter of blood at day 21 were lower in the severe model. Moreover, number of regulatory T cells (Tregs) was decreased in the severe model (p=0.0151). We then investigated whether rapamycin administration could prevent GVHD in the severe model. All (n=8) mice treated with PBS (placebo) died a median of 32 days after transplantation, while 6 of 8 mice given 1mg/kg/day i.p. rapamycin survived beyond day 52 (p=0.0012). Number of Tregs/µl was higher at day 21 in rapamycin-treated mice than in PBS-treated mice (p=0.0796). Moreover, number of naïve CD4+T (p= 0.0089) and effector memory T cells (EMT) (p= 0.0125) were higher in rapamycin mice. Finally, proliferation of EMT (assessed by flow cytometry using Ki-67) was higher in PBS than in rapamycin mice (p=0.0474).

Conclusion
We have developed a mice model of severe cGVHD. Interestingly, rapamycin prevented death from cGVHD in that model, perhaps through in vivo expansion of Treg.

O.07. Retrospective analysis on the impact of iron chelation therapy on survival and leukemia progression in transfusion dependent MDS patients in Belgium

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UZ Leuven, Belgium

Background/methodology
While appropriate iron chelation can prolong survival in patients with thalassemia major, this remains highly debated in MDS. This study aims to investigate the potential effect of iron chelation treatment (ICT) on overall and leukemia free survival and to examine treatment modalities, transfusion needs and chelation practices in transfusion dependent MDS patients. Follow-up data was collected from 186 patients, previously identified in a Belgian cross-sectional study performed in 2008 (Delforge et al., 2011).

Results
Of the patients, 38% were still alive and 4% lost to follow-up. AML progression was reported for 18% of patients. At the time of diagnosis, 68% of patients were classified as low-intermediate1 IPSS score, 9% with intermediate2-high IPSS score, whereas no IPSS score was available for 23%. ICT was started on average 3.6 yrs after diagnosis and 1.4 years after the first RBC transfusion. At initiation of ICT, the mean serum ferritin was 2302±2607 µg/L. 40% never received ICT. Median survival for low-intermediate1 IPSS patients was 87 months. Patients within this group, who received ICT had a longer median survival than non-chelated patients (123 vs. 37 months; p<0.001). Moreover, the intensity of ICT was associated with outcome: patients having received more intense ICT had a longer survival than patients receiving Desferal bolus injections (126 vs. 52 months; p:0.001), whereas no significant survival difference was observed between Desferal bolus injections vs. no ICT (52 vs. 37 months; p:0.322). Similar differences were observed for AML-free survival. In Cox Proportional Hazard models the use and intensity of ICT appeared to be the most prominent factors impacting survival, followed by calculated "transfusion intensity".

Summary/conclusion
Although we cannot exclude a patient selection bias, this study confirms, in an independent fashion, the previous findings of the GFM (Rose et al., 2010) and of the Dusseldorf registry (Fox et al., 2010): patients who received ICT have a better outcome. Prospective randomised trials remain necessary to confirm the benefit of ICT.

O.08. Quantitative and qualitative analysis of metabolic response at interim FDG pet-scan is highly predictive of outcome in diffuse large B-cell lymphoma (DLBCL)

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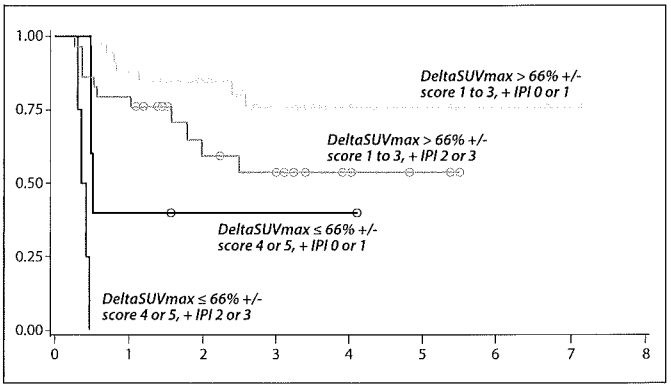
We investigated whether mid-treatment metabolic response had prognostic value in 74 DLBCL pts (median 60y; 47M/27F; IPI: low/low-int 50%, high-int/high 50%) treated upfront with anthracyclin-containing regimens. Qualitative analysis was done using Deauville's criteria, and quantitative analysis by comparing baseline and interim metabolic activity (ΔSUV(max)). Survivals are at two years. Deauville's score was 1 in 34%, 2 in 23%, 3 in 15%, 4 in 18%, and 5 in 10%. Median ΔSUV(max) was 85%. 18% had a ΔSUV(max) ≤ 66%, a highly discriminating threshold. Outcome in pts with Deauville's score of 1 to 3 ("negative") was better than in pts with score 4 or 5 ("positive") in EFS (79% vs 36%, P<.0001), PFS (83% vs 47%, P.0006), and OS (91% vs 58%, P.0003). Pts with a ΔSUV(max) > 66% or ≤ 66% also had different EFS (73% vs 41%, P.009), PFS (78% vs 50%, P.02), and OS (88% vs 56%, P.008). Pts with a positive qualitative pet and a ΔSUV(max) ≤ 66% had an OS of 20%. Pts (n=33) combining aaIPI 0 or 1, and negative pet by any criteria showed excellent outcome (EFS: 85%, PFS: 88%, OS: 94%).

Figure 1. EFS according to results of interim pet with aaIPI.



Deauville's and ΔSUV(max) response independently predicted for EFS (HR 4.3; HR 4.3), PFS (HR 3.2; HR 3.5), and OS (HR 3.6; HR 4.2, respectively). IPI did not retained independent prognostic value. Combination of a favourable IPI (0, 1) and a negative interim pet by any criteria yielded a negative predictive value (PV) of 96%, while positive PV remained poor (50%). In this retrospective study, analysis of metabolic response at mid-treatment, using adapted criteria, was

highly and independently predictive of any outcome (EFS, PFS, OS) in DLBCL. The high NPV of this analysis should foster prospective de-escalation strategies.

Abstracts posters red cell biology and transfusion P.01 – P.04

P.01. Infusion rate of Multigam in a day care setting

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Intravenous immunoglobulin's (IVIG) are routinely used in the hospital in various diseases. The dosage will be adapted to the clinical condition of the patient. Patients who never received IVIG before must be carefully watched for side effects during the first infusion. To avoid side effect an initial slow infusion rate is advised: first 15 minutes 0,55 ml/min. If no reactions, infusion rate can be increased to 2,75 ml/min. On the prescription notice the weight of the patient was not taken in account. For instance 2.75 ml/min means 4.12 ml/kg/hour in a patient with a weight of 40 kg and 2.75 ml/kg/hour in a patient of 60 kg. Our experience urge us to say that in patient who did not react during the first IVIG administration this precaution of initial infusion rate of 0,55ml/min as well 0,55 ml afterwards is to restrict if. To formalize our experience in the hospital we retrospectively analysed our infusion rate of Multigam In 100 consecutive patients. All patient did not expired side effects during previous IVIG infusions. The mean infusion rate was 4,4 ml/min, after a slow initial start on 2,2 ml/min. Taken the weight of the patient in account the infusion rates varied from 6,52 ml/Kg/hour in a patient with 40,5 Kg and 2,72 ml/Kg/hour in a patient of 97 Kg. No infusion related side effects (chills & fever & headache & low blood pressure) was noticed. On this data we could advise that the infusion rate of Multigam can safely be adapted to 4 mL/kg/hour.
This is not only a safe procedure but means also a substantial time saving for the patient, and resources saving for the hospital.

P.02. Metastatic breast cancer with Microangiopathic Hemolytic Anemia: a dreadful association

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Case report
A 52-year-old female presented to casualty department for severe anaemia and thrombocytopenia. She had an history of progressive metastatic breast cancer (invasive grade 2 ductal carcinoma, with strong hormone receptors and no over expression of c-Erb) , treated since six years with surgery, chemotherapy and radiotherapy. On admission she was still on hormone therapy (letrozole 2.5 mg a day) with bisphosphonate treatment for bone secondary localisations. Physical examination showed purpuric features with gingival hemorrhages and reduced intensity of breath sounds. There was no jaundice. Laboratory tests found a reduced hemoglobin (7.4 g/dl, normocytic normochromic) and platelet count (22.000/ml), showing hemolytic features with collapsed haptoglobin, elevated reticulocytes and presence of schizocytes. There were signs of diffuse intravascular coagulation, renal and liver failure. The direct Coombs test was negative. All the tumoral markers were raised. There was no Adamts 13 acquired deficiency. Cranial MRI showed numerous abnormalities, including multiple subcortical infarcts and bilateral subdural hematomas. The patient was denied for plasma exchange, and was treated with best supportive care including all symptoms management and steroid therapy, but nevertheless died rapidly of acute respiratory failure probably due to multiple pulmonary embolisms.

Discussion
Non immune hemolysis occurs in cancer from thrombotic microangiopathy that includes thrombotic thrombocytopenic purpura, hemolytic uremic syndrome and HELLP syndrome. This seems related to cancer cells circulating and accumulating in small blood vessels, causing intraluminal thrombi and development of pathologic endothelial cells. Mucin-producing adenocarcinomas seem to be more at risk of causing thrombotic microangiopathy, with mucin having a detrimental effect on endothelial cell functions. Cancer related thrombotic microangiopathy remains incurable for the majority of the patients whose conditions allow rarely treatment of their underlying cancer , still the most important way of stopping the pathophysiology of this disease.

P.03. An unusual cause of hemolysis in Paroxysmal nocturnal hemoglobinuria

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Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired, potentially life-threatening hematologic condition characterized by complement-induced intravascular hemolytic anemia, hemoglobinuria and thrombosis.
We report the case of a 57-year-old female presenting with a progressive increase of her red blood cells transfusion needs in the setting of a paroxysmal nocturnal hemoglobinuria (PNH) associated with medullary hypoplasia, treated by eculizumab for 15 months.
She complained of dyspnoea and cough. Routine blood tests were normal except for inflammatory syndrome , increasing anemia and stable hemolysis. Chest X-ray was normal. Bone marrow aspirate excluded a leukemic transformation.
CT scan of the lungs showed a large mass originating from the left atrium, expanding into the left ventricle, resulting in a dilatation of the left atrium and pulmonary veins, without evidence of pulmonary embolism. Echocardiography confirmed the presence of a mass appended from the interauricular septum, protruding into the left ventricle through the mitral valve, associated with an important pulmonary hypertension. Surgical resection was performed, confirmed a benign myxoma of the left atrium and resulted in raise of hemoglobin to previous values, and disappearance of inflammatory syndrome and transfusion needs.
This observation deserves our attention because increase of transfusion need in PHN may result from other causes than leukemic transformation, bone marrow failure, iron deficiency or antibodies to eculizumab.

P.04. A new disease: TEMPI Syndrome: telangiectasias, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting

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We report a patient with a novel syndrome that has been termed TEMPI: telangiectasias, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting. In the Case Records of the Massachusetts General Hospital (NEJM, July 29 th; 2010) the case of a man with unexplained erythrocytosis and perinephric fluid collections was published. We were following a strikingly similar case that shared five characteristics constituting the TEMPI syndrome. At the same time one similar patient was reported in Los Angeles. Our patient was a 36-year-old female when she was seen in the dermatology clinic (in 2005) for the development of telangiectasias. Laboratory analysis identified erythrocytosis with a hematocrit of 64% (hemoglobin 22g/dl) and work up identified an elevated erythropoietin of 50 mU/ml. Extensive imaging did not reveal a source of erythropoietin production and evaluations including hemoglobin electrophoresis, hemoglobin oxygen affinity, and VHL gene were negative. The erythropoietin level gradually rose to values greater than 5000 mU/ml and the telangiectasias increased and were most prominent on the

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

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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

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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

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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

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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

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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

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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

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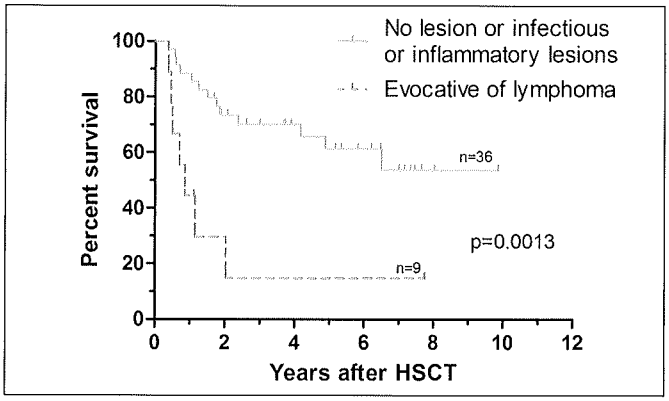
Background
PET scan is increasingly used in the follow-up of lymphoma patients given allogeneic hematopoietic cell transplantation (Allo-HCT). However, whereas several studies addressed the question of the impact of PET positivity after autologous transplantation on transplantation outcomes, very few 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.

Conclusion
A positive PET scan on day 100 post-transplant is predictive of poorer OS. However, there is a noteworthy incidence of false-positive PET scans after non-myeloablative allo-HCT. We therefore recommend that every suspicious lesion should be explored at least by CT scan and/or biopsy.

Figure 1. Overall survival according to 1st PET.



P.12. Is Hematopoietic Cord Blood Transplantation an option for patients with severe infantile osteopetrosis?

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Introduction
Autosomal recessive osteopetrosis (ARO) is a congenital disorder characterized by impairment of bone remodeling due to osteoclast dysfunction, resulting in impaired bone resorption with decrease of the bone marrow space. Clinical consequences include bone marrow failure and compression of cranial nerves leading to different neurological deficits. Though genetically heterogenous, over 50% of ARO patients have mutations in the TCIRG1 gene, encoding the osteoclast-specific H+-ATPase proton pump A3 subunit. So far the only available curative treatment is Allogeneic Hematopoietic Stem Cell Transplantation (allo-HCT). Here we describe the characteristics and outcome of 2 patients with ARO who received Cord Blood (CBU) as grafts.

	Patient 1	Patient 2
Histological Characteristics	Not done	Osteoclast poor
TCIRG1 Mutation	p.R669X/p.R669X	p.R669X/p.R669X
Age at allo-HSCT	8 months	8 months
Conditioning	Busulfan po 16 mg/kg Fludarabine 140 mg/m² Melphalan 140/m²	Busulfan iv according to weight Fludarabine 140 mg/m² Melphalan 140/m²
Graft vs. Host prophylaxis	ATG (Thymoglobulin®) 4x 2.5 mg/kg (during conditioning) Cyclosporine A up day -1 Prednisone 1 mg/kg/dag (day+1 to day+28)	ATG (Thymoglobulin®) 4x 2.5 mg/kg (during conditioning) Cyclosporine A up day -1 Prednisone 1 mg/kg/dag (day+1 to day+28)
CBU Characteristics	1st CBU Match 5/6 (B MM) CD34 cells infused 2.1 x 10⁵/kg (30% viability) 2nd CBU Match 5/6 (B MM) CD34 cells infused 5,63 x10⁵/kg	Match 6/6 CD34 cells infused 9 x 10⁵/kg
Engraftment of Neutrophils >500/µL (day +)	30	13
Engraftment of Thrombocytes >50 000/µL (day +)	66	53
Donor chimerism	Full donor (1st infused CBU)	Full donor
Clinical evolution	Good with bone remodeling.	Good with bone remodeling.
Complications	Ongoing mild/moderate immune hemolytic anemia, clinical very good with low dose steroids	no complications
Follow-up	4 years	1 year

Patients and methods
Two patients with confirmed TCIRG1 ARO received allo-HSCT with CBU as graft in our Center. Patients age, transplant and CBU characteristics are summarized in Table 1.

Patient 1 received 2 CBU, not as part of double CBU program but due to low viability (30%) of the 1st infused CBU.

Discussion
Patients with osteopetrosis are characterized by having a high risk of graft failure, which has been more notorious using CBU as graft. However, in patients with ARO a prompt allo-HSCT is needed to prevent irreversible neurological impairment. We performed unrelated CBU in two patients lacking a HLA- matched donor. The allo-HSCT was successful in both cases without major complications. This suggest that allo-HSCT with CBU as graft is feasible and should be evaluated in those patients without a good matched donor. The CBU must have a good cellularity and the conditioning regimen should be fully myeloablative, for which we suggest the use of Busulfan (full dose), Fludarabine and Melphalan.

P.13. Impact of the use of antithymocyte globulin (ATG) on cellular immune reconstitution after allogeneic Hematopoietic Stem Cell Transplantation

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Introduction
GvHD affect quality of life of patients after allo-HSCT. To prevent GvHD, ATG is used during the conditioning regimen. We performed an analysis of the lymphocytes subpopulations after allo-HSCT to evaluate the impact of the use of ATG on immune reconstitution.

Material and methods
The cellular immune reconstitution after allo-HSCT was studied in 32 pediatric patients, with a minimum survival of 3 months. Evaluations were performed at regular time points or until death or relapse. Patients with diagnosis of T-cell immune deficiency and patients transplanted after CD34 positive selection of the graft were excluded. ATG Thymoglobulin® in most of the cases at a dose of 10 mg/kg was used in 21 pts and 11 pts did not receive any ATG during the allo-HSCT.

Results
Three months after allo-HSCT, the median lymphocyte count is 597/µL. Within the lymphocytes, the population of CD4+ cells is the most severely affected with a median of 122/µl and only 12% of the patients achieving normal CD4 values for age. Patients who received ATG have even lower age-related CD4 levels than those who did not receive this kind of immune modulation of the graft (p=0.02). CD8+ cells are less affected, with normal CD8 counts and NK cells in 62% and 50% of the patients respectively. Nine months after allo-HSCT, there is an improvement in reconstitution of the T-helper cells and normal CD4 counts for age in 60%. One year post allo-HSCT 80% of all patients have normal T-helpers counts. T-cell suppressors are normal in 90% of the patients and all patients have normal NK cell levels.
Conclusion
It is well known that patients after allo-HSCT with full myeloablative conditioning have a severe immunological impairment. The use of ATG in the conditioning regimen retards the immune reconstitution of T- helpers during the first 3 months after allo-HSCT, putting this

patients at higher risk for viral infections. We do not find major differences for T-suppressors cells or NK cells.

P.14. Resolution of immunotactoid glomerulopathy after autologous stem cell transplantation

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An immunotactoid glomerulopathy is a form of non-amyloid fibrillar glomerular immune deposition disease. It can be distinguished from fibrillary glomerulopathy, which is characterized by deposits of randomly arranged fibrils with a diameter of 16-24nm. In immunotactoid glomerulopathy fibrils are typically larger (30-50nm) and organized as microtubules. However, separation of these pathologic entities is still debated. Immunotactoid and fibrillary glomerulopathy can be classified as monoclonal immunoglobulin deposition diseases (MIDD) like light (heavy) chain deposition disease, type 1 cryoglobulinemic glomerulonephritis and AL amyloidosis . The deposits are mostly composed of IgG. Most patients with immunotactoid glomerulopathy are older adults and present with proteinuria (often nefrotic range), hematuria and arterial hypertension. Half of patients have renal insufficiency at diagnosis.
Small case series published so far showed promising results of high dose melphalan followed by autologous stem cell transplantation in patients with MIDD.
Recently we showed a sixty year old man with a medical history of MGUS IgG Kappa presented with dysmorfic hematuria and proteinuria of 5.28 g/l on a routine visit. His GFR was 34 ml/min/1,73m2). A renal biopsy showed a nodular glomerulopathy with sub endothelial and mesangial amorphous eosinophilic deposits. These deposits were Periodic Acid Schiff (PAS) positive, non-argyrophilic and Congo red negative. Immunofluorescence microscopy was positive for IgG. Electron microscopic analysis showed structured deposits of fibrils with a diameter of 70-80 nm, some showed a microtubular organization with a recognizable hollow lumen. The diagnosis of an immunotactoid glomerulopathy was obvious.
In the presented case an analogous treatment with high dose melphalan (200mg/m^2) followed by autologous stem cell transplantation was used . Treatment was complicated by neutropenic fever caused by micrococcus sepsis, which was successfully treated with broad spectrum antibiotics. Treatment was well tolerated by the patient. He could be discharged after 19 days. His proteinuria decreased from 5.28g/l to 1.27g/l. Renal function recovered to a GFR of 70ml/min/1.73m^2.

P.15. Human primitive type blood cells are generated independent of Notch signalling in vitro

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Introduction

Hematopoietic cells are generated during two spatio-temporally separated waves of hematopoiesis. During the first wave, termed the primitive wave, enucleated red blood cells and myeloid cells are generated in the Yolk Sac. During the second wave, termed the definitive wave, hematopoietic progenitor cells (HPC) and hematopoietic stem cells (HSC) are generated in the aorta-gonado-mesonephros region. These then migrate to the fetal liver (FL) and finally populate the bone marrow (BM). Notch signaling is a key regulator during formation of the hematopoietic system. However, currently little is known about the generation and characteristics of early blood cells in the human system.

Materials and methods

We used the hESC stromal cell differentiation method previously described by Timmermans et al. 2009. In short, hESC are transfered onto stromal cell layers in the presence of serum and are allowed to differentiate during 12 days in the presence of the γ-secretase inhibitor DAPT, a well known inhibitor of Notch signalling.

Results

Using the above described culture system, we were able generate hematopoietic progenitor cells, phenotypically characterised by the CD34/CD43 expression profile (Vodyanik et al. 2006). By PCR analysis we show occurrence of both waves of hematopoiesis. Definitive multipotent HPC show erythroid, myeloid and lymphoid differentiation potential, while primitive blood precursor cells show a more restricted potential. To gain more insight on factors influencing formation of these first primitive blood cells, we tested dependence on Notch signaling. As shown here, primitive blood cells can be generated in the absence of Notch stimulation.

Conclusion

Here we describe a culture system to generate blood cells, of both primitive and definitive type. In this system primitive hematopoietic cells can be generated in the absence of Notch signalling.

P.16. Expression of the hemogenic marker Runx1 in in vitro generated human hematopoietic progenitor cells

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Introduction

During postnatal life, all blood cells are generated from hematopoietic stem cells (HSC) in the bone marrow (BM). In contrast, in the embryo, hematopoietic cells are generated in two waves of hematopoiesis. A first wave of primitive hematopoiesis in the yolk sac, characterized by nucleated red blood cells and macrophages, and a second wave of definitive hematopoiesis resulting in the generation of myeloid, lymphoid and enucleated red cells. HSC are as well generated during this definitive wave, when hemogenic endothelium in the aorta-gonado-mesonephros (AGM) region rounds up and starts budding from the endothelial wall of the dorsal aorta. Next, HSC migrate to the fetal liver where they expand and populate the BM hematopoietic niches after birth.
Although the knowledge about early hematopoiesis in mouse and zebrafish has vastly expanded recently , the exact mechanisms of the earliest human hematopoiesis remain largely unknown. Human embryonic stem cells (hESC) are a convenient model to study these earliest developmental stages.

Materials and methods

We induced in vitro hematopoiesis in hESC using an OP9 coculture system based on the protocol from Timmermans et al (2009). Upon day 12 of coculture hematopoietic zones (HZ) become microscopically visible. These HZ were removed and different cell populations were sorted using flow cytometry based on CD34, CD43 and CD45 expression. We performed rt-qPCR expression analyses on these subpopulations for several hematopoietic key genes.

Results

The HZ generated in the coculture system clearly showed CD34+ endothelial cells surrounding CD43+ hematopoietic cells and CD34+CD43+ hematopoietic precursor cells (HPC) and/or HSC.
We detected expression of the transcription factor Runx1, a master regulator in HPC/HSC function, in both CD34+CD43+CD45- and CD34+CD43+CD45+ populations of HPC and, as expected, expression decreased in the more differentiated CD34-CD43+ population.

Conclusion

Our findings are in agreement with the data obtained from different animal model systems and show in vitro Runx1 expression. Moreover, these preliminary experiments confirm that in vitro hematopoietic differentiation of hESC represents a valuable tool to study human hematopoiesis.

P.17. Retroperitoneal Fibrosis as a late complication of allogeneic stem cell transplantation for Chronic Myelogenous Leukemia

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Chronic graft-versus-host disease (GVHD) represents a major cause of late onset disease unrelated morbidity and mortality in the setting of allogeneic stem cell transplantation (ASCT). Here, we present an unusual case of retroperitoneal fibrosis following ASCT presumably caused by chronic GVHD. In 2004, a 53-year-old male with Philadelphia+ chronic myelogenous leukemia in chronic phase underwent a matched related ASCT after conditioning with total body irradiation and cyclophosphamide. His immediate post transplant course was uncomplicated. Over time, he developed a chronic GVHD with cutaneous, mucosal, hepatic and pulmonary involvement that required prolonged corticosteroid and ciclosporin A administration.

In January 2011, he complained of right sided back pain, with neither dysuria nor hematuria. Physical examination was normal except for extensive scleroderma. Blood and urine tests were irrelevant except for a creatinin level of 2.7 mg/dl. Abdominal CT-scan revealed bilateral hydronephrosis secondary to retroperitoneal fibrosis. Ureteral stents were placed for a 6 months period. No biopsy was performed. As GVHD was suspected to be the cause of this fibrosis, immunosuppressive treatment was switched to MMF and corticosteroids, and resulted in a rapid improvement of renal function and scleroderma.

This case deserves our attention since retroperitoneal fibrosis has been invariably reported as part of a broader inflammatory condition. Chronic GVHD may affect several tissues, particularly the skin and liver, but few cases of GVHD leading to retroperitoneal fibrosis have been reported. The dramatic response to immunosuppression supports the hypothesis that chronic GVHD is the most likely cause of retroperitoneal fibrosis in the present case.

Abstracts posters lymphoproliferative disorders and MM P.18 – P.31

P.18. Evidences of senescence in Multiple Myeloma Bone Marrow Mesenchymal Stromal Cells (MM BM-MSC)

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The Multiple Myeloma (MM) is a malignant disorder of post-germinal center B-cell characterized by a monoclonal expansion of secreting plasma cells (PC) in the bone marrow (BM) compartment. It is now well established that the BM constitutes a microenvironment required for differentiation, maintenance, expansion, and development of drug resistance of the MM cell clone. Indeed, Bone Marrow Mesenchymal Stromal Cells (BM-MSC) strongly support MM cell growth, notably, by producing a high level of Interleukin-6 (IL-6), a major MM cell growth factor. Previous studies suggested that direct and indirect interactions between MM-PC and BM-MSC resulted in constitutive abnormalities in BM-MSC. Our study further analysed these abnormalities by demonstrating that MM BM-MSC presented a senescent profile with expression of senescence-associated β-galactosidase, reduced proliferation capacities and characteristic expression of members of the senescence associated secretion profile (SASP). The senescent state of supportive cells has already been linked to cancer promotion in skin, breast, prostate and pancreas. We also observed alterations in the osteoblastogenesis, hematopoietic support and immunomodulation activities of MM BM-MSC compared to Healthy Donors BM-MSC.

P.19. Integration of phenotypic and genetic markers in the diagnosis and monitoring of multiple myeloma

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Multiple Myeloma is a plasma cell malignancy that results in physiological disorders with a variable evolution. The choice of biomarkers that improved care of patients represents a major challenge for the future. Two categories of markers will be used: the genetic and phenotypic markers identified respectively by FISH and cytometry analysis.

We have compared 4 selection kits allowing the enrichment of plasma cells in order to increase the FISH analysis sensitivity: the negative selection kit RosetteSep™ Human Multiple Cell Enrichment Cocktail from Stem Cell Technologies, the EasySep™ Human Whole Blood CD138 Selection Kit (Stem Cell Technologies), the EasySep™ Human CD138 Positive Selection Kit (Stem Cell Technologies) and the MACS CD138 Human MicroBeads separation (Miltenyi Biotec).

	Number of samples	Enrichment rate	Average of purity (%)
RosetteSepTM Human Multiple Cell Enrichment Cocktail	9	2.2	43.5
EasySepTM Human Whole Blood CD138 Selection Kit	5	5.8	27.6
EasySepTM Human CD138 Positive Selection Kit	12	4.01	22.8
MACS CD138 Human MicroBeads	20	3.99	65

The MACS separation kit allows the highest degree of purity and requires fewer manipulations and so greater plasma cell viability.

We compared the classical FISH results (50 patients) with those of the post enrichment MACS method (40 patients) to prove the usefulness of plasma cell selections. Cytogenetic abnormalities with poor prognosis were observed: t(4,14), del(13q14) and del(17p13). With classical FISH, the genetics informations were detected in only 11% of patients in contrast with the post enrichment MACS method which identify aberration in 60% of cases.

We then estimated and compared the cytogenetics aberrations occurrence frequencies on a panel of 41 multiple myeloma reported patients. t(4,14) were observed in 40% of cases in comparison with 15% with del(13q14) and 7.5% with del(17p13). The antigenic profile of tumoral plasma cells was analysed by flow cytometry with a multiparametric staining method CD38/CD138/CD45, CD38/CD45/CD19/CD56, CD38/CD138/CD28 and CD38/CD138/CD20/CD117. The distribution of phenotypic abnormalities is shown below:

	CD19-	CD56+	CD28+	CD20+	CD117+
Total of patients (41)	92.5 %	75 %	40 %	42.5 %	62.5 %
Patients with t(4,14) (16)	100 %	87.5 %	31.2 %	43.7 %	62.5 %
Patients without cytogenetic abnormality (20)	80 %	65 %	45 %	45 %	55 %

The lack of expression of CD19 is mainly observed, abnormalities of CD56 and CD117 are also common. In this limited series of patients, we can conclude that phenotypic aberrations seem to be independent of the cytogenetic status of the plasma cells analyzed. The antigen expression profile is similar with or without presence of a translocation. In the future, we will make CGH-arrays on selected tumoral plasma cells with the aim of screening the entire genome and not only analyzed targeted abnormalities. The cytogenetic and phenotypic datas will be compared and confronted with the different stages of the disease.

P.20. Targeting osteoblastogenesis of mesenchymal stem cells by novel multiple myeloma drugs (HDAC inhibitor- Vorinostat/ Proteasome inhibitor-Bortezomib)

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Introduction
Recent reports revealed an important role of histone deacetylase inhibitors (HDACi) in bone turnover by stimulating the osteogenic differentiation of mesenchymal stem cells (MSC). The purpose of this study is to examine the potential of suberoylanilide hydroxamic acid (SAHA), the first epigenetic agent approved for the treatment of cutaneous T-cell lymphoma and currently used in clinical phase I/II trails for MM patients, to induce osteoblast formation in vitro and in vivo. Moreover, the combination with Bortezomib (Bzb), a drug for which it has been proven that it promotes MSC osteogenesis, will be tested.

Methods
MSC were obtained from bone marrow samples of MM patients and control subjects. The effect of SAHA on osteoblasts was investigated by alkaline phosphatase (ALP) staining, Alizarin Red S staining and PCR. Epigenetic modulations were detected by western blot for acetyl-histone 3 (ac-H3) and p21. For the in vivo study, osteocalcin levels were measured by ELISA and colony forming unit (CFU) assay was carried out to detect MSC activity.

Results
In vitro studies showed SAHA increased, in MSC from both MM patients and normal donors, the activity of ALP, which is an early marker of osteoblast differentiation. This osteogenesis-promoting effect was confirmed by PCR analysis for osteogenic markers and matrix mineralization. Importantly, we observed SAHA upregulates Runx2, a key transcription factor of osteoblast formation, and increased ac-H3 and p21 expression of MSC. Synergistic effects on osteoblastogenesis were found by combining SAHA and Bzb in vitro. Additionally, in vivo experiments in naive mice showed higher levels of ALP-positive CFU and serum osteocalcin levels after treatment with Vorinostat 100mg/kg (3x/week for 21days). Moreover, the highest levels were measured by combining Vorinostat 60mg/kg with Bzb 0.3mg/kg (2x/week for 21days).

Conclusion
In conclusion, our data indicate that SAHA, especially in combination with Bzb, stimulates osteoblast formation in vitro. Further studies in naive and 5T33MM mice will be proceeded to confirm the osteogenesis-promoting potential in vivo.

P.21. Sequential genomic analysis of untreated patients with Chronic Lymphocytic Leukemia demonstrates Clonal Evolution

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Introduction
Changes in clinicobiological behavior of chronic lymphocytic leukemia (CLL) can be related to genetic aberrations either present at diagnosis or acquired during disease course [i.e. clonal evolution (CE)]. This study aimed to characterize CE.

Methods
Paired sequential samples of 53 patients with CLL were investigated by karyotyping, FISH and Affymetrix cytogenetics whole-genome 2.7M arrays. Samples were obtained with an interval of at least one year and patients were never treated at both times of sampling.

Results
The median interval between sampling was 41 months. Treatment was initiated in 33 patients one month after time point 2. IGHV was unmutated in 15 and mutated in 32 patients. Karyotyping revealed CE in 17/53 cases (32%): acquired aberrations included unbalanced translocations (n=7), del(13q)(n=4), del(11q)(n=4), del(17p)(n=2), del(6q)(n=2) and balanced translocations (n=2) and various other aberrations (n=5).
In contrast, FISH using the CLL 4-probe panel revealed CE in only 11 cases (21%). All of them showed del(13q) either as a new or additional subclone. The del(13q) was accompanied by a new del(11q) and del(17p), in one case each.
2.7M arrays detected losses in the regions 13q14, 11q22-23 and 17p13 (at time 1 n=25/7/0, time 2 n=32/8/1 patients, respectively). CE was observed in 12 cases (23%), four of whom did not need therapy. Ten/12 cases showed del(13q) either as a new or additional subclone. The del(13q) was accompanied by a new del(11q) and del(17p), in one case each. Loss chromosome Y was observed in one case and gain of 2p and MYC on 8q24 was observed at time 2 in a last case with CE. Of note, due to the selection criteria, i.e. no treatment-history at time of sampling, therapy-related aberrations are excluded. On the other hand, there may have been a selection bias for less aggressive disease, leading to an underestimated prevalence of aberrations and CE.

Conclusion
This study confirms the occurrence of CE in untreated CLL, but the genome of CLL appears to be quite stable over time.

P.22. A rare TTR mutation in a patient with familial amyloidotic polyneuropathy (FAP)

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We report a case of a rare TTR mutation in a patient with familial amyloidotic polyneuropathy (FAP). Case presentation:A 53-y old male presented in spring 2009 with a complex symptomatology of back pain, weakness and myalgia after heavy exercise. Work-up revealed a discal hernia with myelopathy as well as a slight axonal sensory polyneuropathy of unknown origin.The patient father had died with coronary heart disease at the age of 48, and suffered from a neurologic disorder, first thought to be Guillain-Barré. The medical chart, dating from the 80's, was unavailable.The patient underwent neurosurgery, unfortunately, the suggested synchrone nerve biopsy was not realized at that moment.Extensive blood analyses were normal, including light chain assay and immunofixation. Serology of borrelosis was positive, also with western blot and associated with proteinorachia.Post-operatively, the patient noted prolonged sustained fatigue as well aspolyneuropathic symptom worsening. Worsening of the known axonal sensory deficit with axonal motor involvement and proximal extension, in the four limbs, was confirmed by electroneuromyography.A trial of Ceftriaxone brought no benefit, and a trial of polyclonal immunoglobulininfusions was likewise unsuccessful. Finally, sural nerve biopsy was performed in December 2010, revealing amyloid deposits with moderate demyelination; there was neurogenic muscle degeneration. Research of the most common mutation of the TTR gene (ValMet30) was negative. DNA sequencing revealed the rare p.D59V mutation of the TTR gene. The patient finally retrieved medical chart of his father, showing that he also probably suffered from TTR amyloidosis. Conclusion: This is the first report of a p.D59V mutation of the TTR gene in a Belgian patient. To our knowledge, one similar case has been documented in Germany. The patient underwent successful liver transplant in September 2011.

P.23. Protective interactions between chronic lymphocytic leukemia cells and stromal microenvironment investigated by microarray analysis: potential role of interleukin-6

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Background
Chronic Lymphocytic Leukemia (CLL) cells rapidly dye by apoptosis when cultured alone but we previously showed that direct interactions between CLL cells and stromal microenvironment protect them from spontaneous and drug-induced apoptosis. However the whole molecular mechanisms underlying this protection are still poorly understood.

Methods
We investigated the interactions of CLL cells with a mesenchymal stromal cell (MSC)-based microenvironment using Affymetrix technology. Briefly, CLL cells from 4 different patients were cultured overnight alone or on a MSC layer. CD19+ cells were then isolated, RNA was extracted, labelled and hybridized on array covering the entire human transcriptome.

Results
42% of probe sets were differentially expressed between cells cultured alone or with MSC (FDR<10%) indicating that this contact induces dramatical changes in CLL cells. Gene set enrichment analysis highlighted that important signalling pathways were activated: interferon pathway (P<0.001), B cell receptor (P=0.0012), anti-apoptosis (P=0.01223), STAT-JAK pathway (P=0.0104). Genes with the higher fold change being interferon-induced genes, we investigated interferon production by MSCs: our MSC microarray data bring out that MSCs express a high level of interferon beta 2, well known as interleukin 6 (IL6). In addition, IL6 pathways and STAT-JAK signalling genes are overrepresented in the differentially expressed genes. We thus investigated the role of IL6 on CLL apoptosis after 48h: IL6 (100ng/ml) decreases spontaneous apoptosis by 8% (range -2 to 26%, n=23, P<0.0001). Furthermore, Elisa quantification in conditioned medium (CM) showed a 3.6-fold increase of IL6 in CLL/MSC co-culture supernatant compared to MSC-CM (n=7, P=0.0156). Interestingly, BCR stimulation of CLL cells also induced an increase of IL6 production by CLL cells indicating that both cellular types are able to produce this interleukin.

Conclusions
Altogether, our data support the major role of IL6 in the survival stimulus conferred by the microenvironment to CLL cells. These preliminary data suggest that the microenvironment could produce IL6, induce the release of IL6 by CLL cells creating thus an activation loop of JAK-STAT pathway and an increased CLL cell survival.

P.24. Upregulation of miR-135b is involved in the impaired osteogenic differentiation of mesenchymal stem cells derived from multiple myeloma patients

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Previous studies have demonstrated that mesenchymal stem cells (MSCs) from Multiple Myeloma (MM) patients (MM-hMSCs) display abnormalities, including a distinctive gene expression profile, an enhanced production of cytokines and impaired osteogenic differentiation ability compared to normal donors (ND-hMSCs), however, the mechanisms remain unclear. MicroRNAs (miRNAs) are endogenous non-coding RNA molecules, which are involved in many biological processes and their aberrant expression leads to cancer formation and progression, Importantly, miRNAs play a crucial role in regulating stem cells fate and are also involved in the differentiation process of MSCs. In the present study, we observed an abnormal upregulation of miR-135b in MM-hMSCs, which meanwhile showed an impaired osteogenic differentiation potential. The gain and loss of function studies confirmed that miR-135b negatively regulated hMSCs osteogenesis. We also found that MM cell- produced factors could stimulate ND-hMSCs to upregulate the expression of miR-135b. Importantly, treatment with the miR-135b inhibitor promoted osteogenic differentiation in MM-hMSCs. Collectively, our data illustrated, for the first time, that hMSCs from a malignant MM microenvironment have a differential miRNA profile compared to their normal counterpart, which is associated with their aberrant gene expression and differentiation potential.

P.25. Improved risk-stratification and outcome prediction in children with average risk precursor-B acute lymphoblastic leukemia using a nineteen -microRNA signature

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Risk stratification has led to a tremendous improvement of the 5-year overall survival rates in childhood acute lymphoblastic leukemia (ALL). The average risk group (AR) where nor favorable nor unfavorable factors are found is the largest patient group, accounting for more than 85% of patients. Despite the good overall survival rate the total number of relapses observed in this AR group is considerable. Genome-wide microRNA (miRNA) profiling on diagnostic bone marrow samples of patients who experienced relapse and patients in continuous complete remission (CCR) (follow-up>6 years) allowed us to identify a 19-microRNA prognostic signature, predictive for relapse within this group. The signature holds an accuracy, sensitivity and specificity of respectively 77 %, 69 % and 84 %. Currently, the signature is evaluated in an independent validation cohort. Notably, many of the miRNAs present in this signature are known oncogenes or tumor suppressor genes. Absence of any other prognostic parameter within this patient group makes the identified signature a unique and powerful tool for further risk-stratification. The method and signature are suitable for laboratory routine testing, and, will be further evaluated in a prospective study

P.26. Impact of induction chemotherapy regimen on response, safety and outcome in the PRIMA study

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Background
The intergroup PRIMA study demonstrated a significant increase of progression free survival (PFS) in follicular lymphoma patients (pts) receiving rituximab maintenance for 2-years after first line immunochemotherapy (Salles et al., Lancet 2011). We examined the impact of induction chemotherapy on efficacy and safety.

Methods
Induction -chosen by each center- consisted in either R-CHOP (885 pts), R-CVP (272 pts) or R-FCM (45 pts). Pre-induction characteristics were well balanced between the different induction regimen. 1018 eligible pts responding to induction therapy were randomized (stratified by regimen and response to induction) to observation or R-maintenance, 375 mg/m2 i.v. every 8 weeks for 2 years.

Results
At the end of induction therapy, overall response rates (ORR) and complete response (CR) or unconfirmed CR for R-CHOP, R-CVP and R-FCM pts were respectively 92.8/67.2 ; 84,7/53 and 75/61.4 (missing 4.2; 9.7 and 20.5). Serious adverse events occurred in respectively 23%, 22% and 17% of pts, with infections in 6%, 7% and 9% and febrile neutropenia in 2%, 0% and 11%. 3-years PFS for pts randomized in the rituximab maintenance arm or no further treatment, after R-CHOP (n=768), R-CVP (n=222) and R-FCM(n=28) were 78.6 vs. 59.6 (HR 0.51 [0.39-0.65]), 61.6 vs. 50 (HR 0.68 [0.45-1.02] and 78.6 vs. 64.3 (0.54 [0.13-2.24]) respectively. In a Cox regression multivariate analysis adjusted by prognostic factors, a longer PFS was significantly associated with randomization to the rituximab maintenance group (HR 0.55, 0.44-0.68; p<0.0001), an age of 60 years or older (0.68, 0.54-0.86; p=0.0013), female sex (0.76, 0.62-0.94; p=0.013), lower FLIPI score categories (overall p<0.0001), and R-CHOP or R-FCM as induction treatment (0.39, 0.17-0.89; p=0<0029). Overall survival in the rituximab maintenance and observation groups were not significantly different for the 3 induction regimens: 95.6/95.2 (R-CHOP), 93.7/89.9 (R-CVP) and 74.5/100 (R-FCM)

Conclusions
Preliminary results indicate that the R-CHOP had a comparable safety profile compared to R-CVP but was associated with a higher response rate, a better PFS and a more substantial benefit of rituximab maintenance.

P.27. Intravascular large B-cell lymphoma: 4 case reports from a single center

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Introduction
Intravascular lymphoma (IVLBCL) is defined as a rare subtype of extranodal diffuse large B-cell lymphoma (DLBCL) characterized by the selective growth of lymphoma cells within the lumina of vessels, particularly capillaries, with exception of larger arteries and veins. The heterogeneity of clinical presentation hampers timely and accurate diagnosis. This is partly reflected in the fact that an IVLBCL usually presents itself as a widely disseminated lymphoma with an aggressive and generally rapid progressive clinical course. Although literature reported a very low incidence (probably less than 1:1.000.000), we diagnosed four cases in a single center over a period of 10 years.

Case-reports
Our patients presented mainly with B-symptoms, hepatosplenomegaly and only one patient showed lymphadenopathy. Most relevant laboratory findings were anemia, thrombocytopenia and elevated serum LDH levels. Cytomorphologic evaluation of the bone marrow aspirate revealed in all cases the presence of atypical large sized lymphoid cells. Additional histopathologic and immunohistochemic evaluation of the different bone marrow biopsy specimens showed an intravascular lymphoid infiltrate with a B-cell phenotype. Heteroduplex PCR analysis showed clonal IgH and IgK rearrangements. In all cases the IVLBCL was limited to the bone marrow, no central nervous system or skin involvement was observed. However in one patient the neoplastic cells were also found in a nodule from the left jaw. To date, two patients are still alive (after R-CHOP/ACVBP chemotherapy with and without autologous stem cell transplantation), while the two other patients died from disease progression.

Conclusion
These case reports illustrate that the presence of atypical large lymphoid cells on a bone marrow smear may be the first clue pointing to a diagnosis of intravascular B-cell lymphoma . The recognition of these cells may lead to a more rapid diagnosis of a lymphoma entity that is frequently missed by clinicians. The final diagnosis is based on pathology, immunohistochemistry and molecular biology.

P.28. The synergistic anti-myeloma effect of the BH3 mimetic ABT-737 and the IGF-1 receptor inhibitor picropodophylin (PPP)

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Multiple myeloma (MM) cells are strongly dependent on the bone marrow (BM) micro-environment were growth factors are secreted. IGF-1 is one of the most important growth factors in MM and thus forms an attractive target for anti-cancer therapy. Picropodophylin (PPP), an IGF-1R kinase inhibitor, has shown anti-MM effects both in human and murine cells. However, mice eventually relapsed and showed signs of morbidity. Therefore it would be an attractive approach to combine PPP with other cytotoxic drugs. The Bcl-2 family consists of pro-and anti-apoptotic proteins, the balance between which determines whether or not a cell will undergo apoptosis. In healthy cells, the anti-apoptotic proteins prevent apoptosis by binding and sequestering pro-apoptotic proteins. In MM, elevated expression of the Bcl-2 anti-apoptotic proteins has been demonstrated to cause resistance to drug-induced apoptosis. With the use of a BH3 mimetic, the cell survival/ apoptosis balance could be shifted towards apoptosis induction. ABT-737, a BH3 mimetic, has been shown to induce apoptosis in MM cells. However, Trudel et al. have demonstrated a protective effect of growth factors and BM stromal cells on the ABT-737-induced MM cell apoptosis. This data suggests a high likelihood of synergy between BH3 mimetics and IGF-1R inhibitors. Here, we investigated the combination of ABT-737 and PPP in two human MM cell lines and in the 5T33MM model. Both PPP and ABT-737 alone were found to decrease cell viability and proliferation and induce apoptosis dose and time dependently. Moreover, the combination of both agents synergistically decreased cell viability and proliferation and induced apoptosis compared to single-agent treatment. Western blot analyses revealed that combination treatment results in enhanced cleavage of caspases and reduced expression of anti-apoptotic proteins. In conclusion, PPP combined with ABT-737 appears to have synergistic anti-MM activity and may thus promise a new treatment for MM. In future investigations we hope to uncover the underlying mechanisms of the synergistic effects and evaluate the effect on MM tumor development and overall survival in the 5TMM model.

P.29. Diffuse large B-cell lymphoma presenting with numb chin syndrome: case report

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Numb chin syndrome (NCS) is a sensory neuropathy of the inferior alveolar nerve, a branch of the trigeminal nerve. It is characterized by facial and oral numbness restricted to the distribution of the mental nerve. In adults, NCS may be associated with cancers of breast, lung and prostate. It was also described in non-Hodgkin lymphoma and Burkitt lymphoma. We report this syndrome as the initial symptom of a follicular B-cell lymphoma. A 66-year-old woman presented with numb chin syndrome. Physical

examination revealed a painless hypoesthesia of the lower lip and chin, without other neurological symptoms. There was no lymphadenopathy or hepatosplenomegaly. Cerebral MRI was normal. FDG-Pet-scan showed a diffuse infiltration of axial skeletal system. Blood analysis showed: white blood cells 2190/mm³ with 800 neutrophils and 800 lymphocytes, LDH 4066UI/l(normal range: 240-480), b2-microglobulin 2.23mg/L (nr: 0.7-1.9). Lombar puncture was normal. Medullar aspiration was normal. Medullar biopsy schowed a myelofibrosis with at immunophenotuping analysis an infiltration by monoclonal lymphocytes CD20+, CD10+ and bcl-6+. Molecular biology showed a translocation 14;18. Karyotype was normal. The patient was treated by chemotherapy (R-CHOP) with intrathecal chemotherapy with prednisone, methotrexate and cytosine arabinoside. A complete remission was obtained. NCS results from compression of the mental nerve or the inferior alveolar nerve by jaw metastases and intracranial involvement of the mandibular nerve by lesions at the base of the skull. Paraneoplasic NCS is rarely described resulting from antibody production against Gasserian ganglion. The association of NCS with lymphoma is rare. NCS resulting from a B-cell lymphoma should be interpreted as cranial nerve involvement and consequently treated with intrathecal chemotherapy.

P.30. Cardiac failure in a patient with systemic senile amyloidosis and triclonal gammopathy: A diagnostic challenge

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Amyloidosis is a multisystem disease characterized by extracellular deposition of misfolded fibrillar proteins. Involvement of the heart is the primary determinant of prognosis. Cardiac amyloidosis causes a restrictive cardiomyopathy and is seen in association with deposition of light chain amyloid (AL) or transthyretin (TTR). We report a case of a 74-year-old man who presents with symptoms of heart failure since two months. Echocardiogram shows a marked biventricular hypertrophy causing moderate diastolic heart failure. The diffuse subendocardial contrast enhancement on cardiac magnetic resonance scan suggests cardiac amyloidosis. Serum protein electrophoresis reveals no abnormal bands but serum and urine immunofixation shows a triclonal gammopathy consisting of IgG λ , IgG κ and IgA λ. Serum-free light chains reveal a discrete elevation of free kappa and lambda chains, though with normal κ/λ ratio. AL-amyloidosis is suspected. A positive Congo red staining on endomyocardial biopsy confirms the diagnosis of amyloidosis. However, immunohistochemical staining for λ and κ light chains is not conclusive. By contrast, staining for transthyretin is strongly positive. We establish the diagnosis of Systemic Senile Amyloidosis (SSA), which is caused by deposition of the wild-type TTR, in conjunction with a Monoclonal Gammopathy of Undetermined Significance (MGUS). We note a good response with heart failure treatment and there is an exclusive cardiac involvement. Genetic studies to differentiate between heriditary or acquired forms of TTR were not performed because of the absence of a family history and no implications on treatment. This case underscores the importance of histological confirmation of the amyloid protein, since mistaken assumption of AL amyloidosis can result in inappropriate cytotoxic therapy. Given the common occurrence of MGUS, the clinician needs to be aware of non-plasma cell-related types of amyloidosis in patients with isolated amyloid cardiomyopathy and a coexistent low-grade monoclonal gammopathy. Accurate identification of the precursor protein is of paramount importance because treatment and prognosis differ considerably between AL-and TTR-amyloidosis.

P.31. BCR stimulation in CLL, irrespective of mutation status, increases expression of miR-132 and miR-212

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Chronic lymphocytic leukemia (CLL) is a disease with a variable prognosis. The immunoglobulin heavy chain mutation status is a well known prognostic factor and is linked to the B cell receptor (BCR) complex. Several observations support a role for triggering of the BCR in vivo in the pathogenesis of the disease. We evaluated the microRNA (miRNA) expression profile of peripheral blood CLL cells upon BCR stimulation in vitro and compared mutated with unmutated cases and explored their predicted targets. Peripheral blood mononuclear cells cultured in vitro, were BCR stimulated with anti-IgM reagent (Irvine Scientific, Santa Ana, CA, USA) for 3 or 24 hours. Anti-IgA served as a negative control. Genome wide miRNA expression was measured using Megaplex RT stem-loop primer pools (Applied Biosystems), enabling miRNA specific cDNA synthesis for 636 different human miRNAs. Simultaneous, a Human Illumina Gene Expression beadChip was performed to investigate the differences in mRNA profile. Differentially expressed miRNAs and mRNAs were identified using the Rank Products algorithm. In our genome-wide transcriptome analysis after BCR stimulation, we observed clear kinetic modulation of many genes, suggesting a regulated expression. Two of the most significantly upregulated miRNAs (miR-132 and miR-212) belong to the same cluster, and show considerable overlap in predicted target genes. Some of these are EP400 and ZBTB5. Interestingly, both were downregulated after in vitro-stimulation. These two genes repress CyclinD1 expression, EP400 also represses CyclinD2 expression and both are predicted to target the retinoblastoma tumor suppressor, RB1. We conclude that our results point to a transcriptional response promoting cell cycle in in vitro BCR triggered CLL cells. However, BCR triggering in vitro is not sufficient to induce proliferation of isolated peripheral blood CLL cells. Most likely, additional signals that are present in a suitable micro-environment in vivo, such as the lymph node or bone marrow, are missing in in vitro culture systems. The identification of these additional stimuli will be interesting to discover new therapeutic options in this at present incurable disease.

Abstracts posters myeloproliferative disorders P.32 – P.37

P.32. Clofarabine in bad prognosis Acute Myeloid Leukemia

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Clofarabine, a second generation purine nucleoside analog, has shown a considerable activity and an acceptable safety profile in patients with acute leukemia.
We review the cases of 12 patients treated between 01.07.2008 - 30.07.2011 12 with Clofarabine for relapsed/refractory or bad prognosis AML. Median age was 58.9 years (range 29 to 79 years), male:female ratio was 50:50, and the majority of patients had an ECOG status below 2 at btreatment initiation.
4 patients had secondary AML 4 patients had AML linked to myelodysplastic syndrome, 75% had complex cytogenetics, two out of four patients displaying normal caryotype were FLT3ITD+. 9 patients had refractory disease 2 patients had relapsing AML, one patient had de novo secondary AML and had been recently treated with high dose ARA-C and was thus not deemed eligible for conventional induction. Most patients were heavily pretreated.
Clofarabine was used as single agent in 9 patients or in combination with cytarabine in 3 patients or other drugs (platinum salts) in one patient. 30 days mortality was 23% and median overall survival was slightly less than 2 months. Our experience with Clofarabine in relapsed refractory AML shows a relatively low overall survival. This

may be due to the patient's characteristics (old age, heavily pretreated, multiple previous lines of treatment adverse cytogenetics).

P.33. Anti-tumor and anti-angiogenic effects of mithramycin in the 5TGM1 model of multiple myeloma

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Mithramycin (MTM) is a cytotoxic compound that is currently being investigated for its anti-angiogenic activity that seems to be mediated through an inhibition of transcription factor SP-1. In the current study we evaluated its anti-myeloma effects in one syngeneic murine model of multiple myeloma: the 5TGM1 model. In vitro, MTM inhibited DNA synthesis of 5TGM.1 cells with an IC50 of 400 nM. MTM induced an arrest in cell cycle progression at the G1/S transition point. Western-Blotting showed an upregulation of p53 and inhibition of c-myc, while SP-1 was unaffected. Downstream of p53 and c-myc, p21 and p27 were upregulated, while cyclin B, cyclin E, CDK2, CDK4, CDK6 were decreased, which finally resulted in decreased phosphorlyation of Rb. For the in vivo experiment, 20 C57BL/KaLwRij (injected with GFP transfected 5TGM1 cells) were treated twice weekly with vehicle or MTM (0.750 mg). Chronic i.p. treatment with MTM resulted in a decrease in 5TGM1 cells invasion from to 38.7 to 19.8% (p=0.028). In addition, MTM also reduced the myeloma associated neo-vascularization in vivo (by determination of microvessel density on bone marrow sections) and in vitro in a rat aortic ring assay. These data suggest that MTM has anti-myeloma and anti-angiogenic effects that are not mediated by an inhibition of SP-1 but rather through inhibition of c-myc and activation of p53.

P.34. Professional exposure to toxic chemicals as cause of acute leukemia

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Introduction
Diagnosis of acute leukemia related to professional exposure to toxics is rarely evoked, partially due to the lack of evidence and data in the literature regarding certain agents.

Case report
A 53-year-old woman was admitted for an acute myeloid leukemia (AML). She had been previously treated for a breast carcinoma by tumorectomy, adriamycin-cyclophosphamide based chemotherapy, irradiation and hormonotherapy (Letrozole). In addition, she had a personal history of 20 years exposure to chemicals (i.e. trichlorethylene, sulfuric acid and cadmium) as part of her professional occupation as a jeweller, and 35 years of active smoking. Her husband who shared the same occupation, died 10 years earlier from infectious complications related to acute myeloid leukemia secondary to a myelodysplastic syndrome. No link to toxic exposure had been suspected at that time. If the causative role of toxic exposure was also questioned in the present case, the identification of an MLL partial tandem duplication and the short latency favoured the hypothesis of a chemotherapy-induced secondary leukemia.

Conclusion
Professional exposure has been implicated in the genesis of acute leukemia, as the relationship between long-term chemicals exposure and occurrence of hematological diseases is well recognized in the literature. However, as illustrated in the present observation, the etiological diagnosis in this setting is difficult to establish and probably underestimated.

P.35. Clofarabine in acute leukemia: a single center experience

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Nowadays, relapsed or refractory acute myeloid leukemia in adults remains difficult to cure. Few treatments offer a good response rate with an acceptable toxicity and an increase survival.
Clofarabine is a nucleoside analog synthesized to combine the most favourable properties of fludarabine and cladribine. It is an inhibitor of DNA polymerases and synthesis, as a strong inhibitor of ribonucleotide reductase, exhibiting a synergistic effect with cytarabine. Clofarabine has been approved for the treatment of acute lymphoid leukemia in children by the FDA since 2004. It has demonstrated efficacy in monotherapy or in association with cytarabin in relapse/refractory acute myeloid leukemia and is currently under investigation in combination in induction chemotherapy. Between July 2009 and September 2011, 18 adult patients, median aged 49 years (range 20-66), received clofarabine for AML (n=15) or ALL (n=3). Seven patients received clofarabine in a combination therapy as induction treatment, 6 achieved complete remission (CR). Grade 3/4 toxicities were frequent, with prolonged aplasia over 40 days seen in 4 patients. With time, all 6 remained in CR. Three patients (2 ALL, 1 AML) received clofarabine in a relapse/ refractory setting, as a bridge before a second allogeneic stem cell transplantation (ASCT). All 3 achieved CR, 1 died of infection after 2 months, the 2 others remaining in CR after 5 and 20 months respectively. In our experience, combination chemotherapy with clofarabine seems an acceptable option in young adults with relapsed/refractory acute leukemia, especially as a bridge to ASCT. In first line therapy, his potential benefit is still under investigation.

P.36. Concomitant JAK2 V617F + ET and BCR-ABL + CML masked by Glivec therapy for GIST

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A 72-year-old man was admitted to the emergency department with a perforation of the small intestine. Biopsies of the resected segment showed a gastrointestinal stromal tumor (GIST). The patient also had a leukocytosis of 13,140 x109/l (without immature granulocytes or basophilia) and a platelet count of 968 x109/l. Imatinib mesylate (Glivec) 400 mg daily was started as an adjuvant therapy during 6 months. After this period, the leukocytosis had normalized but the platelet count remained high. Hematological advice was sought, with a bone marrow examination. The biopsy was compatible with essential thrombocytosis (ET): an increased number of megakaryocytes with hypersegmentation and bizarre nuclei. Erythropoiesis and granulopoiesis appeared normal. PCR for Janus Kinase 2 (JAK2) showed the V617F mutation (21 %). PCR for BCR-ABL was positive at an unusually low level (0,90%, p210 transcript). Karyotype was normal, but interphase FISH confirmed the BCR-ABL fusion in 1,5% of cells and 1/10 metaphases. Therapy with Glivec was restarted and a reevaluation after 3 months showed apparent reduction of BCR-ABL levels to 0,14% with a relative increase of JAK2 V617F (54 %). Anagrelide (Xagrid) was added to reduce platelet count. The coincidence of a JAK2 V617F positive myeloproliferative disorder and a BCR-ABL positive chronic myeloid leukemia (CML) is rare. So far, about 14 cases have been reported in the literature. This patient's bone marrow and peripheral blood showed typical features of ET. However, the normalization of the WBC count after therapy with Imatinib for GIST suggests that an underlying CML was masked, as witnessed by the very low levels of BCR-ABL at the hematological diagnosis. The question remains if this is a case of two separate myeloid malignancies or a secondary event (BCR-ABL fusion) in a primary JAK2 V617F + ET. In conclusion, we present the rare case of a patient with concomitant JAK2 V617F positive ET and BCR-ABL positive CML masked by Glivec therapy for a GIST.

P.37. Sarcoïdosis and POEMS: one train can hide another one

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We report the case of a 46 years-old patient referred for suspicion of myeloproliferative disorder based on the presence of erythromelalgia and thrombocytosis. He was treated since 2006 by intermittent courses of corticosteroids +/- lederthrexate for an histology proven thoraco-mediastinal sarcoidosis. Besides classical symptoms of sarcoidosis (cough, joint swelling, rash), he presented atypical symptoms of the feet finally recognised as erythromelagia (redness, burning pain relieved by cold). These symptoms improved with corticosteroïds but evolved independently of sarcoïdosis. Clinically, the patient had red but cold feet, with « drumstick-like » deformation of the toes and gynecomasty. The biology showed a mild thrombocytosis, a slighty increased lysosyme and ACE, and presence of an IgA lambda component. JAK2 mutation was absent and VEGF is ongoing. There was no myeloma on bone marrow aspiration/ biopsy. The electromyography revealed a severe axonal and demyelinising peripheral polyneuropathy of the four limbs. The lower part of L1 body was condensed on standard radiography. Multiple sclerosing and moderately hypermetabolic bone lesions, polyadenopathies and splenomegaly were reported on PET-CT. The association of a monoclonal gammapathy of λ type, polyneuropathy and osteosclerotic lesions is highly suggestive of POEMS syndrome. It is a rare paraneoplastic syndrome due to the underlying plasma cell disorder. The acronym refers to Polyradiculoneuropathy, Organomegaly, Endocrinopathy, Monoclonal plasma cell disorder and Skin changes. Important features not included in the acronym are sclerotic bone lesions and thrombocytosis/erythrocytosis. To our knowledge this is the first report of an association between sarcoidosis and POEMS. This case illustrates the diagnosis pitfalls and emphasises the importance of thrombosis prevention in POEMS.

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P.38. Malignancy-associated thrombotic microangiopathy: a monocentric retrospective analysis

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Background
Malignancy-associated thrombotic microangiopathy (MA-TMA) belongs to the group of the secondary thrombotic microangiopathies. Because of its poor outcome and its unresponsiveness to therapeutic plasma exchange (TPE), it is important to distinguish this entity at an early stage to initiate prompt and appropriate anti-cancer therapy.

Methods
All patients diagnosed with MA-TMA at the University Hospitals Leuven between 2005-2010 were identified and their medical files were retrospectively reviewed for analysis of patient-, malignancy- and TMA- related characteristics.

Results
Of all 52 patients diagnosed with TMA and treated TPE, we identified 13 patients (25%) with MA-TMA. The median age at the time of TMA diagnosis was 66 years (range 45-77). The median time between the diagnosis of the malignancy and the development of MA-TMA was 7 months (range 0-135). Presenting characteristics included: fever (n=5), Coombs negative haemolytic anemia (n=9; in 4 patients direct antiglobulin test was not performed), thrombocytopenia (n=13), neurological symptoms (n=3) and renal involvement (n=10). Only one patient presented with the typical pentad. Other associated symptoms were bone pain (n=6), respiratory symptoms (n=9) and symptoms of anemia (n=12). Laboratory results showed an excess of schistocytes in all patients. Therapeutic plasma exchange was given to all 13 patients, 5 of them (38,5%) also received chemotherapy. All patients except for one died after the diagnosis of MA-TMA. Of these, 8 patients (66.7%) died with TMA, whereas 4 patients (33.3%) died due to progressieve malignant disease with complete resolution of TMA. The median survival time following diagnosis of MA-TMA was 35 days (range 5-449).

Conclusion
In our retrospective analysis we described 13 patients with malignancy associated thrombotic micro-angiopathy and found that this entity does not typically present with the known pentad of symptoms. Despite treatment with TPE with or without chemotherapy, prognosis was very poor with only 1 long-term survivor.

P.39. Spontaneous intraocular bleeding as an initial presentation of Factor XI deficiency

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Hemophilia C is a rare deficiency affecting essentially persons of Ashkenazi Jewish ascendency. Spontaneous bleeding are rare and bleeding episodes mostly occur after invasive procedures.We report the case of a 79 year old patient without previous bleeding history except for slight bruising who presented for blurry vision of the left eye. Fundoscopy revealed severe retinal bleeding in the absence of trauma. Bioloogy showed spontaneously elevated aPTT (74seconds normal range 24-36 secondes). Factor analysis showed isolated decreased FXI at 1%. No FXI inhibitor could be found. The patient was subsequently treated with Factor XI infusions which allowed a reduction of the bleeding and normal eyesight within four days. Spontaneous intraocular bleedings in the old age are rare inaugural signs of mild bleeding disorders.

P.40. Acquired hemophilia

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Acquired hemophilia is a rare potentially lethal acquired bleeding disorder. We report the case of a 35 year old male presenting with macroscopic hematuria. Laboratory tests showed spontaneously elevated aPTT and a factor VIII level below 1%. Testing for factor VIII inhibitor using the Bethesda methods showed a high titer of FVIII inhibitor. Radiologic findings displayed a renal mass corresponding to a hematoma. Heamorragic diasthesis was treated with recombinant factor VIIa infusions. The patient was subsequently treated with corticosteroids for three months (1mg/kg/day during the first month then dose tapering during two months) and cyclophosphamide 100mg/day. No relapse happened to this date.

P.41. Isolated and transient FVII deficiency during infectious episodes in a patient with pulmonary neoplasm

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Introduction
Isolated and transient FVII deficiency in sepsis is poorly described. Several hypotheses of the underlying causes may be raised but the mechanisms are still unknown.

Patient and methods
We report the case of a 53-year-old man with a pulmonary neoplasm. The prothrombin time (PT, Innovin') and the Factor VII activity (Innovin ' and Deficient VII Stago) were evaluated on the STA-R evolution (Stago) during his hospitalization.

Clinical case
At the diagnosis of the neoplasm, the complete blood count and the routine coagulation tests were normal. A first infectious episode occurred (CRP: 29,4 mg/dl, WBC: 6, 7. 103/µl) and simultaneously the PT was found low (44%). During a second sepsis the PT decreased to 36% and the factor VII was only 13%. The activity of other coagulation factors was normal and no hemorrhagic sign was observed. In both cases the patient had fever but no source of infection was found. Antibiotics improved the inflammatory syndrome, PT and FVII activity. A factor VII inhibitor was excluded and the incubation of a mixture of the patient's plasma and a normal plasma for 15 hours at 37°C did not show any decrease in FVII activity. This isolated decrease in FVII during sepsis led us to explore the polymorphisms of FVII gene known to modulate FVII levels.

Discussion
Incubation tests exclude the hypothesis of inhibitor antibodies and that of a plasmatic proteasic activity due to the inflammatory syndrome. The activity of a cellular surface protease may not be excluded. Two mechanisms involving FVII polymorphisms may explain the decreased level. Our patient is heterozygote for the insertion « -323insCCTATATCCT » but doesn't have the R353Q. This genotype may contribute to the decreased FVII level but this effect is probably associated with other mechanisms.

Conclusion
A decrease in PT during an inflammatory syndrome should be followed by an individual measure of coagulation factors. Molecular biology alone does not explain the decrease in FVII during sepsis. Other mechanisms should be explored too.

P.42. Influence of pathogen inactivation of platelet components with INTERCEPT on the use of platelets and red blood cell concentrates in hemato-oncology patients

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Background
In May 2009, the SFS who supplies the blood bank of the Bordet Institute, has introduced INTERCEPT for the pathogen inactivation in platelet concentrates. This study looks at the influence of the introduction of INTERCEPT on the use of platelets (PLT) and red cell concentrates (RBC) in a population of hemato-oncology patients treated at Jules Bordet Institute.

Study design and methods
This is a retrospective analysis of transfusion data extracted from electronic blood bank and clinical laboratory records. The Test period included the 12 months following the introduction of INTERCEPT (01/06/2009-31/05/2010), and was compared with the Control period, including the 12 months before the introduction (01/05/2008-30/04/2009).

Results
Transfusion records were analyzed of 157 patients in the Control period and 136 patients in the Test period. Between Control and Test period, the median number of PLT transfusions per patient increased from 4 to 5 (p= 0.1877), and the total PLT dose per patient rose from (15,0 x10 ^{11} to 22,5 x10 ^{11}) (p = 0,0674). The total of the periods of PLT support per patient also increased between two periods mean (13.6 vs 14.6 days); median (4 vs 6 days) (p= 0,2429).This increase obviously is not linked to the quality of the PLT product, but to the treatment regimen. The number of transfusions per day of PLT support (1,0 Control vs 0,9 Test) (p=0,4907), remained very similar, and such did the dose of PLT per day of PLT support (3,7 x10 ^{11} vs 3,6 x 10 ^{11}) (p=0,4256).There was no increase in the RBC use in PLT recipients between the two periods (11.7 vs 13.9) (p=0.3317) , even when the comparison was focused during the period of PLT support (6.0 vs 8.1) (p=0.1412). No serious adverse effects on PLT transfusions were observed in the two periods.

Conclusion
Pathogen inactivation of PLT components had no adverse impact on blood components use by hematology patients at Jules Bordet Institute.

P.43. Characterisation of Microparticles by Atomic Force Microscopy : a promising diagnostic tool

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Introduction
Circulating microvesicles (MVs) are potential biomarkers for the thrombotic risk encountered by a variety of patients. No device currently allows the size and expression analysis in the nanometer scale. Yuana and coworkers first used the Atomic Force Microscopy (AFM) in analysis purposes in 2010. Three major information are brought with AFM: a number of particles, an estimation of their volume and a selection regarding to an antigenic expression. Objectives: to validate the use of AFM to quantify Tissue Factor (TF) MVs.

Material and methods
Mica sheets are activated with ehanoalmine (55% in DMSO) and glutaraldehyde (7% in PBS). They are abundantly rinsed in PBS and water and than coated with a drop of 10µg/µl antibody (anti CD142 = TF and anti CD141) and rinsed. MVs are added on the surface that is than washed and dried under nitrogen before AFM observation. Twelve millions of MDA-MB-231 breast cancer cells are suspended in 1mL PBS and placed at 37°C for 45 minutes. Cells are eliminated with a 5 minutes centrifugation at 500g. Whole blood is centrifuged twice at 2500 g for 15 minutes.

Results
From AFM images the number of CD142 antibodies on the surface was estimated to approximately 2200/µm2, which is 100-fold larger than obtained by Yuana et al. The molecular diameter was about 20 ± 4 nm. The mean size of MVs released by MDA-MB-231 cells, as estimated from 1200 MVs, was 38 ± 9 nm. The TF-MVs concentration produced by 12 x 106 MDA-MB-231/ml was between 420 x 1010 and 420 x 1011 MVs/ml. When imaging the healthy donor-PFP on TF coated surfaces, only four particles (60 nm to 100 nm) among whole platelet derived MVs were found

Conclusion
The AFM is suitable to analyse TF-MVs. The use of AFM to detect TF-MVs in human plasma should be validated in order to use MVs as biomarker of the prothrombotic risk in different diseases such as cancer.

P.44. Assessment of different methods studying the impact of carbon nanomaterials on platelet function

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Objectives
We aimed to validate an universal, fast, accurate, reliable and relevant toxicological preclinical screening test to measure the potential impact on primary haemostasis of nanoparticles (NP) whatever their physicochemical properties.

Materials and methods
Four types of carbon nanoparticles (Carbon Black (CB), Fullerenes

(C60), Single Wall Carbon Nanotubes (SWCNT), Multi Wall Carbon Nanotubes (MWCNT)) considered as promising in medical applications were investigated. The interference of these nanoparticles on Light Transmission Aggregometry (LTA), Whole-blood Impedance Aggregometry (Multiplate®), Platelet Function Analyzer-100 (PFA-100®) and Impact-R® was studied before the assessment of their effect on platelet function (adhesion, activation and aggregation). Interference and functional impact were also analyzed by transmission- and scanning electron microscopy.

Results
Maximal concentrations of C60, CB, SWCNT, MWCNT that may be tested with optical methods like LTA are 500, 10, 500 and 100 µg/ml, respectively. Each nanoparticle interferes by flux obstruction with PFA-100® at concentration higher than 10 µg/ml. Whole-blood impedance aggregometry was not considered as a suitable method because of the interaction between negatively charged nanoparticles and the impedance measures. Impact-R ® showed absence of interference of C60, CB, SWCNT, MWCNT up to 250, 500, 500 and 250 µg/ml, respectively. Furthermore, the addition of Bovine Serum Albumin 7,4g/l (final concentration) to mimic human blood viscosity abolishes the interference of C60 and MWCNT. Below cut-offs without any interference, none of the nanoparticle has a significant effect on the platelet function, whatever the method used.

Conclusion
Impact-R® is the most adapted test to assess the effect of manufactured NPs on platelet function.

Abstracts posters laboratory haematology P.45 – P.61

P.45. Brief evaluation of Xpert BCR-ABL assay

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Introduction
Chronic myeloid leukemia patients treated with tyrosine kinase inhibitors are routinely monitored for treatment respons by using serial analyses of BCR-ABL mRNA levels by reverse transcriptase real time quantitative Polymerase Chain Reaction (RTQ-PCR). Standardised, accurate and reproducible molecular analyses are essential for clinicians to refine therapeutic stratification and make clinical decisions. Variations in methods used to quantify BCR-ABL make it difficult to compare results between laboratories. Harmonisation by reporting results on an international scale (IS) using method specific conversion factors (CF) is ongoing. Our aim was to investigate whether the automated, cartridge-based XpertBCR-ABLassay, alignedtoISbythemanufacturer, givescomparable results to our time-consuming in house RTQ-PCR assay (EAC method).

Methods
Twenty-four follow-up and two diagnostic peripheral blood samples were analysed both by the Xpert BCR-ABL assay run on a GeneXpert system (Cepheid) and by our in house RTQ-PCR method (EAC method). Ratios (<10% IS) detected by the Xpert assay (n=17) were investigated for bias against the in house assay using the Bland and Altman method. The primers and probes used in the Xpert assay are designed to detect only the major BCR-ABL breakpoint. Concordance for MMR (≤0.1% IS), no MMR (>0.1% IS) and CMR (complete molecular response, no BCR-ABL detected) was defined as the ratio of samples attributed to a given molecular status by both methods divided by the number of samples attributed to the same molecular status by either method.

Results
Only a small constant bias on the log scale (0.05 ; 95% CI= -1.05 - 1.14) was shown. Concordance was found to be 66% for CMR (2/3), 89% for MMR (16/18) and 86% for no MMR (6/7).

Conclusion
Our evaluation shows the Xpert BCR-ABL assay gives quantitative results and stratifications of molecular response comparable to the in house RTQ-PCR assay. For twenty-six patient samples only 2 yielded a discordant molecular status. The discordances can be ascribed to inherent assay variability and would not have affected clinical decision making.

P.46. Rare translocations in acute leukemia: report of two cases

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Introduction
Cytogenetic analysis in acute myeloid and lymphoblastic leukemia has revealed numerous non-random chromosomal abnormalities. Some of the involved genes have been shown to be implicated in leukemogenesis and are used as diagnostic and prognostic markers. Here, we describe rare translocations in two cases with acute leukemia.

Case 1
A 77-year-old man was diagnosed with acute monoblastic and monocytic leukemia (AML-M5a). Diagnostic work-up identified a translocation t(4;11)(p14;q23), involving the mixes-lineage leukemia (MLL) gene and the precocious dissociation of sisters 5A (PDS5A) gene. RT-PCR and direct sequencing revealed an in-frame fusion between exon 8 of MLL and exon 17 of PDS5A. The resulting fusion protein consists of the N-terminal portion of MLL fused to the C-terminal portion of PDS5A. The MLL breakpoint is located within the repression domain. Due to loss of the SET domain, histone methylation of the HOXA9 and HOXC8 promoters cannot occur. As a consequence, HOX gene expression is deregulated. Moreover, the loss of the plant homeodomain fingers in combination with the gain of an activation domain of another partner protein is likely to convert the fusion protein to a constitutive transactivator. This leads to constitutive overexpression of MLL target genes that block stem cell commitment and promote stem cell renewal, probably the first step in MLL-related leukemogenesis.

Case 2
A 57-year-old man was diagnosed with common B-cell acute lymphoblastic leukemia (ALL-BII). Diagnostic work-up, including karyotyping and FISH, identified a translocation t(3;9)(p13;p13), involving the forkhead box P1 gene (FOXP1) and paired box gene 5 (PAX5). The PAX5-FOXP1-translocation has been reported in only 4 patients so far and gives rise to either in-frame or out-of-frame chimeric PAX5-FOXP1 transcripts. PAX5-fusion products suppress the transcriptional activity of PAX5 in a dominant negative fashion. This may contribute to leukemogenesis by blocking differentiation of hematopoietic stem cells into mature B-cells.

Conclusion
We describe PDS5A (4p14) as a novel translocation partner of MLL (11q23) in AML-M5 and report a rare translocation involving FOXP1 (3p13) and PAX5 (9p13) in ALL-BII.

P.47. Haemoglobinopathies in Charleroi

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Charleroi is currently the most cosmopolitan city in Belgium. On the registers of population, 128 nationalities are listed against 109 in Brussels. Italians represent near half of approximatively 30 000 foreign nationals registered in the municipality, followed by Moroccans, French and Turks. This does not take account of many naturalized immigrants, coming essentially from June 1946, resulted from needs for workforce in mines and collieries of Charleroi. At present, Charleroi undergoes a new wave of immigration caused by the housing pressure, mainly Turks, arabic people or Africans leaving Brussels. We thus decided to evaluate the detection of hemoglobinopathies and to carry out a systematic newborn screening of hemoglobinopathies in two hospitals of Charleroi, the Hôpital Civil, CHU de Charleroi (CHU) and Notre Dame de Grace in Gosselies (CDNG). During one year (November 2010 to November 2011), 1361 cord bloods were collected for CHU. From March to November 2011, 336 samples were collected (319 cord bloods) for CDNG. The two hospitals use a capillary-electropherosis system: Coulter MDQ with Ceofix Hb A2 kit (Analys) for CHU and the Sebia Neonat Hb system for CNDG.
Results are listed in the *Table 1.*

		N	Positve %	Variant %
CHU	Classical	1020	B thal: 4.5% Variant Hb: 8.8%	SS: 0.2%; SC: 0.2%; AS: 6.5% AC: 1.2%; AE: 0.4%; AD-Punjab: 0.1%; G Philadelphia: 0.1%; HbO-Arab heterozygote: 0.1%
	Cord blood	1361	Variant Hb: 1%	AS: 0.5%; AC: 0.4%; AE: 0.1%
CNDG	Classical	102	B thal: 16.6% Variant Hb: 4%	AS: 3% AD-Punjab: 1%
	Cord bllod	336	Variant Hb: 2.1%	AS: 0.9%; AC: 0.3%; Hb Bart's: 0.3%; AD-Punjab: 0.6%

Results of cord blood screening are compared with the classical routine of haemoglobinopathies testing during the same period. Rare variant identifications are confirmed by a reference laboratory (Erasme). The identified variants confirm the worldwide immigration in Charleroi. The CHU obtained 1% (13/1361) positive for the screenings on cord blood against 2% (7/336) for CNDG. This is comparable to that observed in Brussels where systematic screening is performed. Considering the location of hospitals, both in Charleroi, these percentages are surprising. The CHU should have obtained at least an equivalent percentage. These request further investigations. This report is in favor of the institution in Charleroi of systematic newborn screening of haemoglobinopathies.

P.48. Laboratory assessment of Dabigatran

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Introduction
Due to low bioavailability and high inter-individual variability, point measurement of dabigatran may be required in specific situations to prevent the risk of bleeding or thrombosis.

Aims
The aim of the study was to determine which coagulation assay(s) could be used to assess the impact of dabigatran on secondary haemostasis.

Materials
Dabigatran was spiked at concentrations ranging from 5ng/mL to 943ng/mL in pooled citrated human platelet-poor plasma. The following clotting assays were performed: Prothrombin Time (PT); activated Partial Thromboplastin Time (aPTT); Thrombin Time (TT); Ecarin Clotting Time (ECT); Ecarin Chromogenic Assay (ECA); Prothrombinase-induced Clotting Time (PiCT); Activated Clotting Time (ACT); Hemoclot Thrombin Inhibitor (HTI) and Thrombin Generation assay (TGA).

Results
A concentration-dependent prolongation of PT, dPT, and aPTT was observed while aPTT being the more sensitive. The results varied mostly due to the clotting reagent. For aPTT, Actin FS was the most sensitive reagent. HTI, ECT and TGA were the most sensitive tests but are not available 24h a day. In addition, HTI showed a linear correlation with a good reproducibility. Dabigatran induced a concentration-dependent delay and inhibition of tissue factor-induced TGA. Cut-offs related with higher risk of bleeding were defined for aPTT and HTI. For aPTT, cut-off values for each reagent were proposed taking into account the inter-reagent variability. Nevertheless, for one particular reagent, aPTT varies according to the lot number. Consequently, each lab should calibrate each lot of aPTT reagent on each coagulometer. In addition, aPTT is also influenced by lupus anticoagulant, acquired or hereditary factor deficiencies.

Conclusions
aPTT could be used for point measurements of dabigatran after calibration and as screening test for the risk of overdose but specific cut-off for each reagent must be used. However, because of its higher sensitivity, good reproducibility, excellent linear correlation, its simplicity of use and possibilities of automation, HTI should be considered as the gold-standard. Standardization of the time between the last intake of dabigatran and the time of blood collection is mandatory.

P.49. Laboratory assessment of Rivaroxaban

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Introduction
Rivaroxaban does theoretically not require monitoring. Nevertheless, point measurement may be useful to avoid thrombosis and/or bleedings in some specific clinical settings or to ensure compliance of the patient.

Aim
The aim of the study was to determine which coagulation assay could be used to assess the impact of rivaroxaban on secondary haemostasis.

Materials
Rivaroxaban was spiked at concentration ranging from 22 to 2180ng/mL in platelet-poor plasma. The following routinely used or more specific coagulation assays were performed: activated partial thromboplastin time (aPTT); prothrombin time (PT); dilute prothombin time (dPT); prothrombinase-induced clotting time (PiCT); ecarin clotting time (ECT); reptilase time (RT); biophen direct Xa inhibitor (Biophen DiXaI); Liquid anti-Xa (LAX) and the thrombin generation assay (TGA).

Results
A concentration dependent prolongation of aPTT, PT, dPT, PiCT was observed. PT and dPT were the most sensitive chronometric assays but results varied depending on the reagent. dPT slightly reduce the sensitivity but it also depends on the reagent. For PT, Triniclot PT Excel S® was the most sensitive reagent while for dPT it was Recombiplastin® diluted 1/64. Biophen DiXaI and LAX showed a concentration dependent decrease in OD/min and the highest sensitivity but are not available 24h a day. Biophen DiXaI showed a linear correlation while LAX showed an exponential decrease correlation. Moreover, Biophen DiXaI is insensitive to heparins and fondaparinux. This is an advantage in the evaluation of the concentration in rivaroxaban in case of switching therapy. In TGA, Cmax was the most sensitive parameter with the tissue factor induced pathway.

Conclusions
PT and dPT can be used as screening test to assess the risk of bleedings. Nevertheless, a more specific and sensitive assay

such as Biophen DiXaI using calibrators should be used to determine correctly the concentration of rivaroxaban in plasma. In addition, cut-offs associated with the risk of bleedings should be defined. Standardization of the time between the last intake of dabigatran and the time of blood collection is mandatory.

P.50. AcuStar HIT-IgG and heparin-induced multiple electrode aggregometry: a useful combination for rapid diagnosis of type-II HIT

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Background
Early type II heparin-induced thrombocytopenia (HIT) diagnosis is essential to improve clinical outcomes of this potentially lethal condition but remains challenging. HemosIL® AcuStar HIT and heparin-induced multiple electrode aggregometry (HIMEA) were recently proposed as new rapid methods for diagnosis of type-II HIT.

Objectives
The primary objective of this study was to study performances of AcuStar HIT-IgG(PF4-H), AcuStar HIT-Ab(PF4-H) and HIMEA. The secondary objective was to compare these assays with PF4 Enhanced®, Light Transmission Aggregometry (LTA), 14C-Serotonin Release Assay (SRA) and clinical outcomes.

Methods
Sera HIT-suspected patients (n=104) were studied retrospectively by AcuStar HIT-IgG(PF4-H), AcuStar HIT-Ab(PF4-H). HIMEA was performed on 81 patients. These tests were compared with ELISA (PF4 Enhanced®), LTA, SRA and clinical outcomes data by Chi-Square tests and ROC Curves. Clinical outcomes were available for each patient (including 9 positive type-II HIT).

Results
The cut-off recommended by the manufacturer for AcuStar HIT-IgG and AcuStar HIT-Ab(PF4-H) (i.e. 1 AU) showed positive predictive value (PPV) of only 64.3% and 45.0%, respectively. When clinical outcome was considered as the reference, negative predictive values of AcuStar HIT-IgG(PF4-H), AcuStar HIT-Ab(PF4-H) and HIMEA were 100 %. The PPV reached 75.0 %, 81.8 % and 80.0 %, respectively. The cut-offs were 2.89 AU, 9.41 AU and 276 AU respectively. Seventy-nine patients presented a medium-high pretest probability requiring biological testing. AcuStar HIT-IgG(PF4-H) allowed to exclude the diagnosis of HIT in 65 of these patients. Among the 12 positive AcuStar HIT-IgG(PF4-H), 9 patients were confirmed HIT and HIMEA allowed to exclude the diagnosis of HIT in 2 out of 3 non-HIT patients.

Conclusions
The combination of AcuStar HIT-IgG and HIMEA with optimized cut-offs is useful for rapid and accurate diagnosis of type-II HIT.

P.51. Malaria-associated pseudo-eosinophilia and abnormal WBC scattergrams determined in the SYSMEX XE-5000 automated haematology analyzer

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According to recent studies, flow cytometry-based automated haematology analyzers have been found to be a potential diagnostic tool in the routine laboratory work up of febrile patients in or returning from malaria-endemic countries. Although there isn't a general consent about the diagnostic value of haematology analyzers for the detection of a malaria infection, we can find more and more promising data on this topic. The SYSMEX XE-5000, an haematology analyzer from the Sysmex Corporation (Kobe, Japan), has hardly been tested on this subject. In a prospective study between August-December 2011, we investigated the peripheral blood on the SYSMEX XE-5000 haematology analyzer of all patients suspected for malaria infection that were submitted to our hospital. Atypical features were only detected in two malaria infected patients, both were infected with the Plasmodium Vivax parasite. In one case a pseudoeosinophilia (which is defined as a gap of more than 5% in eosinophil count between the automated and the microscopic count) was found. For this patient, the automated eosinophil count was 24%, while the microscopic eosinophil count was <1%. Morphologic evaluation on the CellAvison DM96 (Sysmex Corporation, Japan), showed the presence of trophozoites. In both cases an abnormal white blood cell (WBC) scattergram was seen. This abnormal WBC scattergrams encountered two atypical eosinophil populations. Pseudoreticolocytosis -which has been reported in the literature to be associated with malaria infection- was not found in our study. Due to the low sensitivity, the haematological analyzers can not be considered as an alternative method for the diagnosis of malaria. However, special attention of the laboratory haematologist to specific abnormalities in the dot plots may be valuable in the early diagnosis of malaria and, because of early treatment, consequently lead to a reduction in patients' morbidity and mortality.

P.52. Successful switching from acenocoumarol to rivaroxaban in a patient with CYP3A4*1G polymorphism

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A 52 years old woman suffering for atrial fibrillation (AF) was previously treated by acenocoumarol to prevent stroke. This prophylactic therapy appeared to be inefficient since a dose of 15mg a day gave an INR of 1. A genetic analysis looking for polymorphism of CYP3A4, VKORC1, CYP2C9, F7, GGCX, CALU or EPHX1 was performed. The results showed a CYP3A4*1G polymorphism associated with a fast-metabolizer profile. Dabigatran was contraindicated due to a weight higher than 110kg. Therefore, we proposed to switch the patient to rivaroxaban and monitor its effectiveness via coagulation assays. Blood samples were taken at different intervals (0.5; 1.0; 1.5; 2.0; 3.0; 4.0; 6.0 hours) after a single dose at the instauration of the treatment and six days later.

Figure 1. Plasmatic rate at day 0.

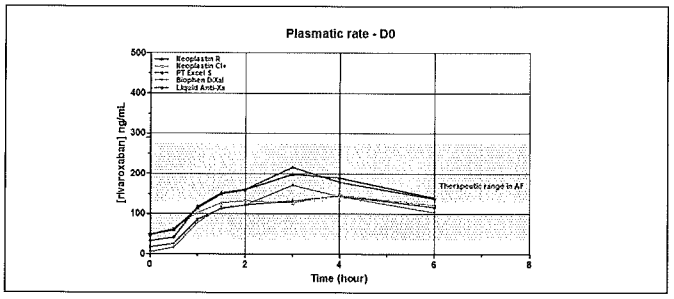
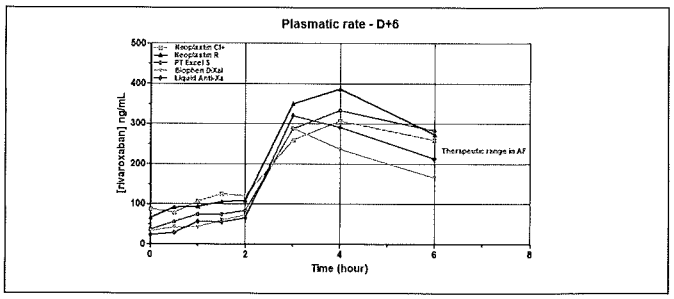


Figure 2. Plasmatic rate at day +6.



The following coagulation assays were performed: Prothrombin Time (PT) (Neoplastin CI+, Neoplastin R; Triniclot PT Excel S), Biophen

Direct Xa Inhibitor and Liquid anti-Xa. Results showed that the anticoagulant effect of rivaroxaban was not affected by a CYP3A4*1G polymorphism. Indeed, using calibrated PT or specific chromogenic assays, the rivaroxaban concentration was within the therapeutic range for AF (mean Cmax = 290ng/mL and mean Ctrough = 32ng/mL). In conclusion, CYP3A4*1G polymorphism is associated with a lack of effectiveness of acenocoumarol but not with rivaroxaban.

P.53. Routine haematological parameters for the screening of hereditary erythrocytes membrane disorders

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Introduction
Hereditary erythrocyte membrane disorders (HEMD), i.e. hereditary spherocytosis (HS), elliptocytosis [3DOTS] are a wide group of haemolytic diseases that are most often the consequence of defects in red blood cells membrane proteins. Clinicians are confronted with a difficulty regarding the diagnosis of these disorders. New routinely available haematological parameters were proposed as a diagnostic tool of HS, the most frequent hereditary membrane disorder in European countries. Our aim was to test routine haematological parameters available on an automated blood cell analyser as useful tools for the screening of HEMD from birth to adulthood.

Methods
From June 2010 to August 2011, all patients screened for erythrocyte membrane disorders and tested by eosin-5-maleimide biding test (EMA) and cryohaemolysis test (CH), were also screened by routine haematological parameters acquired on an automated blood cell analyser Beckman Coulter Unicel DxH800 (Beckman Coulter, Analis, Belgium). This group represents 293 EDTA blood samples. We also included patients with a haemolytic disorder i.e. autoimmune haemolytic anaemia (n=6), patients heterozygotes (n=2) and homozygotes (n=2) for haemoglobin S, unstable haemoglobin (n=1), G6PD deficiency (n= 6) or iron deficiency (n=57). Haematological parameters as haemoglobin level, mean cell volume (MCV), percentage of immature microcytes, microcytic erythrocytes volume (MRV), mean sphered corpuscular volume (MSCV), reticulocyte distribution width coefficient of variation (RDWR), mean corpuscular haemoglobin concentration (MCHC) were included in our study. When a heparinised sample was available and any screening test or clinical picture was in favour of a membrane protein defect, a gel electrophoresis of erythrocyte membrane proteins (SDS-PAGE) was also realized.

Results
The statistical analysis of ROC curve shows us that a delta (MCV-MSCV) cut off at 15 fL allows to include all HEMD tested with 100% of sensitivity and 86.4% of specificity.

Conclusions
Among the new haematological parameters, delta (MCV-MSCV) is the most discriminating parameter to screen for a hereditary erythrocyte membrane disorder. This rapid method could be used routinely as an excellent screening tool of this group of pathologies.

P.54. Belgian survey of the training in hematology compared to 21 European countries : The H-Net/EHA program

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Background
The ECAH project led to the CV Passport (CV-P), a booklet setting out the curriculum for hematologists in training. On behalf of 22 National Societies (NS), E. Hellström-Lindberg, received an EU grant to harmonize Hematology in Europe.

Objective
To achieve this project, each NS performed a survey of recently graduated hematologists in order to validate the CV-P, compare variations between countries, identify gaps in the training program for each NS and revise the CV-P according to the observations obtained.

Population
Volunteer recently graduated hematologists were asked to compare their level of competence with the CV recommendations by ticking off levels of competence.

Results
12/225 (from 22 countries) participated in this survey. Three levels of competence for each topic were compared to the recommendations in the CV-P and to the average in Europe. This confidential analysis identified strengths (malignant hematology, transplantation, professional skills such as evidenced-based medicine, communication, end of life [3DOTS]) and weaknesses (i.e. bone marrow failure, advice on the use of blood products, AIHA, interpretation of coagulation tests). Weaknesses are due to a different means of organization whereby pediatrics, transfusion, coagulation and laboratory hematology are separated in Belgium. In Europe, the length of training is correlated with the level of competence. Three years were considered as the minimal requirement for hematology training, after 2 years of Internal Medicine. An updated CV-P (#2) - revised according to the average in Europe - is available on the EHA website and each trainee is invited to register within the confolio system in order to conduct their self-assessment.

Conclusion
This survey has facilitated the proposal of European guidelines to harmonize the length and the content of hematology training . It was useful to improve the BHS PG course. If we succeed in having all trainees complete the CV passport by the end of their training, Belgium will become a model for the implementation of this program designed to increase mobility of European hematologists.

P.55. Evaluation of the rReceptor for Hyaluronic Acid Mediated Motility (RHAMM) as a tumor specific antigen in AML

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Therapies to eliminate the leukemic stem cell (LSC) could be used to cure Acute Myeloid Leukemia (AML). As chemotherapy often fails to kill the quiescent LSC, the potent Graft-versus-Leukemia effect, observed in hematopoietic stem cell (HSC) transplantation, supports a role for immunotherapy in the treatment of AML, using cytotoxic T-cells recognizing an AML-specific antigen on LSCs. It was shown in vaccination trials that RHAMM generates a cellular immune response in patients with AML. However, it is not clear whether this response actually targets the true LSC. Therefore, we evaluated the expression pattern of RHAMM in LSCs compared to HSCs of healthy donors. We isolated two subpopulations from bone marrow/apheresis of AML patients and healthy donors by cell sorting: CD34+CD38- (LSCs) and CD34+CD38+ from AML patients, and their normal counterparts from healthy donors. qPCR could not demonstrate significant expression of RHAMM in HSCs. Overexpression could be clearly visualized in the AML samples. Strikingly, the overexpression in LSCs was minimal, compared with the CD34+CD38+ fraction of the same patients. Subsequently, cord blood-derived CD34+ cells were isolated and expression of RHAMM was compared in fresh cells versus cells cultured for 7 days: a clear up-regulation of RHAMM was observed in expanded CD34+ HSCs compared with baseline. To evaluate RHAMM expression during in vivo engraftment, we are currently assessing cord blood CD34+ cells in a NOD/scid/gamma (NSG) mouse model.

In conclusion, we were able to confirm that resting HSCs do not express RHAMM. However, our data suggest that immunotherapy targeting RHAMM will not recognize true LSCs and might be capable of eliminating only their progeny. Strikingly, in vitro expansion of HSCs causes a robust up-regulation of RHAMM. This might be a major limitation for immunotherapy targeting RHAMM in the setting of a stem cell transplantation, because RHAMM-specific cytotoxic T-cells might not be able to discriminate engrafting donor HSCs versus LSCs. Therefore, RHAMM is not an ideal AML-specific antigen and we advocate caution to use RHAMM-directed immunotherapy.

P.56. Estimation of activated Partial Thromboplastin Time (aPTT) geriatric reference limits using the indirect Bhattacharya method

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Introduction
To evaluate aPTT results in elderly patients, we estimated geriatric reference limits according to the indirect method described by Bhattacharya (Biometrics, 1967).

Materials and methods
aPTT results were collected from our laboratory information system over a two month period of time. aPTT samples were analysed on a STA-R Evolution using STA-PPT A reagent. Nine thousand six hundred sixty six results were collected (age range: 18 - 90 years). Only the first result for a given patient was included. From this total group of results, different sample groups were selected based on gender (female versus male) and age (subpopulation 18-60, 61-70, 71-80, 81-90 year). Due to the limited numbers of patients > 90 year, reference limits were not calculated for this subgroup. Reference limits were calculated using Excel® and Medcalc® worksheets.

Results
aPTT reference limits are presented for the different age groups in the following order: 18-60 year; 61-70 year; 71-80 year; 81-90 year. For the total population reference limits are: 26,30 to 38,08s; 26,02 to 38,13s; 25,95 to 39,07s and 35,31 to 39,91s. Female reference limits are: 26,20 to 37,55s; 26,00 to 37,18s; 25,53 to 38,56s and 25,21 to 39,11s. Male reference limits are: 26,64 to 38,65s; 26,31 to 38,74s; 26,47 to 39,38s and 25,11 to 41,37s respectively.

Conclusion
Our aPTT reference limits calculated according to the indirect Bhattacharya method are in line with reference limits calculated according the CLSI recommended direct method. Indirect methods remain a valuable, practical alternative to direct methods especially in age groups where healthy volunteers are difficult to recruit. Further analyses are required to confirm these findings.

P.57. Diagnostic potential of CD34+ cell antigen expression in myelodysplastic syndromes

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Background
WHO has introduced flow cytometry (FC) as additional criterium for myelodysplastic syndromes (MDS). Aberrant antigen expression on blasts could be useful to identify "low grade MDS"cases without elevated blast counts, ringsideroblasts or cytogenetic abnormalities.

Aim
To study differences in antigen expression on CD34⁺cells between MDS and secondary cytopenia (SC) patients and to construct a scoring system for MDS.

Materials & Methods
Bone marrow (BM) aspirates of 175 cytopenia patients were classified as MDS (low grade MDS or refractory anemia with excess of blasts [RAEB]) or SC. Expression of stem cell antigens (CD34, CD133), myeloid antigens (CD13, CD33), B-cell antigens (CD19, CD10), growth factor receptors (CD117, CD123) and a chemokine receptor (CD184) was examined. 32 normal adults and 49 AML patients were also examined.

Results & discussion
Percentage CD34⁺cells was significantly higher for low grade MDS, RAEB and AML as compared with SC. CD117-overexpression was the most common abnormality for low grade MDS. Other CD34⁺cell related abnormalities were increased expression of CD133, CD13 and CD123, whereas CD45-expression was either increased or decreased. More advanced disease states (RAEB and AML) showed the same but more pronounced FC aberrations, and also decreased CD184-expression. Sensitivities of our scoring system for low grade MDS, RAEB and total MDS group were 70.4%, 100% and 83.7% respectively, while specificity was 100%. Our findings were confirmed in a validation cohort. Among low grade MDS subsets, CD117-overexpression and aberrant CD45-expression were the most frequent FC alterations, being more pronounced in multilineage dysplasia subsets. CD123-overexpression was also a frequent FC aberrancy in both unilineage and multilineage subsets, but not at all in subsets with ringsideroblasts.

Conclusion
High percentage CD34⁺cells, CD117- and CD123-overexpression, and variable CD45-expression were the best markers for MDS. These phenotypic aberrancies correlated with number of blasts and degree of dysplasia, and were similar to those in CD34⁺AML, reflecting the relationship between these disorders. CD123-overexpression was not found in patients with ringsideroblasts; further studies are needed to elucidate its biological significance.

P.58. An unusual cause of pancytopenia

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Gelatinous transformation of bone marrow, also known as serous atrophy, is a rare condition that can occur in several nutritional deficiencies. We describe the case of a 20-year-old man who complained of fatigue and dyspnoea, occurring after a severe diet responsible for a massive loss of weight of 40 kg over a short period of time. Physical examination was normal except for pallor. Blood tests showed a white blood cell count of 2410/µl, absolute neutrophil count of 1150/µl, hemoglobin level of 11,1 g/dl, platelets count of 105000/µl. Vitamin B12, folates, iron panel, albumin, prealbumin and beta-caroten values were normal, but vitamin D level was low. Whole body CT-scan was irrelevant except from the presence of 2 metallic particles in the small bowel. The patient denied any use of drugs or medications. The bone marrow aspirate and biopsy disclosed few myeloid, erythroid and megakaryocytic precursors, with neither dysplasia nor abnormal cells. Eosinophilic deposits compatible with the diagnosis of a gelatinous transformation were observed. Blood cells count improved with nutritional supplies. Gelatinous transformation of the bone marrow is a rare cause of pancytopenia, characterized by extracellular deposits of a gelatinous substance made of acid mucopolysaccharides It is usually observed in the context of anorexia nervosa but should also be suspected in case of massive weight loss, after exclusion of vitamin or iron deficiencies and drug toxicities.

P.59. Comparison of the use of TFPI blockage and plasma dilution to increase the sensitivity of thrombin generation assay to measure the procoagulant activity of microvesicles

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Introduction
Patients with cancer have a 7- to 10- fold increased risk of developing venous thromboembolism (VTE). Circulating microvesicles (MVs) could be a predictive biomarker for VTE in cancer. Thrombin generation

assay (TGA) is a useful technique to detect procoagulant activity (PCA) of MVs. However, TGA suffers from a lack of sensitivity due to the presence of Tissue Factor Pathway Inhibitor (TFPI) in plasma.

Aims
To improve the sensitivity of TGA to Tissue factor by limiting the interference of TFPI.

Methods
Serial dilutions of MDA-MB231 cells were incubated for 45 min at 37°C to generate MVs. Samples were then centrifuged or not and cells or the supernatants (Sup) were used for TGA assay. Normal pooled plasma was incubated with TFPI inhibitors or is diluted two times to decreased plasma level of TFPI. Lagtime is used as surrogate marker of TGA to detect PCA of MVs. Serial dilutions of cultured breast cancer cells MDA-MB 231 were incubated for 45 min at 37°C to generate MVs. Cells or their supernatants (Sup) were used as TGA activators. Before TGA was performed, plasma was either incubated with different dilutions of a TFPI inhibitor or diluted two times to decrease plasma level of TFPI.

Results
Use of TFPI inhibitor decreased two times the cells concentration needed for a significant reduction of lagtime. Moreover, the use of TFPI inhibitor decreased 2.4-fold the intra-assay variability.
The plasma dilution decreased two times cells concentration needed for a significant reduction of lagtime by cells. However, plasma dilution did not increase the sensitivity to MVs in the supernatant in comparison to undiluted plasma .

Conclusions
TFPI inhibitor increased sensitivity to MVs PCA and decreased variability of TGA. Plasma dilution is less effective than the use of TFPI inhibitor to increase sensitivity to MVs TGA.

P.60. Gain-of-function WAS mutations lead to a disorganized actin cytoskeleton and loss of cell orientation

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Gain-of-function WAS mutations are the cause of X-linked neutropenia (XLN), a rare subtype of Severe Congenital Neutropenia (SCN). How these mutations are leading to the phenotype of neutropenia is poorly understood, but as for other subtypes of SCN, increased apoptosis is supposed to be the main mechanism. The objective of this study was to investigate the effects of activating WAS mutations on cell shape, movement, cycling and viability in XLN cell models and to explore potential differences or similarities between two XLN mutations p.L270P an p.I294T. Molecular cloning techniques were used to make different WASP constructs, which were transfected or transduced into the cell lines U937, HT1080 and A7r5. Our studies in these cell models suggest that apoptosis might not be the key event underlying XLN. We demonstrate that the constitutive activation of WASP, by both p.L270P and p.I294T WASP, alters the cytoskeletal organization of the cells. In the rat fibroblast-like smooth muscle cell line A7r5, the classical stress fiber pattern is replaced by a diffuse actin pattern (figure 1). Podosomes are formed and retracted in a seemingly uncoordinated manner. In addition, the cell volume is decreased, and cell movement is altered, with a loss of coordinated, logical and directional cell movement (figure 2). The migratory nature of hematopoietic progenitor cells is essential for their maturation and trafficking to the appropriate bone marrow niche allows myeloid progenitors to reach the adequate micro-environment, that provides the essential conditions for their normal development. We believe that the loss of directional movement could be an important mechanism by which gain-of-function WAS mutations lead to neutropenia.

Figure 1. Disturbed actin cytoskeleton in mutant WASP cells.

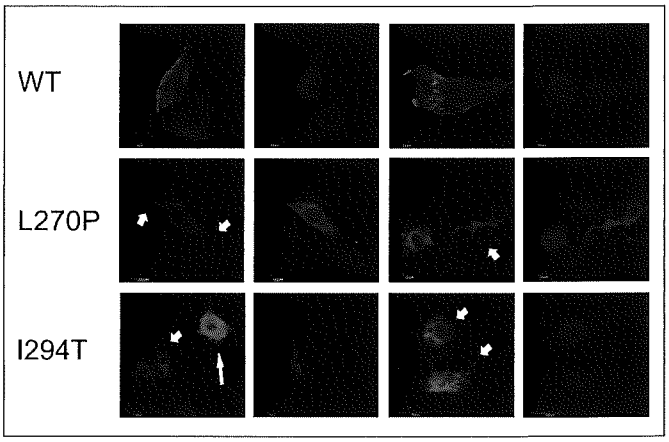
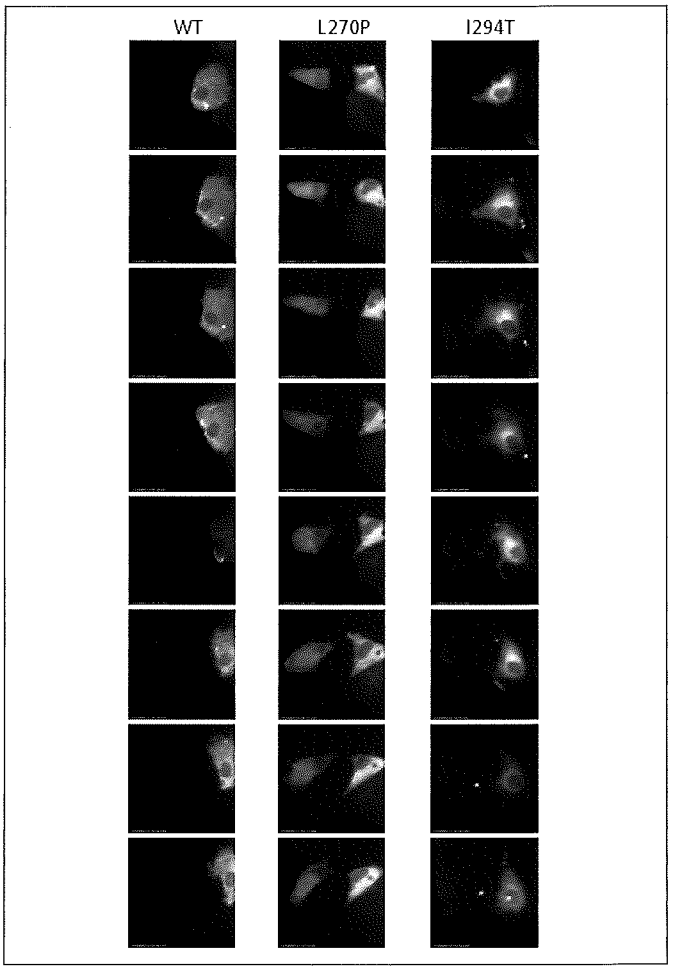


Figure 2. loss of directional movement in mutant WASP cells.



P.61. Implementation of HemoFAXS ®, an automated cell-locating device in the routine laboratory: experimental design and results of a preliminary evaluation

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Background
HemoFaxs® (TissueGnostics, Siemens, Germany) is a device, which automatically scans and classifies human blood cells on peripheral

blood smears. However, the constitution of a database is still a mandatory step at the laboratory level.

Objective
To extend our previously established database of normal blood cells by scanning blood smears from abnormal blood samples and next, to test it in our daily practice.

Methods
Blood smears were performed and stained with the automate Cell-Dyn ® (Abbott, USA).We scanned 200 abnormal blood smears of patients with various pathological conditions such as infections, chronic and acute leukemias and lymphomas as well as leucopenic samples Comparison was performed with the visual manual reclassification cells on the Hemofaxs® screen. Accuracy of the device blood cell classification was assessed globally and for each individual blood cell category.

Results
The global device classification accuracy was 76.4 ± 13.1% before database updating and 90.3 ± 6.2% after. With the enriched database the accuracy of the five most usual cell categories ranged from 95 to 99% except for the monocytes class with an accuracy of 83%. For abnormal cell categories, false negatives were < 20% and only 4 and 2% respectively for blast cells and NRBC. The completion of 8 differentials on the Hemofaxs including manual reclassification allowed us to spare 5 to 10 minutes time compared to the microscopic classification, depending on the technician's experience.

Discussion and conclusion
By updating our database we were able to improve the device performance not only for pathological slides but also for normal slides. However, a lot of false positive was still observed for abnormal cell categories. More scanning of abnormal blood smears is thus still necessary to improve those results. However, the detection of blasts and NRBC by the device is efficient, as attested by the low rate of false negative results for those cell types. Of particular interest also is that the Hemofaxs® differential is faster than the microscopic differential and certainly less tedious.