

**ABSTRACTS STATE OF THE ART LECTURES AND
NEW HORIZONS IN HAEMATOLOGY AND
THE P. STRYKMANS MEMORIAL LECTURE**

BIOLOGY OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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ALL is a neoplastic disorder that develops from one single hematopoietic B- or T-cell precursor that acquired oncogenic anomalies during its maturation process. It is a heterogeneous disease comprising several clinico-biological entities. If the cure rate of childhood ALL is now relatively high (> 80%), it remains low in adults (< 40%) and treatments are responsible for acute and late side effects, particularly when a transplantation is needed for cure.

During this lecture, we will mainly focus on biologic characteristics that are relevant for therapy and show how defects of specific genes disrupting key signaling pathways lead to leukemogenesis following a multi-steps process.

The heterogeneity of ALL is partly explained by differentiation arrests occurring at different stages of B- or T-cell development. Analysis of lineage-associated antigens of lymphoblasts allows to classify the disease according to the degree of maturation. Gene expression-array studies identify unique ALL subgroups with profiles corresponding also to specific arrest of differentiation and that are characterized by specific chromosomal rearrangements. These are usually translocations that activate transcription factor genes encoding proteins at the top of important transcriptional cascades involved in the control of cell differentiation. Translocations result in oncogene expression either by transcriptional activation, usually the consequence of translocations involving *TCR* or *IgH* loci, or by gene fusion which leads to the expression of chimeric proteins. Chromosomal deletions, often cryptic, gene amplifications and mutations are other mechanisms of oncogenes activation. About 25% of childhood B-ALL harbor the *TEL-AML1* fusion gene, generated by the t(12;21)(p13;q22) chromosomal translocation. Both *TEL* and *AML1* are important regulators of hematopoietic-cell development. In adult B-ALL, the most frequent chromosomal translocation is the t(9;22), generating the *BCR-ABL1* fusion gene. It encodes a constitutively activated chimeric tyrosine kinase protein that interacts for transformation with many other elements involved in differentiation, proliferation, and survival. In 10% of T-ALL, the class II homeobox gene, *TLX1 (HOX11)*, is aberrantly expressed as a result of its juxtaposition with promoter elements of *TCRA* and *B* following the t(10;14)(q24;q11) and the t(7;10)(q34;q24) translocations, respectively.

Beside genetic anomalies leading to differentiation arrest, cooperating mutations are needed to generate a fully transformed leukemic phenotype. Normal T and B-cell development requires a fine regulation of differentiation, self-renewal, proliferation, survival and death signals and it is now clear that a combination of mutations targeting each of those different cellular processes is required to give rise to a clinically overt acute leukemia. T-ALL is a paradigm of such multi-step leukemogenesis: defects in cell cycle control are a universal phenomenon, mostly due to loss of the *INK4/ARF* locus; mutations activating the *NOTCH1* signaling pathway are found in nearly 70% of cases, providing cells with self-renewal capacities; constitutive activation of tyrosine kinases (*LCK*, *FLT3*, *ABL1*, *JAK1/2*) or other signaling components (*RAS*) associated to response to growth/survival signals give a proliferation advantage to 30-40% of T-ALL. In B-ALL, genome-wide approach of childhood cases has shown a high frequency of mutations targeting *PAX5* and *IKAROS*, two key regulators of B-lymphocyte development.

The interest of accumulating data on the molecular mechanisms resulting in neoplastic transformation of hematopoietic cells resides in the development of rationally designed therapies targeting specific elements that are essential for B- and T-lymphoblast survival. For example, therapies targeting kinase proteins are already used for Philadelphia positive B-ALL and are probably useful in some T-ALL with ABL1 fusion variant proteins such as NUP214-ABL1. The challenge now is to understand how these cooperative genetic lesions interact to alter the proliferation, differentiation, and survival of lymphocyte progenitors leading to their leukemic transformation. It will provide the molecular rationales needed to select new therapeutic targets and to develop and combine inhibitors with high levels of anti-leukemic specificity.

Another important aspect of the biology of ALL from a therapeutic point of view is the pharmacogenetics of leukemic and normal host cells. Results from global gene-expression profiling studies have identified genomic determinants of treatment responses that could be used to develop polygenic models for optimization of treatment for ALL in order to avoid over- or undertreatment of individual patients.

To improve efficacy and decrease toxicity of the treatments, the future of therapy for ALL patients probably resides in individualized treatments based on the genetic features of the malignant cells and the own unique pharmacogenomics of the patient.

TYROSINE KINASE INHIBITORS IN HEMATOLOGY: PRESENT AND FUTURE

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Protein tyrosine kinases are an important family of signaling proteins that regulate cellular proliferation, survival, differentiation and motility. The human genome contains 90 protein tyrosine kinase genes, many of which have been identified as proto-oncogenes over the past 25 years. A variety of tyrosine kinase fusion genes and tyrosine kinase mutations are involved in the pathogenesis of hematological malignancies and are believed to represent novel targets for therapy. Indeed, the successful application of imatinib (gleevec/glivec) for the treatment of BCR-ABL positive chronic myeloid leukemia and FIP1L1-PDGFR α chronic eosinophilic leukemia demonstrates the efficacy of tyrosine kinase inhibitors for the treatment of chronic leukemia. The introduction of tyrosine kinase inhibitors in the treatment regimen for acute leukemias may be more difficult, in part by the development of resistance and the more complex genetics of these leukemias.

An overview will be provided of the tyrosine kinases that are implicated in the pathogenesis of hematological malignancies, and the recent developments in the use of kinase inhibitors.

PET IMAGING UPDATE IN HEMATOLOGY

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Metabolic imaging with fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) is now the most widely used and accepted functional imaging modality for the assessment of patients with Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL). FDG-PET has major impact on staging, restaging, risk-stratification and patient management in routine clinical practice. However, physicians should still interpret the result of these studies cautiously. The lack of specificity for tumoral tissue is only one important issue. An even more important problem is that we do not know whether more aggressive chemotherapy is really indicated if FDG-PET shows more advanced disease. All our current treatment recommendations concerning the systemic therapy are based on staging performed by conventional imaging techniques. FDG-PET is the best non-invasive imaging modality for early response evaluation. However, we need prospective randomized trials that demonstrate that the prognosis of PET-positive patients after a few cycles of chemotherapy can be improved by intensifying the treatment before using PET routinely in this indication. Posttreatment FDG-PET is a powerful predictor of outcome in both HD and aggressive NHL. The recently proposed, revised response criteria for lymphoma integrate metabolic and conventional anatomic imaging. FDG-PET should be done for end of treatment evaluation in all patients in clinical complete remission. Nevertheless, it is important to remember that PET findings suggesting residual disease should always be correlated with clinical data, other imaging modalities and/or a biopsy before starting salvage therapy. The role of FDG-PET for post-therapy surveillance without clinical, biochemical or radiographic evidence of disease remains controversial. Routine surveillance by PET is currently not indicated.

HODGKIN'S LYMPHOMA: AN UPDATE IN 2009

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Although classical Hodgkin lymphoma (cHL) is a germinal centre B (GCB) cell lymphoma that shows VDJ rearrangement, class switch and somatic hypermutation, most of the gene expression program of GCB cells is silenced in the tumor cells. Several lines of evidence suggest that epigenomic events, especially promoter DNA methylation, are involved in this silencing of many B-cell-associated genes. Although primary cHLs have a brisk inflammatory infiltrate, there is little evidence of an effective host antitumor immune response. The molecular signals and endogenous factors responsible for creating and maintaining the Th2-skewed immunosuppressive microenvironment in cHL remain to be defined, but galectin-1 may play an important role.

Current therapy for cHL is aimed at high cure rates and optimal survivorship. The optimal treatment for early-stage is still the subject of intense debate but 2-4 cycles of ABVD plus 30Gy involved field radiotherapy is considered as the gold standard for both favourable and unfavourable patients. To reduce radiotherapy late effects, Canadian and GELA/EORTC trials have tried to omit radiotherapy but this led in an unselected population to a significant lower EFS. For advanced disease, the gold standard is ABVD. The BEACOPPesc regimen resulted in higher rates of cure and survival compared with COPP/ABVD in the HD9 trial, therefore this regimen was compared to ABVD in the ongoing GELA/EORTC trial for advanced disease. The BEACOPPesc has not been so far universally adopted by physicians due to the attendant increased risks of early and late complications.

Early PET scan after 2 cycles of chemotherapy appeared to be excellent surrogate of the risk of relapse, it is currently used in early stage trial to customize the treatment and will also be used in the next trial for advanced disease in Belgium. Although enthusiasm for PET imaging is great, the challenges for using this diagnostic tool for risk-adapted therapies are substantial and it should not be used outside clinical trial.

Despite high cure rate, 10-15% of early stage and 25-30% of advanced stage patients will fail to respond or relapse after primary treatment, rescue can be proposed with alternative chemotherapy generally followed by high-dose chemotherapy with stem cell support. The role of allogenic stem cell transplantation in this situation remains controversial. New drugs including targeted therapies are evaluated for relapsing or refractory patients.

HOW TO MANAGE NEUTROPENIC FEVER?

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Today, the management of febrile neutropenia requires a risk-adapted strategy since it has been clearly shown that all the patients with febrile neutropenia do not have the same risk of complications and/death. The MASCC scoring system identifies safely the patients with a low (< 5%) risk of severe complications and minimal (< 1%) mortality; these patients represent roughly 70 % of an unselected population of patients with febrile neutropenia. A significant (50 %) proportion of these patients can be treated with orally administered antibiotics and can be discharged early from the hospital. The low risk patients who can not benefit from such a simplified therapy, those at a high risk of complications and death, and also those with prolonged or recurrent febrile neutropenia represent special and specific challenges for which continued research is mandatory.

IMMUNOTHERAPY OF CANCER: WHERE ARE WE?

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Most if not all human cancers express tumor antigens that can be targeted by T lymphocytes. These antigens are peptides of 9 to 15 amino acids, presented to CD8+ lymphocytes by HLA class I molecules, or to CD4+ lymphocytes by HLA class II molecules. These peptides are encoded either by genes mutated in tumor cells, or by genes such as the cancer-germline genes (e.g. MAGE genes) expressed in tumours but silent in normal tissues, or in the case of melanoma, by genes encoding melanocyte differentiation antigens such as *tyrosinase*, *gp100^{Pmel17}* and *Melan-A^{MART-1}*.

In the early nineties, when the first of these antigens were identified, it was thought that the immune system of cancer patients failed to recognize them, and that their use in vaccines would trigger T cell responses able to destroy the tumor cells. Clinical trials have been set up, in which patients with advanced cancer, often metastatic melanoma, were immunized against specific tumor antigens. Various types of therapeutic vaccines, adjuvanted or not, have been investigated, such as synthetic peptides, proteins, naked DNA, recombinant viruses and autologous dendritic cells. In general, these treatments have been very well tolerated. Tumor regressions have been observed with most of these vaccines, indicating an anti-tumoral effect. However, objective tumor responses have been limited to a small minority of vaccinated patients.

Increasing experimental data, including detailed analysis of immunological and tumoral material from vaccinated patients, indicate that cancer patients mount spontaneous immune responses against their tumor during its progression. These responses pressure the tumor to select variants that have become resistant to T cell mediated destruction. This resistance would explain the vaccine ineffectiveness observed in most patients. The molecular nature of the resistance mechanisms remains poorly understood. Their characterization will define new therapeutic targets. Counter-measures to tumor resistance coupled to cancer vaccines are likely to improve the clinical results of tumor immunotherapy.

CANCER STEM CELLS: NEW THERAPEUTIC TARGETS?

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The existence of Cancer Stem Cells (CSCs) with self renewal potential was first documented in leukemias (Bonnet et al, *Nat Med* 3:730, 1997), but has later been extended to solid tumors, including breast cancer (Al-Hajj et al. *Proc. Natl. Acad. Sci.* 100:3983, 2003). As these rare CSCs are both required and sufficient to reconstitute a new tumor, they have immediate and important clinical implications. Recent studies have shown that CSCs, as normal somatic stem cells, express high levels of multidrug resistance (MDR) pumps that efficiently efflux cytotoxic drugs, making them particular difficult to target with cytotoxic therapies. Similarly, they appear to be less immunogenic than more differentiated cancer cells in the tumor and therefore also more difficult to eradicate with immunotherapy. Thus, it has become evident, that CSCs might prove not only to be the most important but also difficult cancer cells to eliminate with conventional therapies, and that a specific monitoring and targeting of the elusive CSCs could become an important tool towards identification and characterization of improved cellular and molecular targets for development of improved cancer therapies.

Despite of the clear importance of CSCs in the genesis and perpetuation of cancers, little is currently known about the biological and molecular properties that make CSCs distinct from normal stem cells, the developmental/cellular origin of CSCs, the mechanisms responsible for their emergence in the course of the disease, and identification of candidate molecular targets for therapeutic intervention.

The isolation and characterisation of CSCs was first demonstrated for AML. Specifically, transplantation of primary AML cells into NOD/SCID mice led to the finding that only rare cells, termed SCID leukemia-initiating cells (SL-IC), are capable of initiating and sustaining growth of the leukemic clone *in vivo*, and serial transplantation experiments showed that SL-IC possess high self-renewal capacity, and thus can be considered to be AML stem cells. Existing cancer therapies have been developed largely against the bulk population. The lack of durable response in most cases, suggests that the treatment used may not effectively target the CSC population. Indeed, the failure of the current therapeutic regimens is likely related to the resistance and persistence of CSC. Thus, the identification of CSC has important implications for future research as well as for the development of novel therapies. Research focused on identifying and characterizing the rare cancer-initiating cells, should allow the identification of specific targets for CSCs, which if specific should provide effective cure and prevent disease relapse. The seminar will summarise our knowledge of Cancer Stem Cells notably in AML and will try to propose few new avenues that might be taken to eradicate these population of cells.

REGULATORY T CELLS IN TRANSPLANTATION

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Regulatory T cells (Tregs) represent specific T-cell subsets that play a key role in inducing and maintaining immunological tolerance. The CD4⁺ Tregs have been categorized into two major subgroups based on their ontogeny. The natural occurring Tregs, which develop in the thymus and are present in normal naive mice and healthy individuals since birth, and the inducible Tregs that are generated in the periphery under various tolerogenic conditions. Among the inducible Tregs, the T regulatory type 1 (Tr1) cells represent one of the most extensively characterized subsets. They are induced in the periphery by chronic exposure to antigen in the presence of IL-10 and they are defined by their unique cytokine production profile (*i.e.* IL-10⁺⁺, IL-5⁺, TGF-β⁺, IL-4⁻, IL-2^{low}, IFN-γ^{low}). Tr1 cells can suppress undesired immune responses mainly through production of IL-10 and TGF-β.

Tr1 cells were originally described *in vivo* in severe combined immunodeficient (SCID) patients after allogeneic HLA-mismatched hematopoietic stem cell transplantation (HSCT). The presence of host-reactive Tr1 cell clones correlated with the absence of graft versus host disease (GvHD) and with long-term graft tolerance without the need for immunosuppression. These data strongly supported the use of Tr1 cells generated *ex vivo* as immunomodulatory cell therapy in T-cell mediated diseases.

We are currently performing at our Institute a clinical trial in which donor T cells anergized *ex vivo* against the host cells in the presence of IL-10 are infused post-transplant in hematological cancer patients undergoing HLA-haploidentical HSCT. The goal of this cellular therapy with anergized donor T cells is to induce immune-reconstitution without GvHD. The administered cells are indeed anergic towards host-antigens, contain precursors of host-specific Tr1 cells able to differentiate in fully competent suppressor cells, but also include T cells able to respond to infectious agents and possibly to provide a graft versus leukemia effect. An alternative protocol to generate Tr1 cells *ex vivo* more efficiently is currently under development. Host dendritic cells differentiated in the presence of IL-10 can indeed generate anergic donor CD4⁺ T cells highly enriched in allo-Ag specific Tr1 cells.

Cellular therapy with alloantigen-specific Tr1 cells can be envisaged in the context of HLA-haploidentical bone marrow transplantation but also in allogeneic unrelated bone marrow transplants and in cell/organ transplantations in which there is high risk of graft rejection.

HYPOMETHYLATING AGENTS IN MYELOYDYSPLASTIC SYNDROMES

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Myelodysplastic syndrome is a hematopoietic stemcell disorder leading to an impaired hematopoiesis and inevitable to acute leukemia if the patient has not died due to the complications of the cytopenias. The risk of developing acute leukemia depends largely on the number of cytopenias, the existence of transfusion dependency but foremost on the presence or absence of certain cytogenetic abnormalities.

The prognosis of patients with a MDS can therefore be predicted by several risk scoring systems with the IPSS - and the WHO risk score as the most used ones. These risk scores can be used to help the physicians in making the right treatment choices. The only curable options is allogeneic stem cell transplantation, which of course can be used only in younger patients. RIC transplantation in patients up to 70 years is still a experimental approach. Since most patients with MDS are elderly, often with co-morbidity, transplantation is not an option and supportive care was the only possibility to improve the quality of the remaining live time. This option might be reasonable in those with a low change of disease progression. Erythropoietin eventually combined with G-CSF is important in the treatment of these patients However in patients with high risk MDS changing the natural course of the disease is the only possibility to improve both survival and QoL.

During the last decade a few drugs are developed that seems to change the course of the disease. Indeed Lenalidomide has shown activity in up to 70% in patients with a 5q minus syndrome. Not only does it correct the anemia but in many cases even a complete cytogenetic response was observed. When together with the 5q- other chromosomal abnormalities are present the response rate is remarkably lower although List e.a. still claim improvements of 50% of the erythropoiesis. In higher risk MDS this drug might probably not such a good choice.

Another new class of drugs are the azanucleosides; 5' Azacytidine (Vidaza) and 5'-2-deoxycytidine (Decitabine). It is thought that these drugs, when given in low concentrations, work via epigenetic pathways. Probably these drugs can work via demethylation of promoters of tumor genes that are hypermethylated during the course of the malignant stemcell. Indeed in vitro and in vivo they can induce demethylation. It was shown that hypermethylated tumorsuppressor genes like p15 could be demethylated with the re-expression of the proteinsynthesis. It is still unclear if patients with hypermethylated genes are the only if better responders. Phase II studies and phase III studies have revealed that when used in the now known treatment schemes response rates are seen in up to 40 till 50% of the patients with a remarkable high number of complete cytogenetic responders. Both drugs have shown a Progression Free Survival benefit in high risk MDS patients. In a large multicentre study Fenaux e.a. reported a median survival benefit for patients treated with Vidaza of almost 10 months when compared with patients who get either supportive care or low dose Ara C or intensive chemotherapy. Decitabine has shown in a recent EORTC study to improve the PFS substantially in a group of very high risk MDS patients. These drugs have shown to be usefull in patients with myeolodysplastic/myeloproliferative syndromes like CMML as well. It use in elderly patients with slowly progressive AML might also be good candidates. It is clear that the optimal use of these drugs is still not known but since the adverse effects of these drugs are extremely low and the treatment approach is so promising and exciting we can expect a lot of new information for these patients who have such a bad life expectancy with such a low quality of life.

INNOVATIVE COAGULATION DIAGNOSTICS THROUGH MEASUREMENT OF THROMBIN GENERATION

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Thrombin is the key biologically active product of the haemostatic mechanism, as is apparent from its many functions, including pro- and anticoagulant activities and pro-inflammatory effects. Thrombin generation analysis therefore is a physiological function test of the haemostatic capacity of the isolated organ blood (-plasma). Unlike clotting times (PT, aPTT, WBCT) it also detects hyper-function, i.e. a tendency to thrombosis, which is clinically much more relevant than bleeding tendency is.

The capacity of a plasma to generate thrombin is reflected in the thrombin generation curve (Thrombogram) and notably in the endogenous thrombin potential (ETP), which is the area under the thrombin generation curve and is a direct measure of the amount of "enzymatic work" that thrombin can do. The currently available thrombin generation assay (Calibrated Automated Thrombogram, CAT) allows routine, quantitatively correct measurement of the thrombin generation curve. Alternative devices are semi-quantitative and/or require addition of polymerisation inhibitors that strongly influence normal thrombin generation.

Monitoring of the thrombogram allows (i) to detect ongoing thrombosis; (ii) to detect increased risk of thrombosis (iii) to install and monitor antithrombotic treatment; (iv) to detect bleeding disorders and monitor their prophylaxis and therapy. Although arterial and venous thrombosis show important differences it seems likely that thrombin generation can cover all these indications. Especially when thrombin generation is not only carried out in platelet poor plasma but also in platelet rich plasma – which is already possible - and in whole blood, which is being developed.

THE TREATMENT OF T-CELL LYMPHOMAS

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Systemic peripheral T-cell lymphomas (PTCL) are uncommon and, with the exception of alk-positive anaplastic large cell lymphoma (ALCL), carry a worse prognosis than the B-cell non-Hodgkin lymphomas. There is no uniformly recognized standard therapy for PTCL. Although the standard chemotherapy for aggressive NHL is with anthracycline-containing combination regimens, less than 30% of patients with PTCL have a complete response (CR) to such therapy. Furthermore, relapsed and chemotherapy-refractory disease has a very poor prognosis.

Autologous stem cell transplant (ASCT)

ASCT has been shown to have a favourable impact on relapsed aggressive B-cell lymphomas and has consequently also been adopted as rescue strategy in PTCL. The role of intensified treatment schedules consolidated by upfront ASCT as 1st line therapy in PTCLs is debated. Recently, a German phase II study of 83 previously untreated PTCL patients evaluated the impact of 4-6 series of CHOP q 3 weeks followed by a mobilizing course of Dexamethasone-Beam and TBI-based ASCT. The final analysis showed a 3yrs OS of 50% with a 3-yrs PFS of 35%. The Nordic Lymphoma Group designed a prospective multicenter phase II study to evaluate the impact of a dose-intensified induction schedule (6 courses of two-weekly CHOEP) consolidated in 1st PR/CR by BEAM conditioning and ASCT in previously untreated systemic PTCL. With a total of 166 patients enrolled this is, by far, the largest prospective PTCL clinical trial completed to date. Newly diagnosed systemic PTCL cases aged 18-67 yrs were included. Cases of ALK-positive ALCL, primary cutaneous, leukemic and lymphoblastic T-NHL were excluded. Of the 166 PTCL cases, 160 were histologically confirmed at referral center level: PTCL unspecified (n=62; 39%), ALK-neg ALCL (n=31; 19%), angioimmunoblastic type (AIL) (n=30; 19%), enteropathy-type (n=21; 13%), panniculitis-like (n=6; 4%), T/NK nasal-type (n=5; 3%), hepatosplenic (n=5; 3%). The M/F ratio was 2.0 and the median age 57 yrs (range 22-67 yrs). The majority of the cases presented with stage III-IV disease (81%), B-symptoms (59%) and/or elevated s-LDH (62%). Nevertheless, 71% of all patients had a good performance score (WHO 0-1) at inclusion. The final analysis of the study is currently in progress.

Immunotherapy

Early studies on T-PLL and cutaneous T-NHL demonstrated that malignant T-cells often express high levels of CD52, making targeted therapy with CD52-specific antibodies a rational therapeutic strategy. Recent reports in systemic PTCL have suggested that only a fraction of cases (30-40%) expresses CD52 when assessed by immunohistochemical technique on paraffin tissue. The possibility of artifacts influencing the expression of the antigen in the context of formalin fixed, paraffin-embedded tissue is still debated. Indeed, the humanized anti-CD52 monoclonal antibody, alemtuzumab (ALZ), has been shown to be an effective monotherapy for patients with heavily pre-treated refractory systemic PTCL, resulting in an overall response of 36% in one early pilot study. These results led to several phase II studies investigating ALZ either as a consolidation treatment following CHOP-like induction therapy in aggressive untreated PTCL or as part of the primary treatment regimen combined with CHOP/CHOP-like or fludarabine-containing regimens. Some of these phase II 1st line trials, achieved high CR rates (60–75%). As for rituximab

in B-NHL, ALZ may, in PTCL, owe its efficacy to the ability to enhance the cytotoxicity of chemotherapies by a complementary mode of action. A potential outcome benefit of the addition of ALZ to CHOP chemotherapy in the first-line setting is currently being explored in an ongoing international, multi-centre, phase III trial (ACT trial). Other monoclonal relevant antibodies relevant in PTCL have been tested in clinical trials. Clinical results with anti-CD4 and anti-CD30 will be shown.

New compounds

Several new compounds have been tested in the relapsed/refractory setting. Among these, the folinic acid inhibitor pralatrexate, a methotrexate analog, histone deacetylase inhibitors (e.g. romidepsine) and antiangiogenic drugs (e.g lenalidomide) have shown promising preliminary clinical results.

AMYLOIDOSIS

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Amyloidosis results from the extracellular deposition of fibrillar amyloid protein. Amyloid is defined by the tinctorial properties of binding of Congo red dye and green birefringence under polarized light. The symptoms of the disorder, including fatigue, edema, and weight loss, are vague and are generally not helpful in the formulation of an appropriate differential diagnosis. Amyloidosis must be considered in the differential diagnosis of monoclonal gammopathies and in any patient presenting with nephrotic range-proteinuria, unexplained cardiomyopathy, unexplained hepatomegaly, or peripheral and autonomic neuropathy.

Suspicion of amyloidosis should lead to immunofixation of the serum and urine as well as an immunoglobulin-free light chain assay. One of these 3 tests will be abnormal in 99% of patients with immunoglobulin light chain amyloidosis.

The diagnostic verification of amyloidosis requires a biopsy. Although patients with amyloid nephrotic syndrome, cardiomyopathy, hepatomegaly, or neuropathy will have the diagnosis established by renal, cardiac, liver, or nerve biopsy, respectively, most patients do not need to have this invasive, potentially risky procedure. The diagnosis can be established by doing Congo red stains on a bone marrow biopsy specimen, which will detect amyloid deposits in 50 to 60% of patients. The subcutaneous fat aspirate is a convenient, noninvasive technique that demonstrates amyloid deposits in 70% to 80% of patients. Biopsy of the minor salivary glands, gingiva, rectum, and skin can reveal deposits at little risk to the patient. Immunohistochemical staining of tissues with commercially available antisera helps confirm the nature of the amyloid as AL.

The ultimate prognosis of amyloidosis is determined in large part by the extent and functional impairment that results from cardiac amyloid infiltration. Assessment of cardiac function with 2-dimensional echocardiography and use of cardiac biomarkers, troponin and brain natriuretic peptide, can help assess the severity of cardiac involvement and the ultimate prognosis for a patient.

The treatment of immunoglobulin light chain amyloidosis (AL) remains inadequate. Often, the diagnosis is made late in the course of the disease. When advanced organ dysfunction is present, particularly of the heart, effective suppression of light chain synthesis will not result in recovery of impaired organ function, and the natural history of the disease cannot be altered. Even when diagnosed early, amyloidosis remains a therapeutic challenge. Initially, therapy for amyloidosis was based on the use of colchicine because of the drug's efficacy in preventing the secondary amyloidosis of familial Mediterranean fever. Since then, however, all therapies have paralleled those used for multiple myeloma. This is reasonable since both amyloidosis and multiple myeloma result from a clonal proliferation of plasma cells. In most patients with amyloidosis, the clonal plasma cells are nonproliferative, and overt multiple myeloma does not coexist. Multiple myeloma produces symptoms as a result of tumor mass, impairment of erythropoiesis, and bone destruction, etc. Symptoms of amyloidosis are rarely related to tumor mass and are a consequence of either the deposition of insoluble light chains or heavy chains as β -pleated sheets into the tissue or the production of toxic soluble intermediates that directly interfere with organ function.

Thirty years ago, the combination of melphalan and prednisone in oral formulations was introduced for the treatment of amyloidosis. Prospective randomized studies demonstrated survival benefit. Unfortunately, measurable response rates in that era were only in the 15% range, and the median survival of treated patients was only 17 months, although nearly 5% can survive 10 years.

The Southwest Oncology Group demonstrated the activity of dexamethasone in the treatment of amyloidosis and reported a hematologic response rate of 24% and an organ response rate of 45%. Dexamethasone has subsequently been incorporated into melphalan-containing regimens with hematologic response rates of 67% and organ response rates of 48%. In multiple myeloma, autologous stem cell transplantation has been shown to produce survival benefits in carefully performed phase 3 studies. Therefore, it was logical to apply this technique to patients with amyloidosis (another plasma cell disorder). However, the management of amyloidosis with high-dose chemotherapy is distinct from that of multiple myeloma. Patients with multiple myeloma have abnormal bone marrow but generally have good organ function, and mortality rates at Mayo Clinic are between 1% and 2%. Amyloidosis, however, is characterized by bone marrow that is only minimally involved with tumor, but organ dysfunction can be widespread, leading to a wide range of posttransplantation complications, including dialysis-dependent renal, cardiac, and hepatic failure. At Mayo Clinic, the overall day-100 mortality rate associated with transplantation since the start of our program is 12%, but in calendar year 2005, it was decreased to 8%, presumably a result of increasing experience with autologous stem cell transplantation and appropriately modifying conditioning chemotherapy dosage.

Data has been reported from the International Blood and Marrow Transplant Registry on 107 patients who underwent autologous hematopoietic stem cell transplantation to treat amyloidosis. Issues regarding patient selection by inexperienced centers might account for the 30-day treatment-related mortality of 18%. The 30-day mortality rate for autologous hematopoietic stem cell transplantation in the Mayo program is 7.4%. Only 10% of patients experienced disease progression after transplantation. The survival data, however, must be viewed carefully since the median projected survival is 47 months, but the median follow-up is only 30 months. Of note, there were 304 patients in the registry but sufficient data in only 107, and the loss of two thirds of the population may lead to unanticipated reporting bias. The role of transplantation experience in improving outcomes is seen as the only predictor of survival in this study because performing transplantation within the past 5 years was associated with decreased early mortality. Therefore, it remains unclear whether transplantations for amyloidosis should be performed only in specialized centers with extensive experience or can be done at centers with transplantation experience in nonamyloid disease. An Eastern Cooperative Oncology Group study suggested no difference in mortality in patients with amyloidosis between centers.

Stem cell transplantation has not been proved to be superior therapy for amyloidosis. Patients eligible for autologous transplantation represent a highly selected group by virtue of younger age, a lower proportion with advanced cardiac involvement, and fewer numbers of organs involved. This selection of the "cream of the crop" has been demonstrated in analyzing the outcomes of patients eligible for transplantation but did not undergo transplantation. These patients had a much better outcome than other patients who would not have been suitable for transplantation. One case-matched control series has suggested an improved outcome for amyloidosis, but a recently reported phase 3 trial

involving 100 patients with amyloidosis was unable to demonstrate survival benefit of transplantation. However, in that study, transplant-related mortality at day 100 was 24%, and at the end of the study, only 29 patients undergoing transplantation were evaluable for response. A determination of the ultimate value of stem cell transplantation will depend on a phase 3 study with a larger number of patients. Until that time, the best treatment for amyloidosis remains uncertain.

There have been reports that the natural history of amyloidosis varies among centers, and the number of patients referred for consideration of autologous transplantation cannot be known from a registry study. At Mayo Clinic, no more than a quarter of patients referred ultimately undergo stem cell transplantation. Nonetheless, the report by Vesole et al represents the largest number of patients reported from multiple centers and is a realistic appraisal of the feasibility and risks associated with autologous transplantation for amyloidosis. Outside of a study, it is difficult to routinely recommend stem cell transplantation as clearly superior based on the current body of knowledge, and patients should be enrolled in scientifically conducted clinical trials.

Current protocols using novel agents in the treatment of multiple myeloma, such as lenalidomide and bortezomib, are under way. Whether these agents will ultimately affect the decision to administer myeloablative chemotherapy to patients with AL remains unknown.

Suggested Reading

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P. STRYCKMANS MEMORIAL LECTURE RECENT IMPROVEMENTS IN MULTIPLE MYELOMA

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While there has been almost no improvement in the prognosis of Multiple Myeloma (MM) until the nineties, in the past 20 years, the management and the survival of patients with MM has dramatically changed for a variety of reasons

I A better understanding of the pathophysiology of MM

- The malignant clone has been better characterized, which has important consequences in clinical practice. For instance, the malignant plasma cell phenotype has a prognostic impact. It is now possible to evaluate minimal residual disease by multiparameter flow cytometry and immunophenotypic complete remission is associated with a better outcome. Genetic abnormalities are multiple, including immunoglobulin gene rearrangements and their knowledge has provided information about the clonal origin of the disease and the natural history of myeloma development, and has become a useful tool for prognosis, and classification of MM. Fish analysis of large series of patients have determined the incidence and the prognostic value of del (13), t(4;14) or del (17p) which are currently the most relevant in clinical practice. Gene expression profile or more recently SNIP-arrays also provide useful prognostic indications
- IL-6 as well as other cytokines plays a major role in MM cell proliferation and survival. Signal transduction pathways are potential targets for new agents under development. Moreover it has been shown that the interaction between the MM cell clone and the bone marrow microenvironment is a major factor of MM cell survival and resistance to therapeutic agents.
- The pathophysiology of bone disease has been studied and the role of the RANK-L/ osteoprotegerin ratio in osteoclastic hyperactivity has been emphasized which, again, will have important therapeutic consequences

II Major therapeutic advances

- The first improvement occurred in the eighties when it was shown that high doses of melphalan supported by autologous stem cells infusion could induce a high response rate, including complete responses. Randomized trials have shown that autologous stem cell transplantation (ASCT) improved the response rate, the progression free survival and in some studies the overall survival. ASCT is currently considered the standard of care for patients without renal failure up to the age of 65. However despite high-dose therapy, relapses eventually occur in most patients and further intensification with double ASCT induced only marginal benefit. Moreover ASCT is usually not proposed to elderly patients while median age at diagnosis is round 70
- The next step was the introduction of novel agents that are active not only on the MM cell but also on the microenvironment or on the immune system. Three agents have been approved in the past few years (thalidomide, bortezomib and lenalidomide). They were first developed in relapsed MM and provided new therapeutic possibilities in addition to alkylating agents and corticosteroids. Their sequential use in relapsed MM already increased median overall survival by 2 years. (from 3 to 5 years)

- These agents are currently widely used as part of frontline therapy in two different settings. In patients who are not candidates for ASCT (over the age of 65) the addition of thalidomide or bortezomib to the classic melphalan-prednisone combination is significantly superior to the combination alone and these two agents were recently approved in Europe in this indication. Moreover they yield complete or very good partial remission and progression-free survival rates that are quite comparable to those achieved with ASCT in younger patients. Same encouraging results are seen with long-term treatment with the combination of lenalidomide plus dexamethasone. These non-intensive approaches are therefore challenging high-dose therapy. However, these three novel agents can also be used in the context of high-dose therapy. When thalidomide is used after ASCT it increases complete remission rate, progression-free survival and overall survival. Novel agents are also used in induction treatment prior to ASCT. Bortezomib-containing regimens (bortezomib-dexamethasone with or without a third agent, doxorubicin, cyclophosphamide or thalidomide) increase the complete plus very good partial remission rate not only before but also after ASCT, which could significantly improve progression-free survival as well.

The use of novel agents both before and after ASCT could further increase the number and duration of complete remissions and could overcome the poor prognosis associated with t(4;14)

In conclusion, the recent therapeutic improvements using novel agents as well as the better prognostic classification raise a fundamental question: is it possible to cure at least some patients and should the objective of initial therapy be to search for cure or to transform MM in a chronic disease ?

ABSTRACTS ORAL PRESENTATIONS
0.01 – 0.10

O.01

MICRORNA-29C AND MICRORNA-223 DOWNREGULATION HAS IN VIVO SIGNIFICANCE IN CHRONIC LYMPHOCYTIC LEUKEMIA AND IMPROVES DISEASE RISK STRATIFICATION

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Background

Aberrant expression of microRNAs has been recently associated with chronic lymphocytic leukemia (CLL) outcome. Although disease evolution can be predicted by several prognostic factors, a better outcome individualization in a given patient is still of utmost interest.

Methods

In the current study, we investigated the expression of two microRNAs, miR-29c and miR-223, by real-time PCR (qPCR), compared them to other biological or clinical markers and assessed a qPCR score to better predict CLL outcome.

Results

We showed that miR-29c and miR-223 expression levels decreased significantly with progression from Binet stage A to C (n=110), were significantly lower in poor prognostic subgroups defined by cytogenetic abnormalities (n=81), IgVH mutational status (n=104), lymphocyte doubling time (n=93), solubleCD23 (n=91), beta-2-microglobulin (n=78), zeta-associated protein 70 (ZAP70) (n=110), lipoprotein lipase (LPL) (n=110) and CD38 expression (n=104) and could significantly predict treatment-free survival (TFS) and overall survival (OS) (n=110). We subsequently developed a qPCR-score combining miR-29c, miR-223, ZAP70 and LPL (from 0 to 4 poor prognostic markers) to stratify treatment and death risk in a cohort of 110 patients with a median follow-up of 72 months (range, 2-312). Patients with a score of 0/4, 1/4, 2/4, 3/4, and 4/4 had a median TFS of >312, 129, 80, 36 and 19 months, respectively (hazard ratio, HR=17.00, P< 0.0001). Patients with a score of 0-1/4, 2-3/4 and 4/4 had a median OS of >312, 183 and 106 months, respectively (HR=13.69, P=0.0001). Finally, in Binet stage A patients (n=77), this score remained relevant and significant for TFS and OS prediction (HR=18.56, P< 0.0001 and HR=12.5, P=0.0068, respectively).

Conclusions

miR-29c and miR-223 levels were decreased in poor prognostic patient subgroups. A low level of these microRNAs was associated with disease aggressiveness, high tumor burden and poor outcome; we proposed and validated a qPCR score to better predict the evolution of individual CLL patients. This score will help to identify patients who will need early therapy and thus require a closer follow-up.

O.02

MODULATION OF MIR-449A, MIR-213 AND MIR-107 EXPRESSION DECREASES CELL VIABILITY AND INDUCES DIFFERENTIATION IN EVI1 DEREGULATED LEUKEMIA CELLS

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Chromosomal rearrangements involving the EVI1 gene are a recurrent finding in malignant myeloid disorders. EVI1 transcriptional activation is present in 10% of acute myeloid leukemia (AML) patients, and is a prognostic marker of poor outcome. Recently, microRNA (miRNA) deregulation was identified as a major contributor to cancer initiation and progression. Furthermore, miRNA genes were shown to be directly regulated by activated proto-oncogenes.

We performed microRNA profiling on patient samples and on siRNA mediated EVI1 knockdown model systems of the EVI1 rearranged myeloid leukemia cell lines Kasumi-3, UCSD-AML1 and MUTZ-3. Our patient cohort consisted of 38 EVI1 rearranged and overexpressing samples as confirmed by FISH, karyotyping and RT-qPCR, 6 normal bone marrow samples and 2 CD34+ fractions. A total of 360 miRNAs were profiled through high-throughput stem-loop RT-PCR followed by pre-amplification and RT-qPCR of individual miRNAs.

We were able to identify 26 significantly up- and 27 significantly downregulated miRNAs ($p < 0.05$) in EVI1 rearranged patient samples compared to normal bone marrow. Among these, 2 up- and 6 downregulated miRNAs were also identified as being differentially expressed in the EVI1 knockdown model systems.

The expression of 3 selected differentially expressed miRNAs, i.e. the downregulated miR-449a and upregulated miRs-213 and -107, was reconstituted by electroporation of a precursor miRNA or anti-miRNA molecules, respectively, in the Kasumi-3 and UCSD-AML1 cell lines. A decreased cell viability was detected as compared to the controls, with the strongest effects noticed for miR-449a. Furthermore, loss of 'early' myeloid markers such as CD34 and CD117, and an increase of megakaryocytic and monocytic markers such as CD36, CD14, CD41 and CD42b, were indicative for an effect on myeloid differentiation.

Given the poor prognosis of EVI1 rearranged leukemias and limited treatment options, the present finding together with the emerging possibilities of miRNA based therapeutics opens new perspectives for treatment.

O.03

IDENTIFICATION OF A HUMAN NATURAL REGULATORY T CELL MICRO-RNA SIGNATURE AND DEMONSTRATION OF THE MAJOR ROLE PLAYED BY MIR-31 AND MIR-21 IN THE CONTROL OF FOXP3 EXPRESSION.

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Regulatory T cells (Tregs) are the main mediators of dominant tolerance. Their mechanisms of action and applications are subjects of considerable debate at the moment. However, a human micro-RNA Treg signature has not been described yet. We investigated human natural regulatory T cells and identified a signature composed of five micro-RNAs (21, 31, 125a, 181c and 374). Among those five, two were considerably under-expressed (miR-31 and miR-125a). We identified functional target sequences for miR-31 in the 3' UTR of Foxp3 mRNA. Using lentiviral transduction of fresh cord blood T cells, we could demonstrate that two of these miRs had an effect on Foxp3 expression levels. The most important, miR-31 negatively regulated Foxp3 expression, via a direct mechanism, linked to the 3'UTR sites of its RNA. We could next demonstrate that miR-21 acted as a positive, though indirect, regulator of Foxp3 expression. Transduction of the remaining three miRs had no direct effect on Foxp3 expression or on the phenotype and will remain the subject of future investigations. In conclusion, not only have we identified and validated a miR signature for human natural Treg, but we also unveiled some of the mechanisms by which this signature was related to the control of Foxp3 expression in these cells.

O.04

ALL-ASSOCIATED JAK1 MUTANTS ACTIVATE THE JAK/STAT PATHWAY VIA IL-9RALPHA HOMODIMERS.

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Point mutations in the JAK2 tyrosine kinase play a major role in the development of myeloproliferative neoplasms by activating homodimeric cytokine receptors such as EpoR and TpoR. More recently, activating mutations in JAK1 have been shown to be associated with ALL, but little is known about their mode of interaction with cytokine receptors. Here, we studied the ability of several ALL-associated JAK1 mutants to activate the JAK/STAT pathway alone or together with the other components of the IL-9 receptor complex (IL-9R α , γ c and JAK3). Expression of the JAK1 mutants alone failed to trigger STAT activation, but coexpression of the IL-9R α chain promoted JAK1 mutant phosphorylation and STATs activation, even without γ c and JAK3. Mutation of the FERM domain of JAK1, critical for cytokine receptor association, or of the single tyrosine of IL-9R α involved in STAT recruitment abolished this activity. Several lines of evidence indicated that IL-9R α homodimerization was involved in this process. IL-9R α variants with mutations of the JAK-interacting BOX1 region not only failed to promote JAK1 activation, but acted as dominant negative forms reverting the effect of wild-type IL-9R α . Coimmunoprecipitation experiments also showed the formation of IL-9R α homodimers. Interestingly, the effect of IL-9R α was partially inhibited by the expression of γ c, suggesting that overlapping residues are involved in IL-9R α homodimerization and IL-9R α / γ c heterodimerization. Co-expression of wild-type JAK3 partially reverted the inhibitory effect of γ c, indicating that JAK3 can cooperate with JAK1 mutants within the IL-9 receptor complex, even in the absence of the cytokine. Similar results were also observed with IL-2R β , which binds JAK1, but not for JAK2-associated homodimeric receptors such as TpoR or EpoR. Taken together, our results show that IL-9R α and IL-2R β homodimers can efficiently mediate constitutive activation of ALL-associated JAK1 mutants, even in cells lacking the expression of γ c.

O.05

MICRORNA SIGNATURES IN GENETIC SUBTYPES OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes. Leukemic transformation of immature thymocytes is caused by a multistep pathogenesis involving genetic abnormalities providing uncontrolled cell growth. Accumulating evidence suggests the presence of at least 5 different molecular-cytogenetic subgroups in T-ALL linked to TAL/LMO, TLX1, TLX3, HOXA and MYB rearrangement. Recently, microRNAs were discovered as important regulators of protein expression and subsequently shown to be directly implicated in cancer. The role of miRNAs in the pathogenesis of T-ALL has not been studied thusfar. In this study, we investigated whether different genetic subgroups in T-ALL are characterized by distinct miRNA expression patterns. In total 360 miRNAs were profiled using high-throughput stem-loop qRT-PCR in a large T-ALL patient cohort (n=52), including 11 HOXA, 16 TAL/LMO, 11 TLX3 and 5 TLX1 rearranged patient samples. We also profiled sorted T-cell subpopulations which served as a negative control for the identification of deregulated miRNA expression that may be leukemia associated. SAM analysis identified significant differentially expressed miRNAs between the HOXA, TLX3 and TAL/LMO subgroups whereas no significant differentially expressed miRNAs were obtained for the TLX1 subgroup. The HOXA subgroup showed specific up-regulation of miR-196a and miR-196b, which are encoded at the HOXB and HOXA cluster, respectively, but no significantly down-regulated miRNAs could be identified. The TLX3 subgroup was characterized by the up-regulation of miR-99a, miR-125b, let-7c, miR-508 and miR-509, and down-regulation of miR-127 and miR-182. Whereas in the TAL/LMO subgroup up-regulation of miR-424, miR-148a, miR-422, miR-362, miR-148a, miR-502, miR-10a, miR-200c, miR-31, miR-660 and miR-15b, and down-regulation of miR-99b, miR-155, miR-125a, miR-153, miR-135a, miR-34a and miR-193b was observed. In conclusion, this study shows that molecular-cytogenetic subgroups in T-ALL are characterized by a specific miRNA expression signature. This report paves the way for further investigation directed at the role of these miRNAs in the pathogenesis of T-ALL.

O.06

OUTCOME OF T-CELL LYMPHOBLASTIC MALIGNANCIES: PAEDIATRIC AND ADULT COHORT IN ONE SINGLE CENTRE.

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Precursor T-cell malignancies are more aggressive than precursor B-cell ALL, in childhood and in adults. The purpose of this study was to analyse the outcome of paediatric and adult patients with a T-cell lymphoblastic malignancy treated in a single institution.

Patients

Between August 1990 and September 2006, 82 patients were diagnosed with T-ALL (n=50) or T-LBL (n=32), 60 males and 22 females. 40 patients with median age of 6.5 years (range 0.9 - 15.6) were treated in the paediatric haemato-oncology unit and 42 with median age of 31.8 years (range 15.2 - 62.3) in the adult unit.

Results

Median follow-up was 4.6 years (range 0-16.1). One child died at day 3 due to DIC. Three patients never achieved a CR. Two adults died in first CR due to transplant related mortality. All relapses (n=23) occurred in the first 3 years, 20 never achieved a second CR. One adult died 9 years after diagnosis due to a melanoma. Overall survival rate at 5 years was 55.3% for the adult group and 79.3% for the paediatric group (p=0.017). The robustness of this result was verified by correction for gender, disease type ALL or LBL, T-cell immunophenotype, LDH and albumine level, white blood cell count, platelet count and haemoglobin level. There was no statistical difference in OS between males and females, ALL and LBL, or different T-cell immunophenotype. Overall survival after 5 years was 76% for children ≤10 yrs (n=30), 90% for patients 10-25 yrs treated on the paediatric protocol (n=10), 68.8% for patients 10-25 yrs treated on the adult protocol (n=16) and 46.4% for adults >25 yrs (n=26), (p=0.025). The difference in OS between the two 10-25 yrs groups was not significant (p=0.28), but groups and number of events are small.

Conclusion

In high risk T-cell malignancies statistical analysis showed a significant better OS for the patient population treated with the paediatric regimen than with the adult regimen. In adolescents the paediatric protocol led to a higher survival rate than the adult strategy.

O.07

RITUXIMAB IN AUTO-IMMUNE HEMOLYTIC ANEMIA AND IMMUNE THROMBOCYTOPENIC PURPURA: A BELGIAN RETROSPECTIVE MULTICENTRIC STUDY

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Background

Although treatment with rituximab is effective in some patients with relapsing or refractory auto-immune hemolytic anemia (AIHA) or with immune thrombocytopenic purpura (ITP), available data are still limited, especially with regard to the duration of responses.

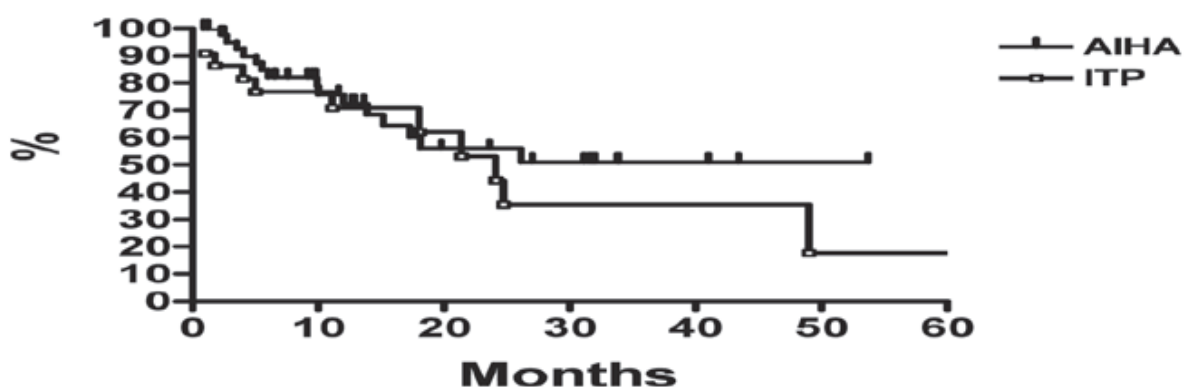
Design and methods

In order to better characterize the effect of anti-CD20 therapy, we retrospectively analysed the use of rituximab in Belgian patients experiencing AIHA and ITP.

Results

68 courses of rituximab in 53 patients with AIHA and 43 courses in 40 patients with ITP were analyzed. All patients were given rituximab after failing at least one previous line of treatment, including splenectomy in 19% and 72.5% of AIHA-patients and ITP-patients respectively. ORR were 79.2% in AIHA and 70% in ITP, with a median follow up since first rituximab administration of 15 months (range 0.5-62) in AIHA and 11 months (range 0-74) in ITP. Progression free survival at one and two years were 72% and 56% in AIHA and 70% and 44% in ITP.

Figure 1. PFS in patients with AIHA and ITP treated with rituximab



In this retrospective analysis we were not able to identify pre-treatment characteristics predictive for response to rituximab. Nine patients with AIHA and three patients with ITP were given one or more additional courses of rituximab. Most of these patients, who had responded to a previous course, experienced a new response comparable to the previous one, both in terms of quality and of duration of response. Finally, the outcome of patients who failed to respond to rituximab therapy was poor both in terms of response to subsequent therapy and in terms of survival.

Conclusions

This study confirms that rituximab induces responses in a majority of previously treated patients with AIHA and ITP. Response duration generally exceeds one year. Retreatment with rituximab in responding patients is most often successful. The outcome of patients who fail on rituximab is poor. We were not able to identify pre-treatment patient characteristics predicting for response.

O.08

NOTCH REGULATES MULTIPLE MYELOMA CELL BEHAVIOR VIA INHIBITION CD147 EXPRESSION

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CD147 is a multifunctional glycoprotein highly enriched on the surface of malignant tumor cells. CD147 promotes tumor invasiveness, metastasis and angiogenesis, by stimulating the secretion of several matrix metalloproteinases and VEGF. CD147 has also been identified as a new subunit of the gamma-secretase complex in Alzheimer's disease amyloid beta-peptide production (Proc Natl Acad Sci USA. 2005,24:7499-504.). Removal of CD147 from gamma-secretase complexes increases the production of Aβ-peptides, suggesting that CD147 is a negative regulatory subunit of the gamma-secretase complex. A very similar or identical gamma-secretase activity is also needed for cleaving the transmembrane domain of the Notch receptor after interaction with a cognate ligand. However, the regulatory relationship between Notch and CD147 is still unclear. In the current study, the function of CD147 and the regulatory relationship between Notch and CD147 in murine 5T33MM multiple myeloma cells were investigated. We demonstrated that CD147 is highly expressed on the 5T33MM myeloma cells. Blocking CD147 with a functional monoclonal antibody induced cell apoptosis, as well as inhibition proliferation, migration and invasion of 5T33MM cells in vitro. Moreover, the secretion of MMP-2 and MMP-9 by 5T33MM cells was also decreased. In addition, we found that the expression level of VEGFa in 5T33MM cells was also down-regulated as measured by Real-time PCR, however we did not observe a significant change in expression levels of Notch downstream genes Hes1, Hes5, Hey1, Hey2 and HeyL, suggesting that CD147 can not regulate Notch signaling via its role as a subunit of the gamma-secretase complex and Notch is thus not a target gene of CD147 signaling. When using the gamma-secretase complex inhibitor L685,458 to block Notch signaling, we observed that the expression level of CD147 was significantly up-regulated on both mRNA and protein levels under both normoxic and hypoxic conditions. Analysis the promoter sequence of CD147 revealed that it harbors 16 Notch downstream gene Hes and Hey response elements. Taken together, our data suggest that CD147 is a downstream gene of Notch signaling regulating multiple myeloma cell behavior.

O.09

BORTEZOMIB IN COMBINATION WITH THE HISTONE DEACETYLASE INHIBITOR JNJ-29481585: EFFECT ON MYELOMA BONE DISEASE IN THE 5T2MM MURINE MODEL OF MYELOMA

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The proteasome inhibitor bortezomib (Velcade) is currently approved as second-line treatment of multiple myeloma (MM). MM related bone disease is one of the most debilitating complications of MM. Besides supportive care with biphosphonates, there is no specific treatment of lytic bone lesions. The present study investigated the effect of bortezomib in combination with a novel hydroxamate based histone deacetylase inhibitor with prolonged pharmacodynamic effects, JNJ26481585, on tumor burden and MM bone disease in the 5T2MM model. The 5T2MM model mimics the human disease closely having a selective growth in the BM, inducing angiogenesis and MM associated bone disease. Injection of 5T2MM cells into C57Bl/KaLwRij mice resulted in a plasmacytosis of 40%, an increase in MVD of 53% and a MM bone disease characterized by 2.5-fold increase in the number of osteoclasts, a decrease in number of osteoblasts of 56%, a decrease of trabecular bone volume of 66% and a decrease of trabecular number of 71% ($p < 0.05$). The 5T2MM mice were treated with bortezomib (0.6mg/kg, twice weekly, sc) according to a therapeutic setting, from the moment of onset of the disease.

Treatment of 5T2MM bearing mice treated with bortezomib significantly reduced tumor burden and angiogenesis with almost 100 % while the MM bone disease was reduced partially (osteoblasts, increase 3.5x; osteoclasts, reduction 18%; trabecular number, increase 2.4x; % bone volume-trabecular, increase 2.3x, compared with vehicle group; $p < 0.05$).

More importantly, the combination of bortezomib with JNJ26481585 (1.25mg/kg, every other day, sc) resulted in a greater reduction of the MM bone disease compared to bortezomib as single agent. (osteoblasts, increase 4.4x; osteoclasts, reduction 71%; trabecular number, increase 4.7x; % bone volume-trabecular, increase 4.6x, compared with vehicle group; $p < 0.05$).

These data suggest that bortezomib has bone remodeling properties which can be improved in combination with low dose JNJ26481585. The study indicates that this combination therapy could be a useful strategy for the treatment of MM patients, especially in those patients with skeletal complications.

O.10 CO-TRANSPLANTATION OF MESENCHYMAL STEM CELLS MIGHT MITIGATE ACUTE GVHD WITHOUT ABROGATING GRAFT-VERSUS-TUMOR ALLOREACTIVITY AFTER ALLOGENEIC TRANSPLANTATION WITH NON-MYELOABLATIVE CONDITIONING

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Background

Allogeneic nonmyeloablative HCT has been an effective treatment for many patients with hematological malignancies who have a HLA-matched related or unrelated donor. However, results of nonmyeloablative HCT in pts with HLA-mismatched donors have been disappointing due to high incidence of graft rejection and severe acute GVHD. Recent studies have suggested that infusion of mesenchymal stem cells (MSC) the day of HCT might promote engraftment and prevent acute GVHD after myeloablative allogeneic HCT. This prompted us to investigate whether MSC infusion a few hours before HCT could allow nonmyeloablative HCT from HLA-mismatched donors to be performed safely.

Methods

20 patients with hematological malignancies were given MSC (1-2 x 10E6 cells/kg) from third party donors a few hours before PBSC from HLA-mismatched unrelated donors, after conditioning with 2 Gy TBI and fludarabine 90 mg/m². Postgrafting immunosuppression included tacrolimus and MMF. HLA-compatibility was assessed at the HLA-A, -B, -C, -DRB1 and DQB1 loci: 13 pairs were mismatched for at least one HLA class I antigen, 1 pair was mismatched for 2 HLA class II alleles, while 6 pairs were mismatched for a single HLA class I (n=3) or HLA class II (n=3) alleles.

Results

One patient with secondary AML had primary graft rejection, while the remaining 19 patients had sustained engraftment. Median donor T-cell chimerism levels on days 28, 100, 180 and 365 after HCT were 90%, 98%, 96%, and 98%, respectively. Grade II, III and IV acute GVHD were seen in 5, 2 and 1 patients, respectively, while 7 experienced NIH moderate/severe chronic GVHD. Three of 7 patients with measurable disease at transplantation achieved complete remission on days 41, 104 and 353 after HCT. Two patients died of non-relapse causes on days 74 and 114 after HCT, while 3 died of disease progression. Projected 1-yr overall and progression-free survivals were 77% and 61%, respectively.

Conclusions

HLA-mismatched nonmyeloablative HCT with MSC co-infusion appeared to be safe, with MSC co-infusion possibly mitigating graft-versus-host alloreactivity without abrogating graft-versus-tumor effects. Survival is encouraging.

ABSTRACTS POSTERS CLINICAL HEMATOLOGY
P.01 – P.58

P.01

IS THE RISK OF DEVELOPING AML OR MDS INCREASED IN X-LINKED NEUTROPENIA ?

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In 2001, we described X-linked neutropenia (XLN) as a novel subtype of severe chronic neutropenia (SCN) with an activating L270P mutation in the GTP-ase binding domain (GBD) of the Wiskott-Aldrich Syndrome protein (WASP). Since then, an XLN case with an S272P and one with an I294T mutation have been described by Ancliff et al. We discovered 3 more XLN families. The first is a large kindred with an activating I294T WASP mutation. This family allowed us to add features as a variable degree of neutropenia -not correlating with the severity of infections-, low IgA and low NK cells to the phenotype. X-inactivation patterns in carriers were inconsistent, suggesting that selection against WASP might not be strong enough to cause consistent skewing. The second new XLN family had an L270P mutation, and is probably related to our original XLN family. The third (Irish) case had also an L270P mutation. The risk of leukaemic transformation in XLN remains unclear. The I294T case described by Ancliff et al. reportedly presented as MDS and 2 out of 5 L270P cases of the original family developed MDS/AML, with (-7), after a prolonged disease course and under G-CSF. CSF3R mutations, which are specific for SCN, were found in leukaemic samples. No MDS/AML cases were described in other XLN families (in which G-CSF administration was less liberal). As XLN can be diagnosed at a later age, the maturation arrest at the promyelocyte/metamyelocyte stage can masquerade as MDS and as XLN sometimes presents as or evolves to AML/MDS, we screened the GBD of WASP in 231 patients so far, with MDS (22%) or AML (78%), including 26% with (-7). No relevant mutations were observed in these paediatric and adult MDS/AML cases. In conclusion, our findings suggest that XLN, like other subtypes of SCN could impose an increased risk for myeloid malignancies, most likely via a final common pathway involving CSF3R and (-7). However, activating WASP mutations do not seem to present as MDS/AML. Therefore, we recommend sparse use of G-CSF, restricted to infectious episodes in XLN.

P.02

A PROSPECTIVE RANDOMIZED MULTICENTER TRIAL OF DARBEPOETIN-ALFA AND I.V. IRON ADMINISTRATION AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background

We conducted a multicenter prospective randomized study analyzing the impact of darbepoetin alfa (DA) with or without i.v. iron on erythroid recovery after autologous HCT.

Patients and Methods

127 autologous HCT recipients with lymphoid malignancies were randomized 1:2:2 between no treatment (group 1, n=25), DA (Aranesp®) 300 µg QOW starting on day 28 after HCT for a total of 7 doses (group 2, n=52), or the same regimen of DA plus i.v. iron sucrose (Venofer®) 200 mg on days 28, 42 and 56 after HCT (group 3, n=50). Primary endpoints included proportion of complete correctors (i.e. patients reaching Hb ≥ 13 g/dL) before day 126 post-transplant and median time to achieve Hb correction in each arm.

Results

In intent to treat analyses, the proportion of complete correctors was 24% in group 1, 81% in group 2 (P< 0.001 compared with group 1), and 92% in group 3 (P< 0.001 compared to group 1, and P=0.099 compared to group 2). Median time to achieve Hb ≥ 13 g/dL was not reached in group 1, 42 days in group 2 (P< 0.001 compared to group 1), and 32 days in group 3 (P< 0.001 compared to group 1 and P=0.127 compared to group 2). Mean ± standard deviation total doses of DA administered were 1,445 ± 489 µg in group 2 vs 1,272 ± 443 µg in group 3 (P=0.06). Ferritin levels at the end of study were 477 ± 597, 393 ± 599 and 479 ± 488 in groups 1, 2 and 3, respectively (NS). Eight patients (2 in group 1, 4 in group 2, and 2 in group 3) required red blood cell transfusions on study, including 4 patients following early disease progression. In per protocol analyses, median time to achieve Hb ≥ 13 g/dL was 190 days in group 1, 44 days in group 2 (P< 0.001 compared to group 1), and 31 days in group 3 (P< 0.001 compared to group 1 and P=0.025 compared to group 2).

Conclusions

This is the first prospective randomized trial demonstrating that DA is safe and highly effective to ensure full erythroid reconstitution after autologous HCT when started on day 28 posttransplant. I.v. iron sucrose tended to further fasten erythroid recovery with a lower dose of DA required.

P.03

LONG-TERM EFFECTS ON BONE MINERAL DENSITY OF DIFFERENT THERAPEUTIC SCHEMES FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) OR NON-HODGKIN LYMPHOMA (NHL) DURING CHILDHOOD

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We studied bone mineral density (BMD) at different sites in 89 patients with completed growth (44 males), aged between 16 and 30 years and in remission since more than five years after treatment for a childhood ALL (74 patients) or NHL (15 patients). The patients had received chemotherapy only (group I; n=41), chemotherapy and prophylactic cranial irradiation (CI) (group II; n=32), or had undergone bone marrow transplantation (BMT) with total body irradiation (TBI) (group III; n=16). Bone mineral apparent density was also calculated at the lumbar spine (BMADLS, g/cm²).

A reduced BMD was observed in 42 (47%) of the 89 patients, more frequently in men (66%) than in women (29%) ($p < 0.001$), whereas there was no significant relationship between BMD and type of disease or age at diagnosis. In comparison with group I, mean BMD was significantly lower at all three sites in group II and at the total hip and femoral neck in group III. Neither mean BMD nor the prevalence of osteopenia were different between patients with or without growth hormone deficiency or hypogonadism. BMADLS was still significantly lower in men than in women ($p < 0.001$) but no difference was longer observed between the three treatment groups.

In conclusions, this study demonstrates that young adult survivors of childhood ALL and NHL have often a low BMD which is related to male gender, previous CI and BMT with TBI. The greater bone impairment in these two treatment subgroups was mainly found at the level of femoral neck and total hip.

P.04 LENTIVIRAL VECTORS WITH EXPANDED LTRS REMAIN FUNCTIONAL IN MULTIPLE MYELOMA STUDIES

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Modifications to the long terminal repeats (LTRs) have contributed a lot to the popularity and biosafety of lentiviral vectors. Sequences cloned into the 3'LTR get duplicated during reverse transcription generating double-copy vectors which produce double amounts of transgene compared to single-copy vectors. Deletions (SIN-vectors) or additions (short hairpin (sh) RNA-, Lox-vectors) of specific sequences have demonstrated that alterations to these LTRs do not inhibit their functionality. Since the added modifications never surpassed the size of the U3-deletion (SIN-vectors), the question whether increased size LTRs are still functional remains unanswered. In this study, we investigated if more drastic (size and extra homology) changes inhibit LTR functionality. Therefore, we gradually added H1 polymerase III promoter-shRNA cassettes to the 3'LTR to increase its size in steps of 300 bp. This way, we generated double-copy vectors with double and triple knockdown cassettes (inserts of respectively 600 bp and 900 bp) with an increased homology within the LTRs compared to single knockdown constructs. Vector productions showed no significant decrease in titer compared to the single constructs. Transduction of the in vitro growing 5T33 multiple myeloma cell line with all constructs separately did not demonstrate any loss of transduction efficiency or stability with expanded LTR-vectors. A genomic DNA PCR amplifying the LTR region of the provirus showed stable integration of the expanded LTR vector. To test the functionality of the expanded LTR, we quantified the knockdown efficiencies of every shRNA-cassette. Our results indicated that all shRNA-cassettes are functional and generate high knockdown efficiencies upon qRT-PCR and Western blot analysis. Besides the conclusion that expanded LTRs remain functional, we offer a rapid single cloning strategy to combine several different shRNA cassettes into one single transferplasmid. This approach enhances the capability of this kind of vector to study interactions of several genes during multiple myeloma development and progression.

P.05 CO-TRANSPLANTATION OF MESENCHYMAL STEM CELLS (MSCS) FAILED TO PREVENT AND TREAT ACUTE GVHD IN A HUMANIZED MICE MODEL

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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 shown that mesenchymal stem cells (MSCs) could exert a potent immunosuppressive effect in vitro and in vivo.

Aim

The aim of the current study was first to establish a humanized model of GVHD in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, and then to assess MSCs ability to prevent and/or treat GVHD.

Methods

200x10⁶ human peripheral blood mononuclear cells (PBMCs) were injected intraperitoneally (i.p.) into NOD/SCID mice. MSCs ability to suppress in vitro CD3T-cell proliferation induced by mitogens (PHA) or anti- CD3/CD28 microbeads was assessed by the ³[H]-thymidine test and by flow cytometric cell cycle analysis. In vivo, 2.10⁶ MSCs were injected in i.p. into NOD/SCID mice were given right at the beginning of PBMCs transplantation (PBMCsT) or on day two or four after PBMCsT to evaluate their capacity to prevent or to treat GVHD respectively.

Results

200x10⁶ PBMCsT into NOD/SCID mice recipients (n=38) induced a weight decrease >10% (n=12) and >20% (n=26), these mice presented severe hunching, ruffled fur, tachypnea, anaemia, and decrease of locomotion. 100% of these mice died before two weeks post-transplantation. CD3T-cells percentages in the mice were 52,42±23,69% in the spleen (n=30), 13,87±14,69% in the BM (n=28), and 51,02±25,37% in the blood (n=20). MSCs cotransplanted with PBMCs in i.p. into NOD/SCID mice (n=15) failed to prevent GVHD. Mice present clinical and pathologic signs of GVHD with a weight loss >20% in 11/15 of these mice. CD3T-cells percentages in these mice were 63,81±23,75% in the spleen (n=10), 21,91±19,31% in the BM (n=10), and 59,08±26,63% in the blood (n=7). MSCs were injected on day two (n=4) or four (n=4) after PBMCsT failed to improved GVHD and human T-cell infiltration.

Conclusions

Although MSCs exhibited potent immunosuppressive properties in vitro, MSCs in i.p. injection failed suppressing GVHD in that model.

P.06

ACQUIRED VON WILLEBRAND SYNDROME: 10 CASES FROM A SINGLE CENTER

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Acquired von Willebrand syndrome (AvWS) is considered as a rare bleeding disorder. vonWillebrand factor deficiency is due to molecule half-life shortening from various conditions and mechanisms. The most commonly associated disease is monoclonal gammopathy (MG), but other lymphoproliferative disorders, myeloproliferative diseases, auto-immune disorders and aortic stenosis are also reported.

The confirmation of the diagnosis may be difficult. The DDAVP test reveals a decrease of the molecule half-live but this can be seen in some variant forms with unstable protein and the test is not recommended in older patients. Further evaluation comprising the vWF propeptide and the demonstration of an anti-vWF antibody can be decisive to confirm the diagnosis. These tests are only performed in highly specialized laboratories, for our patients, they are done in CHRU de Lille (France) since 2000.

Since 1997, we have observed 10 patients with AvWS (M=6, F=4): 5 associated with monoclonal gammopathy, 2 with Waldenström macroglobulinemia, one with CML, one with auto-immune disorder and one with no associated disorder. A hemorrhagic diathesis was present at diagnosis in two patients (1 CML, 1 MG); the others were detected during a preoperative screening or during the investigation of the associated disorder.

Most of the patients exhibit a type 2 phenotype with high molecular weight multimers reduction. The evaluation of the vWF propeptide and anti-vWF antibody was carried out in 7 patients (+ 2 ongoing). For patient n4, the presence of the anti-vWF antibody was crucial for final diagnosis. For 4 patients, the vWF propeptide/antigen ratio was very helpful to confirm or exclude the diagnosis (one was first considered as a congenital form).

In our experience, acquired von Willebrand syndrome is not a rare disease. Many forms may remain subclinical and undiagnosed, or misdiagnosed as congenital forms. Propeptide and anti-vWF antibody can be very useful in the diagnosis. As most of the cases are associated with a monoclonal gammopathy, checking von Willebrand activity seems warranted in the preoperative evaluation of these patients.

Patient	Age at diagnosis	vWF:ag%	vWF:RCo%	F VIII%	vWF propeptide %	vWF inhibitor	Underlying condition
1	52	21	11	28	Ongoing	Ongoing	MGUS IgG
2	55	18	5	35	108	Negative	MGUS IgG
3	52	16	4	31	140	Positive	MGUS IgG
4	47	36	14	61	61	Negative	None
5*	68	68	43	72	108	Positive	Waldenstroem
6	58	40	10	64	412	Negative	Auto-immune
7	65	19	13	49	Ongoing	Ongoing	Waldenstroem
8	41	3	5	7	90	Negative	MM IgG
9	40	80	33	51	Not tested	Not tested	CML
10	60	33	18	38	137	137	MGUS IgG

MM: multiple myeloma CML: chronic myeloid leukaemia MGUS: monoclonal gammopathy of undefined signification.

* lowest values: vWF:ag: 44%, vWF:RCo: 27%

P.07

IMPROVED OUTCOME WITH PULSES OF VINCRIStINE AND STERIODS IN CONTINUATION THERAPY OF CHILDREN WITH AVERAGE RISK ACUTE LYMPHOBLASTIC LEUKEMIA AND NON HODGKIN LYMPHOMA: FINAL REPORT OF THE EORTC RANDOMIZED PHASE III TRIAL 58951

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Background

An overview of trials showed that addition of vincristine (VCR)+prednisolone (PRED) pulses to continuation therapy of childhood ALL, improved DFS (Lancet,96). A recent randomized intergroup trial showed that, when given to intermediate risk (age < 1 or ≥ 6 yrs or WBC > 20x10⁹/L) pts, VCR+dexamethasone (DEX) pulses in maintenance failed to improve DFS and OS (Lancet,07). Methods: In 12/1998, EORTC CLG started the randomized phase III 58951 trial addressing 3 questions: R1) DEX vs PRED in induction; R2) prolonged (24x) vs conventional (12x) ASPA duration during consolidation and late intensification; R3) for average risk (AR) pts: 6 (VCR+steroid) pulses every 10 weeks during maintenance. The steroid of the pulses was that assigned to the patient at R1: DEX (6 mg/sm/d) or PRED (60 mg/sm/d) for 7 d. AR pts were defined as neither low risk nor very high risk. Main endpoint was DFS; secondary endpoints were OS and toxicity. Results: Between 6.1999 and 11.2002, 411 pts (384 ALL + 27 NHL), were randomized. In the Pulses group, 101 vs 101 pts were randomized for PRED vs DEX. At a median follow-up of 6.3 y, there were 19 vs 34 events for Pulses vs No Pulses: relapses (19 vs 32), death in CR (0 vs 2). The 6-y DFS was 90.6% (SE 2.1%) in the Pulses group and 82.8% (SE 2.8%) in the No Pulses group (HR=0.54, 95%CI 0.32-0.94, 2-sided logrank p=0.027). The impact of pulses on DFS was similar in the PRED group (HR=0.56) and the DEX group (HR=0.59). In girls the treatment difference seemed to be more pronounced (HR=0.25, 99%CI 0.04-1.25; p=0.015) than in boys (HR=0.71, 99%CI 0.30-1.66; p=0.30), and also in those randomized for conventional duration of ASPA (HR=0.46, 99%CI 0.18-1.19; p=0.03) than in prolonged ASPA arm (HR=0.87, 99%CI 0.23-3.29; p=0.78). Grade 3-4 hepatic toxicity was lower in the Pulses group; but grade 2-3 osteonecrosis was 4.4% (Pulses) vs 2% (No Pulses) and pancreatitis rate was 4.9% (Pulses) vs 2.9% (No Pulses). Conclusion: VCR+corticosteroid pulses significantly improved DFS, esp in pts having received conventional duration of ASPA. Pulses did not increase toxicity. In future, for AR pts treated according to BFM protocols, pulses should become a standard component of therapy.

P.08

TWO CASES OF PAEDIATRIC KIKUCHI FUJIMOTO'S DISEASE: A RARE CAUSE OF LYMPHADENITIS

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Lymphadenitis is a common problem in paediatrics. It is most frequently caused by virus or bacteria infections. Kikuchi Fujimoto's disease (KFD) or histiocytic necrotizing lymphadenitis associates cervical lymphadenopathy with fever and leucopenia typically in young women. We report here two paediatric cases of KFD. The first case was an 11-year-old girl presenting with firm painless unilateral cervical and sub-clavicular lymphadenitis, weight loss but no fever. The second was a 13-year old girl with fever, unilateral cervical lymphadenitis, weight loss and asthenia. Both patients were initially treated by antibiotics but were referred for symptom persistence. The laboratory tests failed to show leucopenia and elevation of C-reactive protein. The serology screenings for bacteria, virus and autoimmune diseases were negative. The tuberculin skin test was also negative. The Pet-scan displayed hypermetabolic spots localized to the clinically enlarged lymph nodes. A surgical biopsy was performed and revealed the presence of a histiocytic necrosis compatible with the diagnosis of KFD in both children. Few weeks after diagnosis the first patient presented with butterfly erythema and increased antinuclear antibody titer suggesting the development of systemic lupus erythematosus (SLE). She was treated with chloroquine that allowed a rapid resolution of symptoms.

In conclusion, KFD is a rare and benign cause of lymphadenitis that may occur in children. It may be associated with auto-immune diseases such as SLE. It should be considered in the differential diagnosis of prolonged cervical lymphadenopathy where infectious aetiology has been excluded. Histological examination is required to confirm the diagnosis and to exclude malignant infiltration.

P.09 ENDOCRINE AND METABOLIC DISORDERS IN YOUNG ADULT SURVIVORS AFTER HAEMATOPOIETIC STEM CELLS TRANSPLANTATION

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The aim of our study was to evaluate these late endocrine complications in adult survivors of HSCT during childhood, in relation with different therapeutic schemes.

357 children underwent a HSCT; 218 of these are still alive. This study included 68 patients aged 15 yr or older (mean age: 24,3 years) who had been treated by HSCT in childhood and were cured since more than 3 years. Patients were grouped according to the pathology: malignant haematological diseases (group I: n = 42), solid malignant tumours (group II: n = 11), non malignant diseases (group III: n = 15). TBI was given before HSCT in 55% of patients in group I, 64% in group II and 26% in group III. A severe GH deficiency was observed in groups I and II (in 39% and 50% of patients, respectively), but never in group III ($p < 0.001$). Compensated or treated hypothyroidism was observed in 47% of all patients, more frequently in groups I and II (50% and 54% respectively) than in group III (33%). About 3/4 of patients in group I had hypogonadism, as compared with 64% in group II and 53% in group III. Moreover, FSH levels were higher in group I than in groups II and III.

Patients from group I had the worse metabolic profile, with insulin resistance in 49%, compared to only 20% and 27% in groups II and III, respectively ($p < 0.001$). Mean total cholesterol was also higher in group I than in group III ($p < 0,01$). About 18% of all patients were overweighted (BMI > 25 kg/m² (33% in group III), but none had a BMI above 30. Low bone mineral density (z-score < -1 at any site) was observed in 42% of all patients, but more frequently in group II (9/11) than in groups I and III (40 and 38%, $p < 0.001$).

Conclusions

This study reveals a high prevalence of endocrine disorders in young adult recipients of a HSCT during childhood for a malignant disease. Associated treatment with TBI seems to be the most detrimental. Interestingly, metabolic complications appear to be more frequent after HSCT for a haematological malignancy, while bone loss is more frequently observed after treatment for a solid malignant tumor.

P.10

INTEREST OF THE QUANTIFICATION OF THYMIDINE KINASE BY ENZYME-LABELED IMMUNOASSAY IN CHRONIC LYMPHOID LEUKEMIA.

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Introduction

The clinical course of individual Chronic Lymphoid Leukemia (CLL) patients is highly variable. Some patients with low grade CLL at the diagnosis, will develop a life-threatening disease and thus, may benefit from early treatment. Serum thymidine kinase (sTK) could identify such patients.

Unfortunately, until now, sTK was only available by radio-immunoassay (RIA) which is not available in routine labs.

The aims of this study were therefore first to assess the reproducibility of a new enzyme-labeled immunoassay and secondly to compare this new method with RIA.

Material and methods

142 sera from CLL patients were collected and processed with the enzyme-labeled immunoassay LIAISON® TK kit (Diasorin). This immunoassay requires 250µl sample. Reproducibility of the method was tested by measuring consecutively 2 aliquotes of the same specimen for 13 patients. A coefficient of variation based on the difference between those 2 measurements, was calculated. Then, we studied 58 CLL patients with the new method and the gold standard.

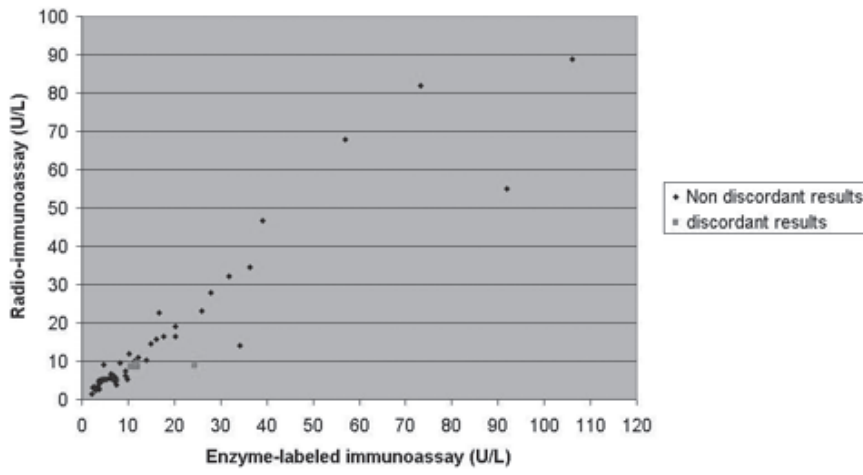
Results

Thymidine kinase for CLL patients ranged from 0,8U/L to 446U/l (mean: 19,0 U/L). In the literature, patients with a higher TK level (cut-off: 7.1-10 U/L) have a shorter average time of progression-free survival. Reproducibility of the method shows a maximum difference of 3,4U/L for 2 aliquotes of the same sample. The systematic difference between immunoassay and RIA was about 10%. Five patients had a sTK concentration above the cut-off of 10U/L with enzyme-labeled immunoassay and below the cut-off with RIA (figure 1). In a near future, the sTK will be compared with other common risk factors and clinical outcome and studied in other malignancies.

Conclusion

The new enzyme-labeled immunoassay LIAISON® TK kit is a quick, automated, non radioactive and reproducible method to measure serum thymidine kinase. Furthermore, it is applicable in all labs and gives similar results to the RIA. Preliminary results show a closest relationship between this new immunoassay and clinical outcome.

Comparison between Radio-immunoassay and Enzyme-labeled immunoassay (cut-off:10 U/L)



P.11 THE LONG-TERM OUTCOME OF ELDERLY PATIENTS WITH PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL) IN THE IMATINIB ERA.

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The use of imatinib combined with chemotherapy has improved the short-term outcome of elderly patients with Ph+ ALL. However, very few data are available on the long-term impact of tyrosine kinase inhibitors on survival. From January 2003 to November 2004, 30 patients aged 55 years or older were treated according to the GRAALL's AFR09 protocol (Leukemia 20; 1526, 2006) which included a pre-phase with steroids, an induction treatment with chemotherapy, a consolidation phase with imatinib and steroids, and 10 maintenance blocks, including two 2-month blocks of imatinib. Overall, imatinib was given for 6 months during this 2-year regimen. Out of 30 patients included in the study, 27 achieved a complete response. At last follow-up (November 2008), median follow-up of surviving patients was 64 months. 7 patients were still alive (23%, 95% C.I.: 10-42%), including 4 patients who had experienced a molecular (n=3) or a hematological (n=1) relapse. Patients surviving after a molecular relapse had been salvaged with various regimens, including imatinib or dasatinib given alone or combined with chemotherapy. In the present cohort, the single patient offered an allogeneic stem cell transplantation relapsed and died. No clinical or biological characteristics (age, blood counts, and steroid sensitivity) at diagnosis allowed for prediction of long-term survival. In conclusion, approximately 20% of elderly patients with Ph+ ALL are offered a long-term survival in the imatinib era. In this study characterized by a relatively short exposure of patients to imatinib, molecular relapses could be successfully managed by additional treatment with tyrosine kinase inhibitors with or without chemotherapy.

P.12

PRELIMINARY RESULTS FROM AN OBSERVATIONAL STUDY ON BORTEZOMIB USE IN BELGIUM FOR PATIENTS WITH RELAPSED MULTIPLE MYELOMA.

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Background

Bortezomib (Velcade) is a proteasome inhibitor for treatment of MM. The eVOBS (electronic Velcade Observational Study) evaluates treatment and clinical outcome with bortezomib in daily practice in the relapsed setting. Enrollment occurs from Oct 06 to Dec 08 in 7 countries, with 3 y follow-up. This report concerns 101 belgian patients included in the latest interim analysis from Nov 08.

Methods

Adults are eligible if scheduled to receive bortezomib within the approved indication. All bortezomib dosages and concomitant treatments are permitted, except investigational therapies. Response criteria are not predefined and may include M-protein, EBMT, SWOG, or others.

Results

101 patients (59% male) are included in this analysis, with median age of 64y, and mean time since MM diagnosis of 3.1 years. Prior number of therapies received was 1 (59%), 2 (27%), and 3 or more (11%). 60% received at least one therapy in the preceding year, with thalidomide and dexamethasone (in combinations or as monotherapy) in 36% and 33% respectively. 97% of patients started bortezomib at 1.3 mg/m² on the usual schedule. By the fifth cycle, 32% percent of patients still receiving therapy had dose reduction. At start of treatment, bortezomib was given in combination with other therapies in 46% of patients, with dexamethasone being the most frequent (35%). By cycle 5, 53% were receiving combination therapy. For the 62 patients with data available for at least 12 weeks, median treatment duration was 5 cycles (with 5 of those patients still on treatment to date). Of the 76 patients that were evaluated for response overall response rate was 71%, with 59% PR and 12% CR/nCR. 8% and 12% of patients reached MR and SD, respectively. 86% of patients experienced AEs as a result of treatment: diarrhea (30%), fatigue (25%), nausea (24%) and neuropathy (PN). AEs led to discontinuation of treatment in 32%. At start of bortezomib treatment 38% of patients reported PN due to myeloma or prior therapy. During treatment, 36% of patients developed a new neuropathy (11% grade 3/2% grade 4) or had worsening of existing PN. PN improved or disappeared for approximately 5% of patients in each cycle.

Conclusion

this preliminary analysis shows that, in Belgium, bortezomib is used most frequently in second line treatment and in combination with dexamethasone. Efficacy in daily clinical practice is high, possibly due to the use of combinations. Adverse events were as expected from previous clinical trials.

P.13 EVALUATION OF DIFFERENT METHODS FOR SENSITIVE JAK2V617F DETECTION

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Introduction

The JAK2V617F mutation has emerged as an essential molecular determinant of chronic myeloproliferative disorders (CMPD), occurring in >95% of polycythemia vera, and >50% of essential thrombocytosis or idiopathic myelofibrosis patients. A correlation between the proportion of mutant JAK2 allele and the propensity to a more symptomatic disease has been described.

Aim

To evaluate the performance of two quantitative real-time PCR (qPCR) methods as compared to an allele-specific (AS)-PCR on DNA extracted from white blood cells. Materials and Methods: AS-PCR reactions, followed by capillary electrophoresis, were performed according to McClure et al*. A qPCR method according to Larsen et al** (wild-type or mutation specific reverse primer, both with intended mismatches), was compared with a Locked Nucleic Acid (LNA)-modified qPCR assay. This LNA-qPCR with TaqMan probes, modified from molecular beacon technology as described by Sidon et al***, enhance allelic discrimination by blocking WT JAK2 sequences from amplification using a LNA oligonucleotide.

Results

All three assays detected 43 JAK2V617F positive patients out of 85 suspected CMPD diagnostic samples (100% overall agreement). 41/42 samples, negative by both AS-PCR and LNA-qPCR, and 19/20 normal peripheral blood samples showed a signal above threshold in the mutation specific Larsen-qPCR, whereas no aspecific amplification was observed in LNA-qPCR. This underlines the need to define a cut-off value in the Larsen-qPCR. With the LNA-qPCR protocol, a limit of detection of 0.1% was obtained whereas AS-PCR achieved 0.8%.

Conclusion We conclude LNA-qPCR is to be preferred above the more laborious semi-quantitative AS-PCR and less specific Larsen-qPCR. Specific, sensitive and quantitative JAK2V617F determination provides an attractive approach to evaluate the prognostic significance of the mutation burden at diagnosis and raises the possibility to monitor disease progression or efficacy of newly developed therapies.

* McClure et al, Leukemia 2006/ ** Larsen et al, Br J Haematol 2007 / *** Sidon et al, Clin Chem 2006

P.14
REPEATED AND PROLONGED EFFICACY OF RITUXIMAB IN
COLD AGGLUTININ DISEASE. REPORT OF ONE CASE RECEIVING
40 INFUSIONS.

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We report the case of a man of 75 years old, followed since 13 years for a cold agglutinin disease with haemolytic anaemia. The bone marrow analysis showed characteristics of a lymphoplasmocytic lymphoma. From June 2004, he has been treated with chlorambucil, plasma exchanges, CVP and erythropoietin without efficacy. He received afterwards rituximab for a total of 40 infusions of 375 mg/m². This treatment was given alone for 36 infusions or with CVP for 4 courses and obtained each time a clinical and biological response. Duration of response was usually 6 months and no infection or toxicity was reported.

Although retreatment with rituximab is frequently used in low grade lymphoma, the optimal schedule was not standardized. Moreover, toxicity for long term treatment comprises neutropenia and herpetic complications. This case is interesting because it illustrates the possibility of prolonged efficacy of rituximab in the cold agglutinin disease with a poor toxicity despite the cumulative dose.

P.15 RECEPTOR-DERIVED CHRONIC LYMPHOCYTIC LEUKEMIA DIAGNOSED SHORTLY AFTER KIDNEY TRANSPLANTATION ON PROTOCOL BIOPSY

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Three months after renal transplantation, a 68-year-old man underwent a routine protocol kidney biopsy. Surprisingly, histological examination revealed infiltration with a small CD5+CD10-CD20+CD23+FMC7-CD79b- lymphoid population, which was also found in the bone marrow. Further prognostic stratification revealed isolated del13q14 by FISH, weak CD38 expression and a mutated IGVH status. Diagnosis of early stage CLL was made. Analysis of STR and the amelogenin locus in the bone marrow aspirate revealed only one profile of recipient origin. In order to definitely prove recipient derived CLL, we explored the status of IGH and IGK rearrangements in a sample posttransplant and in one taken before transplant. Identical rearrangements were found in both samples.

This unequivocally establishes that the CLL was already existing before transplantation. Except for reduction of immunosuppression, a watch and wait approach was initiated. More than two years after the diagnosis of CLL the patient remains in a good condition, without evidence of progressive disease and without signs of rejection of the transplant kidney.

In case of no response after RIS or of very aggressive presentation, transplantation related lymphomas (PTLD) are usually treated with the monoclonal anti-CD20 antibody rituximab either in monotherapy or in combination with chemotherapy. In case of localized disease radiotherapy and surgery can be very effective treatment options. In our patient however, some of these therapeutic modalities would have had devastating consequences.

Conclusion

We describe the case of a renal transplant patient without apparent hematological history, showing diffuse allograft infiltration by a monoclonal small B-cell lymphoid population, leading to a diagnosis of receptor-derived CLL 3 months after transplantation.

Despite the clinical context with profound immunosuppression, differential diagnosis with PTLD is extremely important in order to avoid unnecessary devastating treatment. This case underscores that it is extremely important to distinguish between a pre-existing lymphoma diagnosis after transplantation and a true PTLD as the treatment options of both can be very divergent.

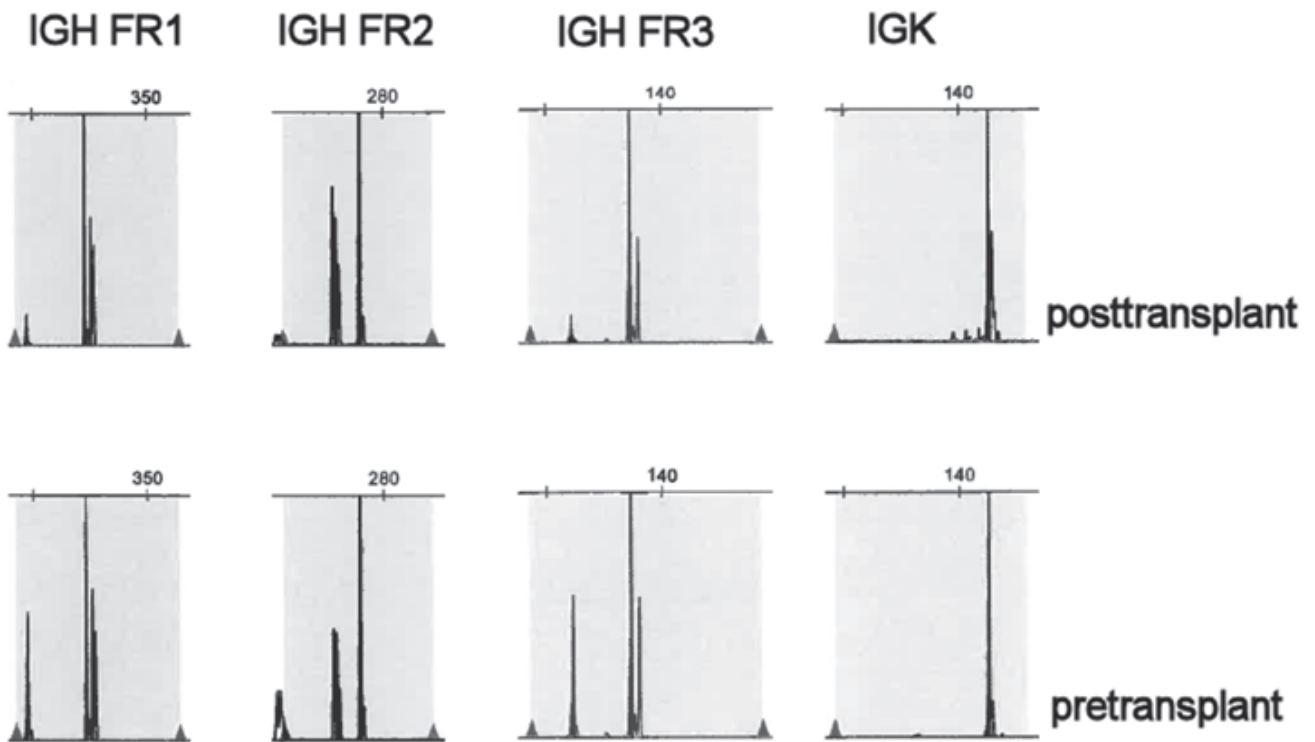


Figure 1. The status of IGH and IGK rearrangements were determined on a diagnostic blood sample obtained after transplant, and a stored pretransplant blood sample. The bottom panel was obtained from a pretransplant sample and reveals identical rearrangements

P.16 THERAPEUTIC MEDICAL PRACTICE IN TTP: A RETROSPECTIVE SINGLE CENTER ANALYSIS

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Background

Currently 80-90% of patients treated with daily TPE survive the initial episode of TTP. However relapses occur in a substantial fraction of patients. To date, there are no randomized clinical trials to provide data for long-term management decisions. The decision to stop or continue treatment is essentially still empirical. Aim. The study aimed at documenting current therapeutic medical practice for TTP and response to treatment in one Belgian University hospital. Methods. Medical records were used for this retrospective observational study. Statistical analysis was purely descriptive. Results. 12 patients with 18 documented episodes were included. Renal and neurological dysfunction were reported in 61% and 33% of the episodes. At baseline platelets and LDH were abnormal in 17, creatinine in 11 episodes. TPE was used in all cases. In 12 episodes adjunctive therapy was given. The mean number of TPE procedures/episode was 18. The number of plasma volume exchanged per day (PE/PV) ranged from 0.1 to 1.5, with a mean patient PE/PV of 1. The outcome of the TTP episodes reported here was: CR in 9, PR in 6 and death in 3 cases. Among the episodes classified as CR, 2 relapses were observed. Normalization of platelets and LDH took an average of 13.1 and 8.1 days respectively, corresponding to 12 and 7.4 TPE procedures. Renal and neurological normalisation was reached after 11 and 5.3 days respectively, corresponding to 10 and 4.7 TPE procedures. On average, relapses (6 in 4 patients) occurred 85 days after last treatment of the previous episode. Conclusion. We performed a retrospective single center study analyzing current therapeutic medical practice for TTP. With this small study we hope to offer a platform for large observational studies to identify the benefit of particular treatment schedules, to define a relevant treatment algorithm, to identify factors of poor prognosis and to determine the place of adjunct therapies. Long-term clinical follow-up studies on the outcomes of patients after recovery from an acute episode of TTP are necessary to document the risk for relapse as well the occurrence of long term complications.

P.17 POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER: CHARACTERISTICS AND OUTCOME IN A BELGIAN UNIVERSITY HOSPITAL

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Background

PTLD is a life-threatening complication of both solid organ transplantation (SOT) and stem cell transplantation (HSCT). Data regarding incidence, which is estimated to be around 2%, are mainly retrospective and are derived from smaller single center studies and larger registration databases. Management is primarily based on retrospective case series and consequently, lacking large prospective randomised trials, there is no general consensus on optimal treatment of the disorder except for reduction of immunosuppression (RI).

Aim. We undertook a retrospective analysis of all patients diagnosed with PTLD following SOT or HSCT between January 1989 and December 2008 at the University Hospitals of Leuven, aiming to obtain information about incidence, pretreatment characteristics, treatment and outcome. **Methods.** Medical records of all patients were used for this retrospective observational study. Information was obtained with regard to baseline patients characteristics (age, gender), transplant related characteristics (underlying disorder, time of transplantation, induction therapy, maintenance immunosuppressive therapy, EBV status before transplantation), PTLD characteristics (time of diagnosis, histological subtype, IPIscore, aalPIscore, PTLDScore, haemoglobin, sites of involvement, relation to EBV), treatment administered (RI, surgery, radiotherapy, antiviral agents, chemotherapy and rituximab) and response to treatment (initial response, final outcome, cause of death). Statistical analysis will be performed in January 2009 and will be available at the Annual Meeting. **Results.** 106 patients were included in this study. PTLD was biopsy proven in 100 patients (with one patient experiencing two different types of PTLD), whereas in 5 patients, all following HSCT, diagnosis was made based on rapid increase of EBV viral load in peripheral blood with positive PETscan. Overall incidence for all transplant types was 1.9%. **Conclusions.** We report a retrospective analysis of 106 cases of PTLD following SOT or HSCT. Statistical analysis will be performed in January and presented at the Annual Meeting of the BHS.

P.18 LONG-TERM OUTCOMES OF ANTERIOR LAPAROSCOPIC SPLENECTOMY IN ADVANCED HEMATOPROLIFERATIVE DISORDERS

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Background

Splenectomy is not very popular in malignant hemopathies because of severe mortality and morbidity. Anterior laparoscopic splenectomy was introduced in the nineties with much lower surgical complications but remains controversial for large spleens.

Material and methods

We retrospectively reviewed the outcomes (mortality, morbidity, haematological recovery) of anterior laparoscopy splenectomy in 30 (23 male and 7 female) patients (pts) (63 ± 13 yo) between 1998 and 2007 to assess the safety and the efficacy of this approach for refractory advanced chronic hematoproliferative disorders.

The pt is submitted to a general anesthesia. The first steps of the procedure is the dissection of the splenocolic and the splenopancreatic ligament which frees the lower pole of the spleen. The spleen is introduced in a plastic bag. The spleen is morcelated (to allow further anatomo-pathological review). Leuco, Hct, Hb, VCM, platelets were measured and analysed by linear mixed model with an autoregressive covariance structure to assess if there is a statistically increasing or decreasing trend before the splenectomy, at time point 0, 1, 3, 6, 9 and 12 months after splenectomy:

Results

Among these pts, 14 pts had Chronic lymphocytic leukaemia (CLL), 8 were non Hodgkin's lymphomas and 3 Hodgkin's diseases and 5 MMM. Indications for splenectomy were hypersplenism (n= 1), transfusion-dependent anaemia (n= 13), idiopathic thrombopenia 14, diagnostic purpose (n= 2). None of the pts died from the procedure (0% operative mortality). Two pts had significant post operative morbidity (one pulmonary embolism and one pancreatic caudal resection). Spleen weight (g) varied from 18 g to 3580 g and the operative time was 60 to 95 min. Anaemic pts became transfusion independent with a significant increase in Hb (p< 0.01) and plt (p< 0.02).

Conclusions

Anterior laparoscopic splenectomy is feasible and safe even with large spleens. This procedure is minimally invasive for malignant refractory advanced hematologyproliferative diseases allowing histological analysis and providing durable improvement in peripheral blood counts.

P.19

STRONGYLOIDES STERCORALIS RELATED HYPEREOSINOPHILIA AND PICA

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We report the case of a 13-year-old girl who presented with fatigue, some gastric discomfort and Pica. Since a few months, she was secretly digging holes in the walls of her bedroom and bathroom, in order to eat plaster. The family thought she had a psychological disturbance. Her general practitioner discovered a high WBC count and a very high eosinophil count (10.410/mm³). She was then referred to our department. The past history revealed that she was born in Ecuador, where she spends every summer in the family house, and enjoys swimming in the river at the back of the garden. During the investigations, we found out that, in addition to the eosinophilia, she was suffering from anaemia due to severe iron deficiency. A stool culture was positive for *Strongyloides Stercoralis*. This parasite was responsible for the eosinophilia, the iron deficiency, the anaemia and the Pica.

Pica is an eating disorder which can occur in patients who chronically lack certain minerals, especially iron. Patients who suffer from Pica usually eat clay, earth, soil, or, as did our patient, plaster. Intoxications are frequent and depend on the substance ingested (the most common is lead intoxication).

The infection by *Strongyloides* is caused by contact with water infested by the parasite, like a river, in an endemic area. The *Strongyloides* usually infests the body through the skin. From there, it goes into the general circulation, to the lungs, the trachea, the pharynx and finally it infests the digestive system after being swallowed. In the respiratory system, the parasite can cause a Loeffler's like syndrome (eosinophilic pneumonia). It reaches its final destination in the gut where it settles and begins to produce eggs. Those eggs can develop into larvae and re-infect the patient or can be expelled within the stools. Diagnosis can be made by stool cultures. Repeated cultures are needed because they are rarely positive.

Our patient was treated by Ivermectine and iron supplementation. The eosinophil count rapidly became normal. Pica has disappeared.

P.20

RAPID DETECTION OF IMMUNOGLOBULIN HEAVY CHAIN CLONALITY USING PCR COUPLED WITH MICROFLUIDIC CAPILLARY ELECTROPHORESIS DETECTION

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Background

Detection of clonal rearrangements occurring in genes coding for immunoglobulin heavy chain (IGH) is an essential step in the initial setup of patients suspected of lymphoproliferative disorders. Classical molecular techniques are long and fastidious (Southern Blot) or require costly equipment (PCR with capillary electrophoresis). This work relates our experience in the determination of IGH clonal rearrangements using PCR coupled with microfluidic capillary electrophoresis (MCE) detection, an innovative technology that allows fast detection of PCR products with limited cost.

Patients and methods

During December 2008, 23 patients (M/F: 14/9; median age (range): 60.7 y (41.7 y-93.1y)) presenting various hematologic diseases were tested for IGH clonality using PCR followed by detection using MCE performed on LabChips (Experion™, Bio-Rad, Hercules, CA). The patients were also screened for IGH clonality using Southern Blot and PCR in a reference laboratory. Flow cytometry as well as bone marrow aspirate and biopsy data were reviewed and compared with the results of our molecular technique. Statistical analysis was performed using SPSS 16.0.

Results

Comparison of results between PCR coupled with MCE detection and flow cytometry showed a Kappa coefficient of 0.721 (substantial agreement). Comparison with consensus result (combination of flow cytometry data and bone marrow aspirate and biopsy results) showed a Kappa coefficient of 0.808 (excellent agreement). To this date, comparison between PCR coupled with MCE detection and Southern Blot could be performed in 12 cases. Interestingly, all cases were concordant except one case that was negative in the Southern Blot technique, but which proved to be monoclonal in PCR coupled with MCE detection.

Conclusion

PCR coupled with MCE detection provides a fast, reliable and cost-efficient technique for clonality detection in the IGH gene. Moreover, interesting features allowed by MCE detection such as peak quantification could be particularly important in the follow up of minimal residual disease.

P.21

IKZF2-ERBB4 FUSION IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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We performed array-CGH screening of 12 T-ALL cell-lines using BAC and high density oligonucleotide arrays to identify novel unbalanced genomic rearrangements that could be responsible for the activation of tyrosine kinases in T-ALL.

We detected a deletion at the 3' end of the IKZF2 gene on chromosome 2 in the KE-37 and HPB-ALL cell lines, resulting in the loss of the polyA signal of IKZF2. As a consequence, IKZF2 became fused to part of ERBB4, located directly downstream in the same transcriptional orientation. The IKZF2-ERBB4 fusion was detected by RT-PCR in KE-37 and HPB-ALL, as well as in 2 other cell lines, and in 9/88 T-ALL patients. While the mechanism of the generation of the fusion transcript is clear in KE-37 and HPB-ALL, the mechanism in the other cell lines and patients remains unexplained, since these cases did not harbor any detectable defect at DNA level in IKZF2, ERBB4 or the intergenic region. Sporadic reports suggest that chimeric transcripts can be generated in normal mammalian cells as a consequence of the transcription of two adjacent genes with the same transcriptional orientation or following trans-splicing mechanisms. Based on these considerations, we could demonstrate that the IKZF2-ERBB4 transcript was also present in normal kidney and brain tissues of different strains of mice. In this case, expression of the IKZF2-ERBB4 fusion was systematically detected together with the normal IKZF2 and normal ERBB4 transcripts, supporting the hypothesis that IKZF2-ERBB4 might exist in some tissues as a kind of alternative spliced variant of these genes. In contrast, in all IKZF2-ERBB4 positive T-ALL cases and cell lines, normal ERBB4 expression was not detected. We hypothesize that aberrant expression of the IKZF2-ERBB4 transcript in an inappropriate context might be oncogenic. Both IKZF2 and ERBB4 have been already linked to cancer through tumor suppressor and oncogenic properties, respectively. The IKZF2-ERBB4 fusion loses sequences of IKZF2 and retains those coding for the kinase domain of ERBB4. Further investigations are needed to understand the mechanisms of generation of the IKZF2-ERBB4 fusion transcript and its potential oncogenic functions.

P.22 TRANSTHYRETIN RELATED AMYLOIDOSIS: REPORT OF TWO CASES

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Introduction

Extracellular deposit of abnormal transthyretin (TTR), a thyroxine and retinol carrier protein, mainly synthesized in the liver, is a rare form of systemic amyloidosis. Mutated TTR cause hereditary amyloidosis, characterized by variable clinical manifestations including peripheral neuropathy, cardiomyopathy, autonomic dysfunction and gastrointestinal disorders.

Case presentations

A 46-year-old man, with a history of bilateral carpal tunnel release, presented with restrictive cardiomyopathy. Lip biopsy revealed the presence of non-AA amyloid deposits without evidence of plasmacell dyscrasia. Immunohistochemistry of the lip biopsy was positive for TTR; TTR gene sequencing revealed the presence of a new mutation which was identified in an asymptomatic relative as well.

The patient was offered a combined heart and liver transplantation. He is currently, almost 1 year after transplantation, in good medical condition.

The second case is a 56-year-old man with a medical history of 12 year recurrent non-AA amyloidosis related gastric ulcerations. He was admitted for severe iron deficiency anaemia and episodes of diarrhea. Work-up confirmed the presence of gastric ulcerations, revealed a nephrotic syndrome and an asymptomatic mild sensorimotor polyneuropathy. Plasma cell disorders were excluded.

Histological examination of duodenal, colon and bone marrow biopsy confirmed the presence of non-AA amyloid deposits, immunohistochemically positive for TTR. Mutation analyses of TTR genes are currently ongoing.

Discussion

We present two patients with TTR related systemic amyloidosis. In contrast with most cases described, in which neuropathy is the predominant feature, our patients presented mainly with cardiac and gastrointestinal involvement, respectively, emphasizing the heterogeneity of disease manifestation. In addition, the clinical outcome is variable, according to the mutation of the TTR gene involved.

TTR amyloidosis must be suspected in every case of non-AA amyloidosis in which plasmacell dyscrasia has been excluded. Diagnosis is confirmed by immunohistochemistry showing TTR deposits and demonstration of a mutation in the TTR gene.

P.23 COMPARISON BETWEEN A ZAP-70 RATIO IN FLOW CYTOMETRY AND IGVH MUTATION STATUS

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Introduction

Two types of prognostics may be distinguished in CLL between the presence or absence of somatic mutations IgVH. B-CLL cells have two types of expression for ZAP-70, one negative in the mutated IgVH group and one other more highly expressed in the non-mutated IgVH group. ZAP-70 was proposed as a substitute marker for IgVH mutation status thinking that it would be easier to use in a clinical laboratory. The aim of this study is to establish a cut-off for expression of ZAP-70 in correlation with the mutational status and clinical course.

Methods

We studied 12 patients with CLL-B, 3 women and 9 men, between 49 and 82 years. Samples were analyzed by a procedure where patient cells were mixed with a normal healthy control with a high lymphocytes count. Blood was stained with CD5-FITC/CD19-APC/CD3/57-PC7. FACS Lysing Solution was used to lyse RBC and permeabilize WBC. Permeabilized cells were then stained with a ZAP-70 PE. The samples were analyzed by flow cytometry and a MFI ratio between pathologic CD19+5+ cells and control cells CD19+5- was established. Samples were all screened for somatic mutations in IgVH gene accordingly to the I.P.G. (2)

Discussion

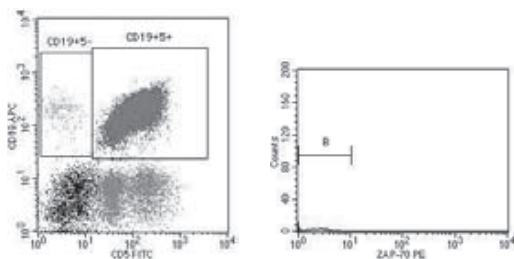
IgVH mutational status was mutated in 7 patients and unmutated in 5 patients and was considered as a gold standard for CLL-B prognostic.

A cut-off of 1,87 for expression of ZAP-70 gives the highest accuracy.

A cut-off of 2,2 gives a sensitivity equal to 100%. From a clinical point of view, 7 patients have an indolent course. All the patients have a mutated IgVH status, but one patient shows a positive ZAP-70. Three patients have an aggressive course, with a ratio upper than 2,08. The two other patients, with ZAP-70 upper than 1,96, are newly diagnosed and haven't any clinical sign of CLL-B yet.

Conclusion

Clinically, a cut-off of 2 is proposed. We propose a simple improved analytical method standardized thanks to a ratio which permits a reliable classification. An inconvenience of our method is for sample with low CD5 expression, where populations of normal B cells and CLL-B cells is hardly dissociable.



P.24

HYPOXIA ACTIVATED PRODRUG TH-302 FOR THE TREATMENT FOR MULTIPLE MYELOMA IN 5T33MM MOUSE MODEL

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Hypoxia is linked to increased metastatic potential and a treatment-resistant phenotype that leads to rapid progression and poor prognosis in solid tumors. Multiple myeloma cells reside in a relatively hypoxic bone marrow microenvironment. Given the contribution of hypoxia to tumor progression and drug resistance, a number of hypoxia-targeted therapeutics are under development. TH-302 is a new hypoxia-activated prodrug (HAP) that is currently being evaluated in the clinic as a monotherapy and in combination with standard chemotherapy regimens for the treatment of solid tumors. In the current study, we first evaluated the effects of TH-302 on human LP-1, MMS-1, RPMI-8226, Karpas cell lines and murine 5T33MMvt cell line. Flow cytometry analysis revealed that TH-302 (0.5-50 μ M) can induce a G₀/G₁ cell cycle phase arrest, and can induce apoptosis of 5T33MMvt cells in hypoxic condition (both 1% and 0% O₂) in a concentration dependent manner, in contrast to that in normoxic condition (20% O₂) (P < 0.001). Further studies conducted in the 5T33MMvv mouse model demonstrated that animals treated prophylactically with TH-302 (12.5mg/kg, 25mg/kg and 50 mg/kg, i.p.) for 3 weeks from Day 1 after tumor inoculation showed impressive improvements in all parameters including serum paraprotein (12.5mg/kg, p < 0.05; 25mg/kg, p < 0.001; 50mg/kg, p < 0.001), liver (12.5mg/kg, p < 0.001; 25mg/kg, p < 0.001; 50mg/kg, p < 0.001) and spleen weight (12.5mg/kg, p < 0.05; 25mg/kg, p < 0.001; 50mg/kg, p < 0.01), compared to vehicle-treated 5T33MMvv mice. The frequency of apoptotic multiple myeloma cells in bone marrow sections was also significantly increased (12.5mg/kg, p < 0.05; 25mg/kg, p < 0.05; 50mg/kg, p < 0.01). Treatment with TH-302 resulted in no adverse events, any observable detriment to the mice or weight loss (p > 0.05). In summary, these results show that TH-302 shows promise for the treatment of multiple myeloma.

P.25 ECONOMIC EVALUATION OF VORICONAZOLE VERSUS CASPOFUNGIN FOR THE TREATMENT OF INVASIVE ASPERGILLOSIS IN BELGIUM

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Objective

To perform an economic evaluation of voriconazole versus caspofungin in first line treatment of invasive aspergillosis (IA). These 2 antifungal drugs have a more favorable toxicity profile than the conventional amphotericin B and have a lower cost than the expensive liposomal/lipid formulation of amphotericin B. No head-to-head comparative study was conducted with voriconazole and caspofungin in IA. Based on the clinical trials of both antifungals, a conservative approach of similar efficacy has been considered.

Methods

The analysis is based on a simplified cost-minimization model with results from the National Health system RIZIV/INAMI perspective (year 2008). Only limited direct costs were considered, namely the drug cost over the episode of treatment. Treatment duration and patients' weight were key parameters. Their values were obtained from the Belgian observational VORIBEL study (Pfizer data on file) for voriconazole treatment. Treatment duration for caspofungin was derived from the EORTC study where almost 50% of the patients were recruited in Belgian centres. Mean cost and incremental cost were calculated. Univariate sensitivity analyses were carried out on weight, treatment duration as well as on route of administration.

Results

In invasive aspergillosis, the weighted cost per episode of fungal infection was 11.996 € with voriconazole treatment (voriconazole IV followed by oral voriconazole) and 13.657 € with caspofungin treatment (intravenous caspofungin only). The incremental saving with voriconazole treatment was 1.661 € per patient. The cost-saving results of voriconazole were confirmed with varying treatment duration within realistic range. 41% of the patients in the VORIBEL study were fully treated with oral formulation. For these patients a saving of 6.375 € was achieved with the use of oral voriconazole.

Conclusion

Voriconazole is a cost-saving option compared with caspofungin in the treatment of invasive aspergillosis.

P.26

DISSEMINATED TOXOPLASMOSIS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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We report a case of disseminated toxoplasmosis in a 60-year-old male, diagnosed in October 2004 with RAI stage I CLL. The patient was previously treated by consecutive therapeutic regimes: FCR (fludarabine, cyclophosphamide, rituximab), chlorambucil and alemtuzumab.

On September 29th 2008, he underwent an allogeneic unrelated HLA-matched HSCT. The conditioning regime consisted of fludarabine, high-dose melphalan and ATG. He received cyclosporin A for GVHD prophylaxis. Due to persistent vomiting Pneumocystis prophylaxis with cotrimoxazole was replaced by pentamidine inhalations. Two months after transplantation, he presented at the emergency ward with fever. Staphylococcus epidermidis was isolated from blood cultures probably due to a catheter infection. He deteriorated very quickly despite antibiotic treatment and catheter replacement. Peripheral blood analysis showed thrombocytopenia, elevated liver enzymes, high ferritin and LDH levels. Bone marrow aspirate showed absence of any hematological malignancy but abundance of Toxoplasma tachyzoites without obvious hemophagocytic syndrome. Qualitative PCR for Toxoplasma gondii showed strong positivity in the patient and was negative in the donor. Pretransplant serological tests were positive for Toxoplasma IgG and negative for IgM. Antitoxoplasmic therapy with clindamycin was initiated immediately. Nevertheless, within 24 hours after start of this treatment his condition evolved towards a septic shock with multiple organ failure and death.

Disseminated toxoplasmosis after SCT is very rare (0.5-5%) and usually develops as a reactivation of Toxoplasma cysts in seropositive patients. Because of its non-specific clinical signs and lethal outcome, Toxoplasma is often misdiagnosed and only revealed at autopsy. Prophylactic treatment and /or regular monitoring by Toxoplasma PCR in high risk patients (positive pretransplant serology, allogeneic transplantation and GVHD) are possible ways to improve the outcome of toxoplasmosis in SCT recipients.

P.27

MULTIPLE MYELOMA PATIENT WITH PANCYTOPENIA AND A CHANGING ELECTROPHORESIS

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A male patient was diagnosed with MM in august 2005. After induction chemotherapy he was autologously transplanted in January 2006. Although a VGPR was obtained a rapid relapse occurred at the end of 2006. No good response was seen after bortezomib alone and bortezomib-dexamethasone, but a prompt reduction of the paraprotein was obtained after adriamycin had been added.

Some weeks after the last cytoreductive regime the planned NMST was blown off because the general condition of the patient worsened. A wasting syndrome developed after the patient had experienced a low grade fever (virology negative, cultures negative) for some weeks. The liver and bone marrow biopsy could not explain the organomegaly, the elevated liver enzymes nor the establishing pancytopenia. Two months later (August 2007) the paraprotein level started to increase again and thalidomide was started and continued till April 2008. During this period the pancytopenia persisted. Although the IgA stabilised a polyclonal increase of Ig G remained unexplained.

Prior to the start of lenalidomide a bone marrow aspirate was done which revealed striking and surprising results. In a hypocellular marrow numerous intracellular parasites were seen in macrophages suggestive for *Leishmania amastigotes*. PCR confirmed the presence of *Leishmania* DNA and the species was identified as *Leishmania infantum*. Travel history revealed a trip to Corsica in april 2006 and to the south of Spain in September 2006. The patient remembered a lot of insect bites on his legs during his stay in Corsica.

Discussion

This MM patient became infected some months after his autologous transplantation when entering the Mediterranean basin where *Leishmania infantum* is endemic. Time between exposure and clinical signs varies from days to years and took probably more than a year in this case. Since an intact cellular immune system is required to control *Leishmania*, Visceral leishmaniasis (VL) occurs preferentially in immunocompromised patients. Our patient showed the five characteristic hallmark features of VL: fever, organomegaly, cachexia, pancytopenia and hyperglobulinemia. This is the first report of VL in a patient with MM.

P.28

ROLE OF FDG-PET/CT IN HODGKIN'S LYMPHOMAS: CLINICAL ASSESSMENT IN A SINGLE CENTER

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Objectives

To evaluate the contribution of FDG-PET/CT in Hodgkin's lymphoma (HL) patients (pts) .

Population and methods

Twenty-six pts with newly diagnosed HL were prospectively studied using FDG-PET/CT for initial staging, early response assessment (ERA), end of treatment (EOT) and post-treatment surveillance (PTS). ERA with FDG-PET/CT was performed after 2 cycles of chemotherapy (ABVD) in 17 pts and/or after an average of 4 cycles, in 16 pts (in 8 pts FDG-PET/CT was performed after 2 and 4 cycles). PTS with FDG-PET/CT was performed every 3 months during the first year, 6 months in the second year and yearly thereafter. Complete remission (CR), partial remission (PR) or progressive disease (PD) were classified according to the IHP criteria (Juweid, JCO 2007). Besides FDG-PET/CT, all pts had clinical and biological follow-up.

Results

Twenty out of 26 pts remained in CR after first line of treatment with a median follow-up of 29 months. Five of the 7 pts with PR on ERA with FDG-PET/CT relapsed; interestingly, the 2 pts who remained in CR had a consolidation with radiotherapy at the EOT. 13/13 patients with CR after 2 cycles of chemotherapy remained in CR during follow-up. Two out of 11 pts in CR after 4 cycles of chemotherapy relapsed during follow-up. One of these 2 pts - also evaluated after 2 cycles - was in PR after 2 months. At the EOT, FDG-PET/CT showed a CR in 23 of 26 pts, a PD in one patient and residual disease (PR) in 2 pts who progressed during follow-up. Three patients in CR at EOT relapsed during PTS (with a median relapsing time of 5 months after EOT). Four of 6 patients with PD on FDG-PET/CT had no clinical evidence of relapse.

Conclusions

Our small series confirms the high predictive value of ERA with FDG-PET/CT in HL patients. The negative predictive value of ERA after 2 cycles (100% CCR) is higher than after 4 cycles for prediction of response . We suggest that FDG-PET/CT at the EOT and during PTS is particularly useful in HL pts with a PR at ERA. In this population, an early relapse can be detected by FDG-PET/CT before clinical evidence.

P.29 PLATELET-FREE PLASMA OBTAINED BY HIGH-SPEED CENTRIFUGATION IS SUITABLE FOR BOTH LUPUS ANTICOAGULANT ASSAYS AND COAGULATION FACTOR ASSAYS

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Introduction

The platelet-poor plasma (PPP) obtained after a routine centrifugation (RC) is suitable for the routine coagulation tests, but the residual platelets in this PPP could compromise the lupus anticoagulant (LA) assays due to the freezing and thawing procedures. Till now, there is no recommendation defined by CLSI how to obtain the Platelet-Free Plasma. The aim of the study is to evaluate if a high-speed centrifugation (HSC) of PPP obtained after first RC is suitable for the LA assays and has no adverse effect on the coagulation factor assays. Methods and Materials: All blood samples were collected in 0.105M sodium citrate tubes and centrifuged at 1500 g for 10 minutes at room temperature. All coagulation assays were performed on MDA II. The HSC were evaluated at 5000, 12000 and 18000 g for 10 minutes in 50 routine samples. The residual platelet counts after each centrifugation were measured by Sapphire. All coagulation factors were analysed in 5 normal and 5 pathological samples after HSCs or only one RC. The LA assays were performed in 50 samples after the HSCs or filter technique. All the plasmas were stored at -20°C for maximally 10 days. Results: We found a mean value of residual platelet counts of 4369/ μ l (1000 -19500) in 50 tested samples after one RC, while the values for these same samples were found all inferior to 1000/ μ l after HSCs at 12000g and 18000g. At 5000g, two values were found superior to 1000. The correlation coefficients (CC) of the coagulation factors between RC and two HSCs are shown in table 1. The CC of the LA assays between HSC at 18000g and filter technique, between two HSCs are shown in table 2 and table 3. Furthermore, APTT and PT obtained from 50 samples before freezing and after thawing with the 18000g preparation showed a CC of 0.96 and 0.99 separately. The von Willebrand factors (antigen and ristocetin-cofactor) tested in 6 samples gave us a CC of 0.99 and 0.98 between the 1500g and the 18000g centrifugation.

Table 1 (n=10)	PT	FII	FV	FVII	FX	APTT	FVIII	FIX	FXI	FXII
1500g vs 18000g	0.99	0.99	0.95	0.99	0.99	0.98	0.96	0.99	0.99	0.98
1500g vs 12000g	0.99	0.99	0.93	0.99	0.99	0.98	0.96	0.98	0.99	0.97

Table 2 (n=46)	PTT-LA	dRVVTs	dRVVTm	dRVVTc	dRVVT _r	TTI
Filter vs 18000g	0.98	0.99	0.98	0.95	0.98	0.98

Table3(n=49)	PTT-LA	dRVVTs	dRVVTm	dRVVTc	dRVVT _r	TTI
18000g vs 12000g	0.98	0.97	0.79	0.95	0.95	0.92

Conclusion

Platelet-Free Plasma obtained by HSC at 18000 or 12000g is suitable for both LA assays and coagulation factors assays. In our study, we have found the results obtained with the HSC at 18000g are better than those at 12000g.

P.30

COMPOUND HETEROZYGOSITY FOR HEMOGLOBIN E AND BETA THALASSEMIA MINOR: A CASE REPORT.

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We present the case of a young woman, referred to our clinic because of the suspicion of thalassemia. Except for fatigue and intermittent headache she did not have any complaints. She originated from North Eastern Thailand. Medical and familial history were unremarkable. Blood analysis showed a mild anemia (Hb 11.6 g/dL) that was severe microcytic (59.8 fL) and hypochromous (19.8 pg). Hemoglobin analysis (HPLC chromatographic analysis and electrophoresis at pH 8.4 and 6.0) showed the presence of HbA, HbF and HbE+HbA2. On chromatographic analysis 18.5% was HbA, 22.7% HbF and 51% HbE+HbA2 (HbA2 and HbE cannot be distinguished by chromatographic analysis). Based on the presence of a low but considerable percentage of HbA, not due to transfusions, and the severe microcytosis, compound heterozygosity for β -thalassemia and HbE was suspected. The patient's mother had indeed β -thalassemia minor: microcytic anemia with 6.1% HbA2 and 2.3% HbF. The father was not available for testing but he is presumed to be at least heterozygous for HbE.

β -thalassemia is due to impaired production of β -globin chains, which leads to a relative excess of β -globin chains.

Hemoglobine E syndrome is due to a specific mutation of the β -globin chain. This mutation leads to substitution of glutamic acid by lysine at codon 26 of the β -globin gene, activating a cryptic mRNA splice site. This results in reduced synthesis of the β -E chain.

The mutation is frequent in Southeast Asia.

The compound heterozygote state of β -thalassemia and HbE is rare in Belgium but it affects a million people worldwide. The phenotype can range from asymptomatic to severe transfusion dependent thalassemia major. The type of β -thalassemia plays a role in the phenotypic heterogeneity. But even patients with the same mutation within one family may show significant differences in clinical severity. The cause of that is largely unknown. Treatment consists of transfusion and iron chelation. HbF modulating agents (e.g. hydroxyurea) can also be used.

As our patient was asymptomatic and had only mild anemia, treatment was not indicated.

P.31 ALVEOLAR HEMORRHAGE AFTER G-CSF STIMULATION IN A HEALTHY STEM CELL DONOR

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We present the case history of a healthy 44-year old male, the HLA-identical haematopoietic stem cell donor for a brother suffering from B-CLL. After a 4-day injection period with G-CSF (Filgrastim 10 µg/kg/d) mobilizing CD34+ cells, the donor developed cough with hemoptysis. A Chest X-ray showed diffuse interstitial infiltrates. On further exploration with HRCT bilateral diffuse groundglass attenuation was found. This clinical picture is compatible with diffuse alveolar hemorrhage, most likely secondary to administration of G-CSF.

G-CSF is widely used to accelerate recovery from neutropenia after chemotherapy. A second major indication is mobilisation of stem cells. Adverse reactions are frequent but mostly mild e.g. bone pain, fatigue (30%). Splenic rupture is a rare but known risk. However, pulmonary toxicity is rare and not widely known. Case reports are found in literature, although only few on pulmonary complications during G-CSF stimulation alone (1,2,3).

The mechanism of this complication is not well known. Some of the unwanted adverse effects of G-CSF can be partially explained by the significant production and the activation of PMN (2). One of the reports proposed a role of interleukin-1β in the development of acute lung injury (3).

Our donor was hospitalised for observation and he received a single high dose of corticosteroids IV. During hospitalisation oxygen saturation levels remained normal. As planned stem cells were collected with sufficient circulating CD34+ cells at presentation. Obviously, additional administration of G-CSF was omitted. Spontaneous and full recovery occurred over the next week.

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P.32

BMSC INDUCE GROWTH AND SURVIVAL DIFFERENTLY IN CELL LINES AND PRIMARY CELLS

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Multiple myeloma (MM) cells reside in the bone marrow (BM) where they receive necessary survival signals by MM-stroma interactions. It has furthermore been shown that BMSC can confer drug resistance by cell mediated adhesion. In this study we wanted to investigate the mechanisms behind the CAM-DR and investigate whether this is a temporal phenomenon. When primary 5T33MM cells were co-cultured with BMSC for 3h, BrdU uptake dropped from 21% to 8%, with a concomitant increase in G1 phase from 58% to 70%. Using an alpha 1 antibody these effects could be blocked by 50% and using a beta 1 antibody by 15%. At this time point, bortezomib seemed to have no effect. After a 24h co-culture, BrdU uptake in the MM cells increased from 10% to 20%, and at the same time the cells were sensitive to bortezomib (5nM induced 70% cell death). When we investigated if these changes in cell cycle progression were reflected in differential expression of cell cycle proteins, we found surprisingly that at 3h of co-culture c-myc expression was upregulated. At 6h of co-culture p27 was decreased and other cell cycle proteins such as Cdk6 and cyclin D2 increased. Similar experiments were performed with the MMS1 cell line. In these cells cell cycle arrest increased over time, with no effect at 3h, an increase in G1 phase from 53% to 63% at 24h, and a large G1 arrest at 48h (68% vs 51%). The blocking antibodies had no effect on these cells. Furthermore, these cells were protected at the later timepoints against bortezomib (20% vs 55% at 24h and 20% vs 85% at 48h). When the cell cycle proteins were investigated we found that Cdk6 and cyclin D2 were upregulated but p27 to a much larger extent, thereby most likely causing the cell cycle arrest. In summary, it seems that BMSC arrest primary MM cells in the first hours of contact, rendering them less sensitive to cytotoxic drugs. During these early hours however, the MM cells are increasing their cell cycle machinery to be able to proliferate rapidly at later time points. This is in contrast with cell lines where the BMSC induce an increasing cell cycle arrest and stronger drug resistance. These findings provide new insights in BMSC-MM interactions.

P.33

FIRST EXPERIENCE WITH SCIG IN A COHORT OF BELGIAN PEDIATRIC PRIMARY IMMUNODEFICIENCY PATIENTS

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In Nov 2005, subcutaneous immunoglobulin (SCIG) was first registered and reimbursed in Belgium for primary immune deficiency (PID) patients.

Aim

To evaluate the efficacy and safety of SCIG.

Material and Methods

All pediatric PID patients treated with SCIG from 11-2005 till 01-2008 were included. Patients and/or their parents were instructed to use SCIG during 5 weekly visits at the day hospital. Afterwards, SCIG was administered at home by the patient/parents. The SCIG dose was based on the calculated weekly IVIG dose resulting in appropriate IgG trough levels or calculated as 100-200 mg/kg/week. Serum IgG and IgG3 trough levels were measured 4 weeks prior to and 4 weeks after start of / switch to SCIG and an infection diary was kept. The Child Health Questionnaire-Parental Form 50 was used to evaluate quality of life while on IVIG and after switch to SCIG.

Results

31 patients, mean age 8 years (range 11m-20y), were treated with SCIG for PID requiring Ig substitution. Of these 31 patients, 21 were switched from IVIG to SCIG because of venous access problems (n=2), low IgG/IgG3 trough levels and ongoing infections despite adequate three-weekly substitution (n=6), severe side effects of IVIG treatment (n=8), personal preference and feasibility of home therapy (n=5). The mean SCIG dose for the total group was 111 mg/kg/week (range 75-238 mg/kg/week). For patients with IgG3 deficiency + SPAD, IgG3 trough levels were significantly higher with SCIG (0.17g/L) versus IVIG (0.14g/L) (p=0.03- t-test). Trough IgG levels were higher with the same dose SCIG versus IVIG (8.86 g/L (SD 3 g/L) vs 10.24 (SD 3 g/L)) (p=0.006 – t-test). For the entire group, IgG trough levels under SCIG were 9.5 g/L (SD 3 g/L) and resulted in a decreased incidence and severity of infections in all but 3 patients. Transient redness and swelling at the infusion site were noticed in all patients. Results of the CHQ-PF50 were available for 9 patients who switched from IVIG to SCIG. Six reported an improvement in health, none experienced any deterioration.

Conclusion

SCIG is an efficient and well-tolerated method of IG substitution for PID patients.

P.34

CONTRIBUTION OF FLOW CYTOMETRY IN THE DIAGNOSIS OF HEREDITARY SPHEROCYTOSIS

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Introduction

The hereditary spherocytosis is a regenerative haemolytic anemia characterised by a loss in proteins in the erythrocytic membrane. On one hand, the high level of reticulocytes interferes with the detection of ankyrin deficiency in SDS-PAGE electrophoresis and, on the other hand, the ektacytometry is not available in Belgium. One test based on the fluorescence of red blood cells after incubation with eosin-5-maleimide was described recently for the diagnosis of hereditary spherocytosis. The aims of this study are first, to study the role of flow cytometry in the diagnosis of hereditary spherocytosis and to suggest eliminating reticulocytes by cell sort to make easier the detection of ankyrin deficiencies by electrophoresis.

Material and method

The test needs 5µl of red blood cells washed from an EDTA sample.

The percentage of lowering of fluorescence intensity of the patient in comparison to the mean of the 6 healthy subjects is calculated. 25 samples suspect of hereditary spherocytosis were tested. The diagnosis is confirmed by electrophoresis or clinical data for 11 patients.

In a second step, for 3 patients with negative electrophoresis unless a high clinical suspicion of hereditary spherocytosis, a separation between reticulocytes and red blood cells is realised by cell sorting.

Results

On the 11 confirmed hereditary spherocytosis, 10 show a ratio higher than 21% (positive cut-off) and the last one is in the grey zone at approximately 20%. Three negative patients in electrophoresis have a ratio higher than 21% with a high concentration of reticulocytes. The high reticulocytosis could mask an ankyrin deficiency and therefore explain the normal result observed in electrophoresis. The elimination of an important fraction of reticulocytes (~75%) should make interpretation of electrophoresis easier.

Conclusions

The flow cytometry is a screening tool useful for the diagnosis of hereditary spherocytosis by his speed, low blood volume need, easiness of interpretation, very high specificity and sensitivity.

Patient	Hb (12-18 g%)	MCHC (32-36.5 g/dL)	Indirect Bilirubin (<1 mg%)	Reticulocytosis (25000 - 75000/ μ l)	$\frac{\text{MFI patient}}{\text{MFI 6 healthy subjects}} \times 100$	Cryohemolysis (0,4-10,1%)	Electrophoresis	Diagnosis
MJI	14.4	36.3	0.99	124,700	19.6	14.5	Unrealized (first diagnosis in 1958)	Hereditary Spherocytosis
CV	8.4	35.1	1.16	252,000	33.3	13.8	Spectrin & ankyrin deficiency	Hereditary Spherocytosis
PMI	8.6	36.7	Unrealized	326,400	21.8	16.3	negative	Hereditary Spherocytosis
LV	8.7	35.4	Unrealized	199,000	33.6	19.7	negative	Hereditary Spherocytosis
JF	16.1	38.2	10.22	426,600	27.6	19.8	Spectrin deficiency	Hereditary Spherocytosis
HM	11.4	37.2	Unrealized	303,000	43.1	Unrealized	negative	Hereditary Spherocytosis
PR	11.2	34.8	Unrealized	202,000	39.9	51.0	Membrane protein deficiency	Hereditary Spherocytosis
LB	13.6	33.9	8.12	514,000	34.3	56.2	Unknown	Hereditary Spherocytosis
BS	12.0	36.5	unrealized	538,000	34.0	unrealized	Unknown	Hereditary Spherocytosis
WW	13.0	33.8	unrealized	178,000	27.0	unrealized	Unknown	Hereditary Spherocytosis
HL	9.5	35.0	unrealized	113,600	34.9	13.1	Unknown	Hereditary Spherocytosis
FFI	11.9	33.1	Unrealized	83,000	6.4	10.2	Unrealized	Isolated Anemia
BZ	10.8	35.2	Unrealized	79,000	16.3	12.4	Unrealized	Isolated Anemia
CA	12.0	32.9	Unrealized	110,000	4.5	15.3	Unrealized	Isolated Anemia
FFII	11.3	33.6	Unrealized	102,300	6.2	10.56	Unrealized	Isolated Anemia
BP	13.7	32.9	Unrealized	30,200	13.7	12.1	Unrealized	Diagnosis not yet known
MJII	10.1	30.1	0.42	200,000	11	7.2	Unrealized	Diagnosis not yet known
PMII	6.4	35.2	0.78	20,000	7.5	1.45	Unrealized	Medullar Aplasia
MJ	10.5	35.5	0.99	157,300	-2.3	Unrealized	Unrealized	Sickle cell anemia
PL	14.2	34.7	0.4	51,900	4.56	Unrealized	Unrealized	Cold agglutinins
FB	11.7	32.8	Unrealized	117,000	4.9	2.1	Unrealized	G6PDH deficiency
MJ	15.7	28.5	0.5	75,900	6.1	unrealized	Unrealized	Brother with Hereditary Spherocytosis
TD	11.4	33.8	2.25	131,000	6.9	Unrealized	Unrealized	Paroxysmal Nocturnal Haemoglobinuria
AB	13.7	34.7	1.16	204,000	-9.6	Unrealized	Unrealized	Diagnosis not yet known
BM	8.1	33.3	Unrealized	41,000	-25.3	Unrealized	Unrealized	Schizocytosis

P.35 BIMODAL ACTIVITY OF MESENCHYMAL STROMAL CELLS ON T-LYMPHOCYTES ISOLATED FROM CORD BLOOD AND PERIPHERAL BLOOD.

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Background

Bone marrow derived human mesenchymal stromal cells (MSCs) is known to have in vitro and in vivo immunomodulatory capacities. In an effort to understand the mechanism of modulation, co-cultures of MSCs with different actors of immune response have been studied. In this study, we investigated the capacity of MSCs to modulate the proliferation of T-cells obtained from peripheral blood (PB) and umbilical cord blood (CB); this later considered as source of naive T-cells. We addressed the impact of MSCs on lymphocyte phenotype, activation and proliferation and investigated the importance of MSCs/T cell ratio, the requirement of cell contact and the role of soluble factors on MSCs activities.

Methods

MSCs were incubated with purified CD3⁺ lymphocytes activated by mitogens, CD3/CD28 agonists or alloantigens for 5 days. T-cell proliferation was assessed by BrdU incorporation or after CFSE labeling. Detection of FoxP3⁺ regulatory T-cells was performed by flow cytometry and qRT-PCR.

Results

MSCs induced a dose- and contact-dependent inhibition of T-cell proliferation, regardless of the stimulus type. This effect was weaker on CB than on PB T-cells, suggesting that naive T-cells are less sensitive to MSC inhibition. The expression of lymphocyte CD38 marker was downregulated. At low ratio, MSCs supported both CB and PB T-cell proliferation. For this stimulatory activity, the contact between T-cells and MSCs was not required and IL-6 seemed to be the major actor of this effect. The % of CD45RO positive cells increased after co-culture with MSCs while CCR7/CD62L positive cells were differently modulated by MSCs. We also observed the expansion of regulatory T-cells in both CB and PB independently of MSC concentrations.

Discussion

MSCs display a bimodal activity on T-cells depending on cell ratios used in the culture. Differences in T-cell subset (RA/RO) sensitivity to MSC inhibitory effect were evidenced. The expansion of regulatory T-cells from both CB and PB occurred at all ratios and seemed not to be related to MSC inhibition. The dual ability of MSCs to either sustain or suppress immune responses should be considered in the context of clinical applications.

P.36

PLASMOCYTOID DENDRITIC CELL LEUKEMIA: A CASE REPORT

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Plasmacytoid dendritic cell leukemias (pDCL) is a very rare proliferative entity mainly occurring in elderly adult males, and characterized by an aggressive clinical course despite sensitivity to multi-agent chemotherapy.

We describe the case of a 70-year-old man who developed a pDCL one year after the diagnosis of chronic myelomonocytic leukemia.

The patient presented with an isolated cutaneous lesion of the scalp, that rapidly evolved to other cutaneous sites; he was in good physical state, without B symptoms except for nocturnal sweats. Blood tests showed cytopenia with bone marrow infiltration by blastic cells, CD4+, CD56+, CD7+, CD36+, CD123+, in the absence of any specific B-, T-lymphoid, NK-cell or myeloid lineage markers. Caryotype showed complex aberrations including loss of chromosome 13 and Y, deletion of the long arm of chromosome 6, t(12;15), add(8q24) and centromeric inv(9); del(5q) was not found. Biopsy of the cutaneous lesion showed a characteristic infiltration of the derm, sparing the epidermis and the vessels by mononuclear cells bearing the same immunophenotype. After an induction chemotherapy including anthracyclines, cyclophosphamide, L-asparaginase and prednisone, the patient achieved a complete hematological remission; however, chromosomal aberrations still persisted on subsequent caryotypes. He is actually on consolidation therapy.

We have the opportunity to describe a case of pDCL, also recognized by the WHO classification as CD4+ CD56+ hematodermic tumours. This observation emphasizes the role of cytometry in the diagnosis of haematological disorders.

P.37

MULTIPLE MYELOMA IN BLACK AFRICANS: DO THEY BEHAVE DIFFERENTLY?

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Multiple myeloma (MM) is the most common hematologic malignancy in African-Americans, with an approximately 2-fold higher incidence compared to Caucasians. However, little is known about the characteristics of the disease in Black Africans.

From December 1987 to October 2008, 705 consecutive cases of MM were diagnosed in our institution. In order to determine differences in clinical features and outcome between Black African (BAMM) and Caucasian MM (CMM) patients, we identified 18 BAMM, and compared them to a control cohort of CMM patients (ratio 1:4) matched for age, sex, stage and ISS at diagnosis, medical history (e.g. infections, HIV status, antecedent of MGUS, ...).

BAMM patients resided in Belgium for professional, political or medical reasons. They were characterized by a peculiar medical history of HIV (n=1), MGUS (n=5), infections (n=9), hypertension (n=9), and by a median age of 44 (34-65), no gender predominance, DS stage I (n=8)/III (n=10), ISS I (n=4)/II-III (n=10), IgG/A/light chain isotypes (n=10/4/4), kappa/lambda/both (n=12/5/1). Treatment was required in 12 patients (DS I (n=1)/III (n=11)).

Front-line therapy consisted of VAD (n=7), VACD (n=1), TD (n=2) or MP (n=1), followed by either single/double/tandem ASCT-RIC/alloSCT (n=6/1/1/1), or MP-Bortezomid (n=1). Patients treated by single/double/tandem ASCT-RIC achieved at least PR (n=5); one treated by alloSCT developed PD at 49m, was subsequently diagnosed with AML, is in CR for both diseases after conventional chemotherapy at 102m; 2 patients did not respond to ASCT but were rescued by a MP-combination. All except one patient treated upfront without ASCT (n=4) developed rapid PD. After a median follow-up of 33.5m, 13 patients (SD I (n=5)/III (n=8)) are alive (1CR, 5PR, 5SD, 2PD) (median 57m, range 11-117m), and 5 (SD I (n=2)/III (n=3)) died of PD (n=2) (1 and 2m after diagnosis) or unrelated causes (n=3) (8, 21, 22m after diagnosis).

Comparison with the Caucasian cohort and epidemiological data from the Belgian Registry for Cancer and the International Agency Research and Treatment of Cancers will be provided.

P.38

ANAPLASTIC LARGE CELL LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Anaplastic large cell lymphoma represents 5-8% of all adults lymphomas and about 10-20% of T-cell lymphomas. The disease comprises three distinct clinical entities : ALK+ ALCL, ALK- ALCL, primary cutaneous ALCL.

We report our experience on 14 patients (5 ALK+ ALCL, 9ALK- ALCL). First line treatment consisting in anthracyclin containing regimen yielded 9 complete responses, 2 partial responses, 3 refractory diseases.

Five year overall survival in the total population was 45%. ALK- status could be identified as an adverse prognostic factor. (5year OS 21% in the ALK negative population). IPI could not be determined as an adverse prognostic factor probably because of the small size of the studied sample. One ALK+ refractory patient displayed t(2;17) characterizing a CTLC-ALK fusion. This patient also had a complex karyotype and c-myc rearrangement on FISH analysis, which could probably explain the bad outcome in this patient.

From the second line of treatment on, global response rate is quite low (15% of complete responses). This could indicate that relapsed or refractory disease carries a bad outcome whatever the ALK status, thus stressing the need for more efficient second line treatments

P.39

RITUXIMAB FOR MULTICENTRIC CASTLEMAN DISEASE: ABOUT THREE CASES

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Background

Multicentric Castleman disease (MCD) is a rare HHV8 associated lymphoproliferative disease affecting essentially HIV+ patients. Although recent reports have stressed the efficacy of monoclonal anti CD20 antibody Rituximab in HIV+ patients, few data exist on HIV- MCD patients. A recent study indicated that flare-up of Kaposi sarcoma was a frequent complication in HIV+ MCD treated by anti CD20 antibody. Little is known about the efficacy of Rituximab retreatment in relapsed MCD.

Case reports

We report our experience of Castleman disease in 3 patients. 2 patients were HIV-, one patient was HIV+: the HIV positive patient showed concomitant infiltration by Kaposi sarcoma.

The first patient presented MCD in the setting of auto-immune disease. He was treated by four doses Rituximab/3 weeks and remained in complete remission (CR) for four years. First relapse was retreated by the same scheme and yielded CR. Response persisted one year. A third course of Rituximab therapy had to be interrupted for severe allergic reaction.

The second patient was treated with four weekly doses of Rituximab and a consolidation therapy of monthly Rituximab injections: CR persists 8 months after initiation of treatment:

The third patient is a HIV+ patient presenting multiple organ failure and respiratory distress due to MCD and Kaposi sarcoma. Liposomal Doxorubicin was added to Rituximab treatment. The treatment allowed rapid reversion of respiratory distress. CR was achieved and maintained after 6 months of treatment.

Conclusions

We describe sustained CR in MCD patients treated with Rituximab in one HIV- patient and 2 HIV+ patients: Retreatment with anti CD20 antibody for relapse seems a feasible option and allowed prolonged CR in one patient. We did not observe any flare-up of Kaposi disease in the HIV patient, as this patient was simultaneously treated by liposomal Doxorubicin.

P.40 SYSTEMIC MASTOCYTOSIS ASSOCIATED WITH CLONAL HEMATOLOGICAL NON-MAST CELL DISEASE: ABOUT 2 OBSERVATIONS

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Systemic mastocytosis (SM) comprises a heterogeneous group of disorders characterized by infiltration of bone marrow and other tissues by neoplastic mast cells. A subset of these patients (20-30%) has associated clonal hematological non-mast cell disease (SM-AHNMD, WHO), usually of myeloid origin, including AML, MDS and MPD disorders, rarely of lymphoid origin, including CLL, Hodgkin and plasmacell dyscrasia.

We report 2 cases of SM-AHNMD.

A 68-year-old woman was diagnosed in 1983 with SM, based on a typical cutaneous and bone marrow infiltration, with osteoporosis. She received interferon-alpha for 2 years, treatment discontinued because of asthenia. Blood count has always be normal. In 2007, she was referred for thrombocytopenia with lymphocytosis (>5000/mm³). Viral and auto-immune serologies were negative. Tryptase was abnormal. Bone marrow was infiltrated by 8% mast cells (CD2+, CD25+, CD117+) and by 20% of monoclonal lymphocytes (CD5+, CD19+, CD38+, CD20neg), with normal megacaryocyte count. C-kit and IgVH mutations were negative. Isotopic platelets survival study was also normal. Thrombocytopenia was attributed to an auto-immune process, no further therapy was needed.

A 68-year-old woman was referred for left upper abdominal pain related to hepatosplenomegaly. Blood test was normal, without cytopenia. Bone marrow showed a large mast cell infiltration with reticulinic fibrosis. Skin was not involved. Bone x-rays exhibited a characteristic alternance of osteolytic/osteosclerotic lesions. Caryotype was normal. With time, she became symptomatic, imatinib was started despite the absence of c-kit mutation. After a 6 months follow-up, splenomegaly was reduced but she developed a neutrophilic hyperleucocytosis with thrombocytosis; complementary genetic analyses revealed a negative bcr-abl status but a positive JAK-2 mutation, suggesting the co-existence of myelofibrosis with SM.

We report 2 cases of SM-AHNMD, with unexpectedly, no D816V c-kit mutations. Association of SM with CLL is extremely rare, with only 3 cases reported in the literature. Co-existence of SM with MPD is less uncommon, and JAK-2 mutation has been described in this setting.

P.41

COMPOSITE LYMPHOMA, ANOTHER JANUS: ABOUT 2 CASES

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Malignant lymphomas are classified into either HL or NHL, based on morphologic, immunophenotypic and clinical particularities. The co-occurrence of NHL and HL in the same patient is rare, focusing on the concept of composite lymphoma, defined as the occurrence of more than one histological pattern of lymphoma in a single patient.

We report on 2 cases of composite lymphoma.

A 62-year-old man was referred for dyspepsia, abdominal pain, weight loss and fatigue. Blood tests were normal. PET-CT revealed multiple hypermetabolic retroperitoneal and mediastinal lymph nodes. Bone marrow was not involved. Diagnosis of stage III follicular lymphoma was made, and complete remission was achieved after 8 cycles of Rituximab-CHVP. Relapse occurred 3 years later, with multiple peripheral adenopathies associated with B symptoms. Biopsy revealed a stage IV sclerodular Hodgkin lymphoma associated with a follicular component. The patient is actually in complete remission after 6 cycles of ABVD.

A 63-year-old man had an indolent right popliteal mass for 3 years; he was referred for homolateral inguinal enlarged lymph nodes; he was in good general status without B symptoms. Blood test showed a high CRP with LDH elevation. PET-CT showed multiple hypermetabolic sites. Bone marrow was not involved. Biopsy showed a stage IV composite lymphoma with co-existence of follicular and Hodgkin lymphoma. Partial response was achieved after 6 cycles of Rituximab-ABVD, suspended for bleomycin-related pneumonia. Relapse occurred 5 months later. A second biopsy identified a follicular lymphoma in blastic transformation. The patient is actually in clinical complete response after 4 courses of Rituximab-CHOP and 4 courses of Rituximab-COP.

In conclusion, we describe the association of follicular and Hodgkin lymphoma in 2 patients, the first occurring sequentially, the second, simultaneously. This uncommon medical phenomenon is reported in less than 5% of lymphomas. Many other associations are possible. This emphasizes the importance of morphological and immunophenotypical analysis in order to treat adequately both components of the disease.

P.42 HAPLOIDENTICAL TRANSPLANTATION IN BELGIUM: PLAYING WITH FIRE?

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Up to 50% of patients needing a hematopoietic transplantation never find a suitable fully compatible matched related or unrelated donor. This has led to the use of alternative transplantation options such as cord blood or haploidentical transplantation. These transplantations are generally more hazardous than standard protocols because of intricate immunological issues when fiddling with HLA compatibility. This can sometimes be a problem when performed small scale. We have retrospectively analysed our transplantation data of the last five years to compare with worldwide results using similar protocols. From August 2003 to September 2008, we performed 17 haploidentical transplantations in Gasthuisberg University Hospital, Leuven (males/ female ratio=11/5, median age at transplantation= 41 years). All patients were considered high risk (AML=11, ALL=4 and NHL=2). Twelve patients were transplanted according to the Aversa protocol (or variant), three patients received haploidentical cells as means of rescue post aplasia and two patients were transplanted in aplasia following reinduction therapy (variant of Kolb et al., ASH 2006). Post transplantation GVHD was relatively prevalent (aGVHD 8/15 patients, cGVHD 4/5 patients). Infections were extremely frequent in the first two years following transplantation (fungus infection 9/17, CMV infection/reactivation 6/17). Yet, we observe a 23% survival rate at one year (fig.1) in this population of high risk patients, similar to many other literature reports. Furthermore, seven out of eight survivors were in complete remission at the latest follow up. The survival plateau beyond one year suggests that transplant related mortality stabilises once adequate immune reconstitution is achieved, reducing the risk for infection. We therefore conclude that haploidentical transplantation is a potential option for patients lacking a compatible matched related or unrelated donor and that the first year following transplantation is critical for determining long term survival.

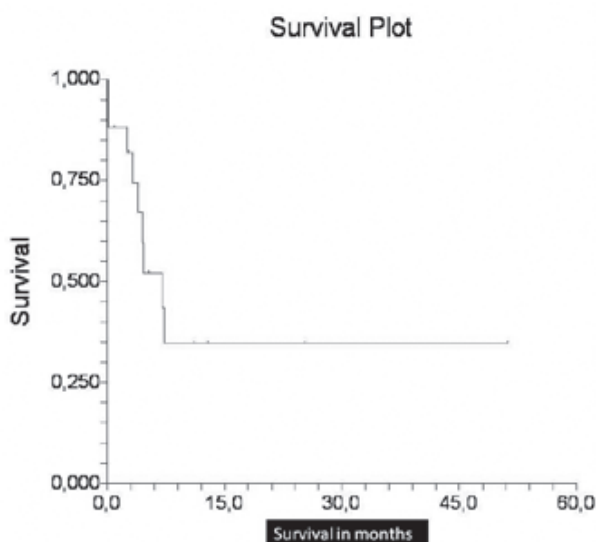


Fig 1. Kaplan Meier survival curve of patients receiving a haploidentical transplantation from Jan 2003 to September 2008 in Gasthuisberg, Leuven. C3 = survival time in months.

P.43

EFFICACY AND SAFETY OF DONOR LYMPHOCYTES INFUSIONS TO TREAT HEMATOLOGICAL MALIGNANCIES IN RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: THE EXPERIENCE AT THE JULES BORDET INSTITUTE

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Background

Adoptive immunotherapy by donor lymphocytes infusions (DLI) can induce remissions in some patients with hematological malignancies in relapse after allogeneic bone marrow transplantation. However DLI can also induce significant acute and chronic graft versus host disease (GVHD) and myelosuppression. In this work, we reviewed the safety and the efficacy of DLI .

Patients and methods

We retrospectively analyzed response and toxicity of 53 DLI administered , between 2003 and 2008 , to treat 22 patients with post allograft relapse of chronic myeloid leukemia (CML; n=2), acute myeloid leukemia (AML; n=5), acute lymphoid leukemia (ALL; n=4), myelodysplastic syndrome (MDS; n=4); multiple myeloma (MM; n=5), non Hodgkin's lymphoma (NHL; n=1) and Hodgkin lymphoma (HL; n=1). Two additional patients received DLI for incomplete chimerism (1 MM and 1 AML).

Results and discussion

Complete remissions were induced by DLI in 7/22 pts(30%) : 1/2 patients with CML, 1/5 patients with AML, 2/4 patients with ALL, 1/4 patients with MDS, 1/5 patients with MM, and in the patient with non Hodgkin's lymphoma. Targeted therapy or chemotherapy was combined to DLI in all the responders excepted in the patient with multiple myeloma. The two patients treated by DLI for incomplete chimerism converted to full chimerism. 9/22 patients developed GVHD grade II or more (II=5, III=1, IV=3) during DLI. 5 of the 7 responders developed acute GVHD grade II or more (II=2, III=1, IV=2). Three of these responders died from acute GVHD. One patient died directly from GVHD, 5 died from infection (4 of these 5 patients had at least grade II GVHD), 5 died from the disease and 1 from unrelated cause.

Conclusion

In accordance with previously reported studies, our results indicate that DLI can contribute to induce remission in hematological malignancies in relapse after peripheral blood stem cell transplantation. However, severe GVHDs account for a high morbidity and mortality. Predictive factors for responses and toxicities will be presented here and discussed in the light of larger published studies.

P.44

RECEPTOR TYROSINE KINASE FUSION PROTEINS ESCAPE UBIQUITINATION AND DEGRADATION

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Chimeric receptor tyrosine kinase genes are created by chromosomal rearrangements associated with atypical myeloproliferative neoplasms. These genes encode fusion proteins in which the intracellular kinase domain of a receptor is fused to the N-terminal part of a partner protein. Constitutive activation of the kinase domain leads to constitutive signaling and uncontrolled cell proliferation. The ETV6-PDGFRB (EP) hybrid protein is produced by the t(5;12) translocation between the genes TEL/ETV6 and the platelet-derived growth factor receptor beta (PDGFRB). It is found in a subset of patients with chronic myelomonocytic leukemia (CMML) associated with eosinophilia. FIP1L1-PDGFR α (FP) results from a deletion on chromosome 4q12 in chronic eosinophilic leukemia. In contrast to wild-type PDGFR alpha and beta, which are quickly degraded upon activation, we observed that EP and FP escaped down-regulation, resulting in accumulation of these proteins in the hematopoietic cell line Ba/F3. This was confirmed in peripheral blood mononuclear cells derived from patients. Similar data were obtained in cells expressing ZNF198-FGFR1, another fusion protein associated with the 8p11 myeloproliferative syndrome.

The normal degradation pathway of receptor tyrosine kinases requires receptor ubiquitination by the ubiquitin ligase CBL. We observed that EP and FP induced a strong phosphorylation of CBL, suggesting that the ubiquitin ligase was activated. However, the ubiquitination of these hybrids was much reduced compared to wild-type receptors. We next determine which domain of EP was involved in this process. An active kinase domain was not required for the stabilization of EP or FP, as kinase dead mutants were even more stable. By contrast, deletion of the pointed (PNT) domain in EP, which is required for polymerization and activation, strongly destabilized the protein.

In conclusion, receptor tyrosine kinase fusion proteins escape efficient ubiquitination and down-regulation, which likely contributes to hematopoietic cell transformation.

P.45 PERSISTENT POLYCLONAL B-CELL LYMPHOCYTOSIS WITH BINUCLEATED LYMPHOCYTES: A CASE REPORT

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Persistent polyclonal B-cell lymphocytosis (PPBL) is a rare entity. This syndrome is described essentially, but not only, in women, usually smokers. It is characterized by a moderate, chronic lymphocytosis ($> 4 \times 10^9/L$). The circulating B-lymphocytes have a binucleated or bilobulated form.

We report the case of a 35-year-old woman. She is a current smoker (20 packs/year). The peripheral blood examinations showed a persistent hyperleukocytosis ($12.9 \times 10^9/L$, normal range : $4.5-10^9/L$) with lymphocytosis ($4.5 \times 10^9/L$, nr : 1-4.5) and atypical binucleated lymphocytes. LDH was normal. Flow cytometry immunophenotyping established a polyclonal CD5+ B-lymphocytosis without kappa or lambda chain restriction. No clonal rearrangement of the immunoglobulin heavy chain gene was found by PCR. The cytogenetic analysis was normal. A CT scan showed a slight splenomegaly (14.8x9.1cm)

PPBL is a rare disease described in young smokers with a female predominance. It is also named « the smoking woman syndrome ». Although not observed in our patient, the karyotype is frequently abnormal with an isochromosome i(3q) and/or premature condensation of chromosomes.

The CD5+ phenotype is uncommon, only described in another case report in a male patient.

Whether PPBL is a real pathology or a simple cytological abnormality is still a matter of debate. The syndrome could be a premalignant disease (abnormal cytogenetics) or a incidental benign finding. The clinical course of PPBL is favorable in the vast majority of cases. A rapid diagnosis is warranted to avoid any aggressive exploration and therapy.

Keywords

Smoking, binucleated lymphocyte, polyclonal lymphocytosis.

P.46

METHEMOGLOBINEMIA DUE TO TOLUIDINE INGESTION: A CASE REPORT

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Toluidine is an industrial component and can pose a serious risk of toxicity when ingested. We report the case of a child who developed an acute cyanosis due to methemoglobinemia (MHG) following toluidine ingestion.

A 2-year-old boy was admitted to the intensive care unit for acute severe cyanosis and dyspnea 24 hours after the ingestion of pure solution of toluidine, a commercially available industrial component kept in home. At admission, blood hemoglobin was 12.7g/dl (normal range: 9.4-15.5), LDH: 660UI/L (nr: 150-500). Arterial gas values were pH 7.36, paO₂ 262mmHg, paCO₂ 38mmHg, base excess -4, O₂ saturation 44%. Methemoglobinemia was 55% and carboxyhemoglobin 0%. Methylene blue was given i.v. at a dose of 1 mg/kg and MHG decreased at 20%. Because of persistent severe hypoxemia and 20% of MHG, a second dose of methylene blue was injected, but without any further effect. A severe haemolytic crisis occurred 3 days later, with a fall in hemoglobin to 7.8g/dl, in haptoglobin (nr: 22-164) to undetectable levels and an increase of LDH to 775UI/L. There was no personal or familial history of hemolytic disease. Coombs test was negative. Hemoglobin electrophoresis was normal. No G6PD deficiency was found. Blood MHG disappeared after 4 days.

In conclusion, accurate substance identification is essential for appropriate management of ingestion of industrial products. MHG is formed by oxidation of heme iron from ferrous Fe²⁺ to ferric Fe³⁺ state. The resulting molecule is unable to bind oxygen and oxygen delivery to the tissues is impaired. Methylene blue therapy reduced MHG. Hemolysis can occur by oxidant damage of other red cell proteins. In this case, prolonged medical observation is needed due to the later manifestation of symptoms.

Keywords

Toluidine, methemoglobinemia, methylene blue.

P.47

IN VITRO GENERATION OF CONVENTIONAL AND UNCONVENTIONAL TCR α -BETA CELLS

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Ex vivo generated T cells offer the possibility of overcoming the immune deficient window period after stem cell transplantation. Notch signalling is essential for T cell development and hematopoietic stem cells undergo T cell differentiation when cultured on the Delta-like-1 expressing OP9 cell line. T cells generated in these cultures have never been fully characterized. We investigated whether the T cells generated on OP9-DL1 cell line, show characteristics of conventional T cells as seen in thymus. HSC isolated from postnatal thymus were cultured on the OP9-DL1 cell line and at the time mature cells were observed, part of the cells were stimulated with PHA and IL-2. This was followed by stimulation with PMA and ionomycin and after 18 hours cells were checked for cytokine production. Another part of the cells were transferred to fresh OP9-DL1, in the presence of IL-15. HSC matured to CD27⁺CD1⁻ T cells and this maturation was faster and more efficient for the TCR $\gamma\delta$ fraction, compared to the TCR $\gamma\delta$ fraction. As expected, mature TCR $\gamma\delta$ cells were mostly CD8 $\alpha\alpha$ or double negative. However, few mature CD4 SP TCR $\gamma\delta$ cells were observed and the mature CD8 SP cells were subdivided into a CD8 $\alpha\alpha$ heterodimer and a CD8 $\alpha\alpha$ homodimer expressing subpopulation. In addition, both the mature TCR $\gamma\delta$ and TCR $\alpha\beta$ cells expressed the IL2R β receptor and proliferated on IL-15 without prior antigen stimulation, the CD8 $\alpha\alpha$ populations being the most IL-15 responsive. Both mature activated TCR $\gamma\delta$ and TCR $\alpha\beta$ produced IFN γ and TNF α and little or no IL-2 and IL-4. This suggests that CD8 SP TCR $\alpha\beta$ cells generated on OP9-DL1 are unconventional CD8 SP cells. However, after PHA and IL-2 challenge, conventional appearing CD8 $\alpha\beta$ cells emerged, with a cytokine production profile similar to that of thymic CD8 $\alpha\beta$ TCR $\alpha\beta$ cells. Our data suggests that OP9-DL1 supports the development of conventional and unconventional CD8 SP TCR $\alpha\beta$ cells. Further experiments are now being addressed to further characterize these cells.

P.48 INTERINSTRUMENT COMPARISON OF CBC RESULTS AT ONE SITE OF A GENERAL HOSPITAL

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Introduction

Nowadays clinical laboratories frequently use different haematology analysers in daily practice. Therefore interinstrument bias should be within allowable limits. We evaluated interinstrument bias of our different haematology analysers and compared them with recommended goals.

Materials and Methods

Sysmex XE 2100, XS 1000 and Poch100i are in use in our laboratory.

Instruments are calibrated according to manufacturer's recommendations and daily quality control is performed in line with current standards. Interinstrument comparison was performed using patient samples. Three times a week, three patient samples with different levels for complete blood count (CBC) are analysed on all three analysers within 4 hours. For all samples, daily results for XS1000 and Poch100i are compared to results of XE2100 that serves as our internal reference instrument. Bias is expressed as a percentage.

Results

Interinstrument bias for wbc, hb and plt between XE2100 and Poch100i was 3.06, 1.85 and 6.5% respectively. Interinstrument bias for wbc, hb and plt between XE2100 and XS1000 was 3.22, 1.2 and 5.6% respectively. Recommended goals for interinstrument bias are 0.3 to 0.5 times within subject variability (CVi). CVi for wbc, hb and plt are 10.9, 2.8 and 9.1% respectively.

Conclusion

Results of our evaluation of interinstrument bias for wbc and hb are in line with recommended goals based on CVi. For plt results, our interinstrument bias frequently was above goals based on CVi, but within limits in use in other laboratories (5-10%; personal communication).

P.49

EVALUATION OF THE CYTODIFF MONOCLONAL ANTIBODY COCKTAIL

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The CytoDiff cocktail consists of a 6 markers/5 colours antibody mixture (CD45-PC7/CD36-FITC/CD19-ECD/CD16-PC5/CD2-PE + CRTH2-PE) which allows a flow cytometric differentiation of white blood cells into 12 types (B-lymphocytes, non-cytotoxic T-lymphocytes, cytotoxic T- and natural killer cells, circulating monocytes, pro-inflammatory monocytes, basophils, eosinophils, neutrophils, immature granulocytes, myeloid blast cells and lymphoid blast cells of T- or B-lineage). With this newly developed method, it is possible to quantify cell types which cannot be identified by a "5 DIFF haematology analyzer", nor by microscopy. It can also give a lineage orientation in certain pathologies, which may be useful for further specific investigations, possibly shortening the time to diagnosis.

We used the Cytomics FC500 flow cytometer to analyze 147 samples with various clinical conditions and also tested a healthy population to set the reference values for the 12 cell types. We determined the reproducibility of the method using the Immunotrol control material. Comparison with our routine methods (ADVIA 2120 haematology analyzer, supplemented by microscopy) was performed with Pearson and Spearman correlation coefficients, concordance correlation, difference analysis and Passing Bablock fit.

Good reproducibility was obtained for cell types with a high prevalence, but it was lower for those with a low prevalence. This could partially be attributed to differences in manual gating adjustment, a problem of which the manufacturer is well aware, and for which he is in the process of developing software for automated gating.

For most cell types, good correlations were observed between the Cytodiff and the "classical" method. We found lower correlation coefficients for immature granulocytes, monocytes and basophils.

Overall, the performance of the antibody cocktail compared to our routine methods was good and due to the more detailed identification of circulating white blood cells it can be of great value in a contemporary haematological environment.

P.50
FIRST-LINE THALIDOMIDE-DEXAMETHASONE IN PREPARATION
FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN
SYMPTOMATIC MULTIPLE MYELOMA.

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Over the last decade, there have been major advances in the management of patients with multiple myeloma (MM) by the introduction of new agents targeting both the tumour clone and the bone marrow environment. The combination of thalidomide with dexamethasone (TD) has been widely used as induction regimen before autologous stem cell transplantation (ASCT). However, the fact that thalidomide might interfere with stem-cell collection has been questioned.

In order to evaluate the impact of induction therapy on PBSC mobilisation, we collected data from MM patients undergoing a first-line ASCT after either TD or VAD as induction regimen, at our institution from March 2005 to June 2008.

Data collected for 12 TD and 15 VAD patients, concerned age, DS and ISS at diagnosis, sex, Ig isotype, number of induction cycles, need for additional mobilization regimen, number of mobilization and collection, CD34+ cells/kg collected and reinfused, engraftment. Patients that received more than one induction therapy before mobilisation were excluded.

Analysis revealed that all patients easily reached the minimum target number of at least 4×10^6 /kg CD34+ cells considered to be adequate to safely perform at least one ASCT. However, the number of CD34+ cells collected with thalidomide was lower (median of 10.489×10^6 /kg)(8.426-17.968), compared to VAD (median of 15.083×10^6 /kg) (4.479-26.879) ($p=0.051$). Nevertheless, because the number of CD34+ cells infused was comparable in both groups, we did not observe any significant differences in terms of engraftment.

Based on these results, it appears that short exposure to thalidomide is not a risk factor for impaired stem-cell procurement and allows collection of sufficient numbers of PBSC to support at least 2 courses of high-dose therapy.

P.51 HIGH PREVALENCE OF ANEMIA AND LIMITED USE OF ERYTHROPOIESIS-STIMULATING AGENTS (ESA) IN CANCER PATIENTS: A SURVEY OF PATIENTS PRESENTING AT BELGIAN ONCOLOGY AND HAEMATOLOGY CENTRES (ANEMIA DAY 2008)

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Objectives

To provide relevant and accurate information on prevalence and R/ patterns of anemia in Belgian cancer (ca) patients (pts).

Data collection and analysis

The Anemia day 2008 survey was a single visit, multi-centre, non-interventional study in adult ca pts under systemic R/ (chemoR/, hormonoR/, immunological and targeted R/) and/or radioR/. Pts signed an informed consent and data were collected, i.e. disease and its stage, ca R/ and anti-anemic treatment, including transfusions and ESA.

Hb values (g/dL) were classified into 4 categories WHO to assess the severity of anemia: no anemia: Hb > 12 g/dL; mild 10 < Hb < 11.9 g/dL; moderate 8 < Hb < 9.9 g/dL; severe : Hb < 8 g/dL. Adequate statistical analyses were performed.

Results

A total of 1403 pts aged 63+/-13 (mean+/-SD) yrs were enrolled in 106 oncology or hematology centres. The mean Hb was 11.6+/-1.8 g/dL and the prevalence of anemia was 55.7 % (95% CI: 53.1-58.3%), resp. 35.9 % mild, 17.8 % moderate and 2.1 % severe anemia. Anemia was more frequent in females than in males (p < 0.001), and in hematology pts (73.4 %) than in those with solid tumors (51.4%) (p < 0.001). Anemia prevalence was higher in hospitalized pts (75.5 %) compared to those seen in one-day-clinic (54.3 %) or in consultation (33.9 %) (p < 0.001), and in pts treated with chemoR/ (61.3 %) compared to those receiving radioR/ (34.4 %) or hormonoR/ (19.5 %) (p < 0.001). There was a clear correlation between severity of anemia and WHO PS (p < 0.001). Among anemic pts, 53.1% received no R/ (mean Hb 10.8+/-0.88 g/dL). Among the anemic pts who received R/ for their anemia (mean Hb 9.69 +/-1.13 g/dL), the most frequent treatments were RBC transfusions (39.4%), ESA (36 %), transfusions + ESA (10.9 %), ESA + iron (9.2 %) and iron alone (4.6 %). Comparison to the previous ECAS survey (2004) shows that there has been no major change in treatment patterns attitude towards anemia management in the last decade.

Conclusion

This survey shows that cancer-related anemia is still frequently observed in ca pts in Belgium. Frequent resorting to transfusions and limited use of ESA's are still prominent.

P.52 THE UNMET NEED IN CHRONIC MYELOID LEUKEMIA (UNIC): BELGIAN RESULTS FROM A EUROPEAN STUDY

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Introduction

Imatinib is standard first-line treatment of patients with Chronic Myeloid Leukaemia (CML). Management of patients showing resistance or intolerance to imatinib represents an unmet need.

Purpose

Quantify the unmet need in the management of CML patients in Chronic Phase (CP) and describe real life disease monitoring patterns.

Methods

UNIC is the largest European multi-centre, observational, cross-sectional study with retrospective chart review of 1493 treated CML patients, recruited Sept. 2006 - March 2007 and classified as resistant or intolerant according to the treating physicians' judgement. Type and frequency of monitoring were recorded. Some data were collected before the publication of the European Leukemia Net recommendations in 2006.

Results

All patients (n=56) were pre-treated with imatinib, with a 92.9% in the past 12 months. 46.4% had experienced resistance and/or intolerance to imatinib. The proportion of observed resistance or intolerance to imatinib was 16.1% or 32.1% respectively. 10.7% stopped imatinib because of toxicity. A median of 2 PCR-analysis was performed within the last 12 months, 10% of patients having 3-monthly PCR, and 10% having no PCR in the last 12 months. No mutational analysis was performed in half of the resistant patients.

Conclusion

A significant number of patients developed imatinib resistance and/or intolerance. Disease monitoring deviated from the ELN recommendations.

P.53 DIAGNOSTIC VALUE OF SERUM FLC FOR THE DIAGNOSIS OF MALIGNANT PLASMA CELL DISORDERS

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Background

Serum FLC K/L ratio is usually interpreted as normal or abnormal based on the reference range (0.26-1.65) established by Katzmann et al.. It is reasonable to assume that the likelihood or probability of a malignant plasma cell disorder increases with increasing or decreasing FLC ratios. To investigate this, we calculated the likelihood ratios (LR) for different malignant plasma cell disorders for various FLC K/L ratio intervals.

Results

The likelihood ratios are given in Table 1. A LR of 10 indicates that a positive result is obtained ten times as frequently in patients with the disease than in patients without this disease. A LR of >10 or < 0.1 indicates an important difference in pre-test to post-test probability. A LR of 1 indicates the test is useless. The LR for intact MM, NSMM, LCMM and ALA-A increases with increasing or decreasing FLC K/L ratio. The LR was > 45 for a FLC K/L ratio < 0.1 and was >1000 for a LR < 0.01 or > 10. For a FLC K/L ratio between 1.65 and 10, the LR was < 5, indicating a small change in pre-test post-test probability for a malignant plasma cell disorder.

The LR for a normal FLC K/L ratio (0.26-1.65) was 0.17 for intact MM, 0.33 for NSMM, < 0.01 for LCMM and < 0.01 for AL-A (Table 1). These results indicate that a normal FLC K/L ratio virtually excludes the diagnosis of LCMM and AL-A, but not the diagnosis of intact MM or NSMM. For the diagnosis of intact MM, there is a modest but substantial difference in pretest-posttest probability ($0.1 \leq LR < 0.2$), while there was only a minor change in pretest-posttest probability for NSMM ($0.2 \leq LR < 0.5$).

Conclusion

The likelihood ratio for a malignant plasma cell disorder increases as the serum FLC K/L ratio is more abnormal. When serum FLC K/L ratio is within the normal reference range, this virtually excludes AL-A and LCMM, but not intact MM or NSMM.

	Intact MM (1) (n=18)	NSMM (2) (n=28)	LCMM (3) (n=223)	AL-A (1,4) (n=5)
< 0.01	>1000	>1000	>1000	>1000
0.01-< 0.10	58	45	90	208
0.10-< 0.26	35	0.01	17	0
0.26-1.65	0.17	0.33	0	0
>1.65-10	4.3	1.1	0.17	0
>10	>1000	>1000	>1000	>1000

Data from:

1. Vermeersch P. et al. Br J Haematol. 2008;143:496-502.

2. Drayson M. et al. Blood 2001;97:2900-2.

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Likelihood ratios for different malignant plasmacell disorders

P.54

QUALITY MANAGEMENT IN STEM CELL TRANSPLANTATION. ADVERSE EVENTS: WHAT SHOULD WE REPORT AND HOW CAN WE IMPROVE OUR STANDARD OF CARE?

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Background

"The quality management plan shall include methods for detecting, evaluating, documenting, and reporting errors, accidents, and suspected adverse events, biological product deviations, and complaints" Jacie International Standards Accreditation Manual, Fourth Edition

Aim of the study

To review and analyse our method of reporting and evaluating adverse events from march 2007 until december 2008.

Method

Each person involved in stem cell transplantation (adult and paediatric clinics, cell collection and processing) is allowed to report an adverse event to the program director. All these events are reviewed monthly by a group of representatives of all departments. We have adapted the adverse event evaluation method described by Fuentes and Lassale (Hôpitaux Sud, Marseille) to our stem cell transplantation program. Adverse events are classified in the following sections : the graft, vigilance, nosocomial infections, therapy, diagnosis, accidents, stay of patient (waiting time, inadequate treatment of pain, fall,...), hospital life (cleanness, parking, behaviour,...), technical problems, transportation, communication, labels, computer technology, environment, misdemeanour. Each of these sections can be further subdivided.

The risk of an adverse event is then evaluated (HFMEA: Healthcare Failure Mode and Effects Analysis) according to a formula taking 3 factors into account : S = seriousness, O = probability of occurrence, D = probability of non detection. The result of $S \times O \times D$ = a risk score. The highest value indicates the necessity for urgent corrective actions.

Results

212 adverse events have been reported and analysed. Thanks to this method, we could point out the fields requiring more attention.

Conclusions

The tools proposed (classification and measurement of a risk score) have helped in the reporting of adverse events. They are also useful to detect which area of the program needs urgent corrective actions.

P.55

SATB1 IS TARGETED BY A NOVEL T(3;14)(P25;Q32) IDENTIFIED IN CASE OF EBV POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA OF THE ELDERLY

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EBV positive diffuse large B-cell lymphoma (EBV+DLBCL) of the elderly has been recently recognized as a new lymphoma entity. This neoplasm occurs in patients more than 50 years old and without any known immunodeficiency or prior lymphoma. 70% of patients present with extranodal disease and more than half have high IPI scores. The clinical course is aggressive, with a median survival of about two years. Genetic aberrations underlying pathogenesis of this lymphoma are unknown.

We report here one case of EBV+DLBCL of the elderly diagnosed in a 65-year-old woman who died two months after the diagnosis. Cytogenetic and FISH analysis of this lymphoma identified a novel IGH-mediated t(3;14)(p25;q32) that was present as an isolated aberration in 17/19 karyotyped metaphase cells. Using FISH with a panel of BAC probes, we mapped the 3p25 breakpoint in the region harboring SATB1. This gene, broadly expressed by immune cells, is a genome organizer that tethers multiple loci and recruits chromatin-remodelling enzymes to regulate chromatin structure and gene expression. SATB1 codes for a nuclear protein that is essential for proper T-cell development. This protein was found to promote breast tumor growth and metastasis. RNA interference-mediated knockdown of SATB1 in highly aggressive cancer cells altered the expression of more than 1,000 genes, including metastasis-associated genes and tumor suppression genes. To analyze expression level of SATB1 in the index case, qRT-PCR studies were performed on RNA extracted from the patient's bone marrow and lymph node cells. Three-fold upregulation of SATB1 mRNA was found in bone marrow cells, but not in lymph node. In comparison with the SATB1-negative K562 cell line, however, the latter sample showed more than 1,700-fold overexpression of SATB1. Further studies aimed at determination of a prevalence of t(3;14)(p25;q32) in NHL, and particularly in EBV+DLBCL of the elderly, are ongoing.

P.56 PRIMARY CEREBRAL LYMPHOMA AFTER CARDIAC TRANSPLANTATION

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Post transplant lymphoproliferative disorders (PTLD) is an infrequent but serious complication of solid organ transplantation. In heart transplantation, higher incidence of PTLD probably reflects a greater intensity and longer duration of immunosuppression.

We describe the case of 39 year-old woman who developed a PTLD, presenting as a primary cerebral diffuse large B cell lymphoma, CD20+ and EBV induced. Diagnosis was made 8 months after a cardiac transplantation for post-partum myocarditis. Immunosuppression (tacrolimus, mycophenolate mofetil, methylprednisolone) was drastically reduced and immunochemotherapy given with R-CHOP and HD methotrexate. Complete response was achieved after four cycles, and confirmed after two more cycles of consolidation. Because of the young age of the patient, cerebral radiation therapy was not done. Immunosuppression has been adapted, using everolimus in association with methylprednisolone 4mg a day. Acute graft rejection occurred, but was successfully controlled with transient high dose corticoids. The patient remains in complete remission one year after the end of treatment.

Despite the fact that cerebral lymphoma has been reported in the post-transplant setting, very few data are available in heart transplantation. Our observation suggests that association of HD MTX with standardized Rituximab-CHOP regimen and appropriate reduction of immunosuppression may modify the usual adverse prognosis of the disease.

P.57 PREGNANCY IN HEMATOLOGICAL MALIGNANCIES: A DIFFICULT CHALLENGE

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Hematological malignancies diagnosed during pregnancy are a difficult clinical condition with important impact on the patient's health and possibly on foetal integrity.

We report two cases we encountered in November 2008.

A 28-year-old woman, in her 20th week of gestation, was referred for fatigue and spontaneous bruises. She had a grade 4 anaemia with circulating blast cells, related to a M2 acute myelogenous leukaemia. After careful consideration and patient informed consent, induction chemotherapy with cytarabine and daunorubicin was initiated, but complicated by a grade 4 neutropenia, with neutropenic colitis; complete remission was achieved. No obstetrical complications were noted, and foetal development remained normal. Second course of chemotherapy was started on her 25th week of pregnancy. Because of adverse genetic prognostic factors, an allogeneic bone marrow transplantation is planned after delivery.

A 32-year-old woman, in her 19th week of pregnancy, had a 6 weeks history of coughing, associated with fatigue, weight loss and progressive dyspnea, related to a stage IV diffuse large cell non-Hodgkin lymphoma. After 2 courses of a Rituximab-CHOP-14 regimen with prophylactic intrathecal methotrexate injections, she is asymptomatic, and foetal monitoring is normal. Chemotherapy is foreseen for a total of 8 cycles. Delivery should occur at the end of treatment.

The decision to give chemotherapy in a pregnant woman has to be taken in consideration of multiple factors. Despite the lack of data concerning the possible adverse effects on the unborn child, there are evidences that administration of chemotherapy in the second trimester of pregnancy can be considered. Nevertheless, radiological monitoring has to be limited, and psychological stress can be difficult to handle by the mother, particularly if treatment has to be upgraded and if interruption of pregnancy has to be considered in a second time. Due to the rarity of pregnancy-associated haematological malignancies, we encourage collecting data in a national registry in order to gain expertise in this field.

P.58

EVALUATION OF THE ACTIVATED CLOTTING TIME WITH ACT PLUS® (MEDTRONIC)

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Object

The Medtronic Automated Coagulation Timer (ACT) is a point-of-care instrument which is used to monitor the response to high doses of standard heparin f.i. in cardiovascular bypass surgery and hemodialysis. In our hospital, kaolin is used as an activator (HR-ACT) and also the cartridges with heparinase (HR-HTC) are available to be able to differentiate with other coagulation defects. The aim of this study is to evaluate the use of the ACT® Plus (Medtronic). This instrument has two measuring channels, a barcode reader and can be connected to the laboratorium information system.

Materials and methods

We tested the within-run-reproducibility within a channel and on both channels on 10 measurements in 3 different concentrations of Li-heparin (see below) with the HR-ACT cups. We tested also the reproducibility of the cups HR-HTC (1st channel contains heparinase and the 2nd not). We compared measurements between the 2 channels, we checked lot to lot variation and compared instrument to instrument (ACT® Plus vs. ACT II). We tested also linearity. Nursing and laboratory staff were asked about the practical issues.

Citrated (106 mmol/l citrate) whole blood spiked with Li-heparin whole blood of the same donor were used for this study. The heparin spiked samples were prepared to obtain concentrations of 0, 1, 2 and 4UI/ml Li-heparin. Immediately before the measurement the samples were recalcified. For the comparison between the 2 ACT instruments whole blood without additives from patients in the intensive care unit was used for this purpose.

Results

CV for within-run reproducibility on 20 samples (both channels) was respectively for citrated whole blood 3%, on heparin spiked blood (HSB) (1UI/mL heparin with a mean value of 315 sec) 3%, on HSB(2 UI/mL heparin with mean value of 435 sec) 2.5%, on HSB(4 UI/mL) with heparinise (mean value 151 sec) 4.5% and without heparinise (mean value 407 sec) 8.2 %. CV for ACTII on citrated whole blood on 20 samples (both channels) was 4.2%.

Comparison on HR-ACT cups channel to channel gave a mean absolute difference of 2.8 sec (range 0-2 UI/mL heparin on 20 samples) with a r² of 1. Comparison on HR-ACT cups lot to lot gave a mean absolute difference of 9 sec with a with a r² of 0.99 (range 0-4 UI/mL heparin on 20 samples). Comparison on HR-ACT cups instrument to instrument (ACT Plus versus ACTII) gave a mean absolute difference of 1.7 sec (range 100-160 sec on 8 samples) with a with a r² of 0.87.

Linearity up to a concentration of heparin of 4UI/ml was tested on HR-ACT cups with a r² of 0.99.

Discussion

The within-run-reproducibility is comparable to the values mentioned by the supplier

on whole blood without additives (normal range CV of 2.6% and high range (4IU/ml heparin) CV of 3.3%). Only for the heparinase cups (HR-HTC) the CV is higher. This can be due to our method used on citrated blood.

The mean ACT value on citrated whole blood and heparin spiked blood is comparable to that reported in the literature (154s±36 for the baseline, 308s±54 for 1UI/ml heparin spiked blood, 393s±72 for 2UI/ml heparin spiked blood) (1). Linearity is good, but was only tested up to a heparin concentration of 4 UI/mL. Linear regression analysis showed an acceptable comparison and linearity between lots and between channels. However between instruments a poorer correlation was obtained but comparison was done on a small number of samples (8 patients) in a small range (100-160 sec). The tests were done on spiked samples in standard laboratory conditions, except for the instrument to instrument comparison which was performed on whole blood patient samples in the intensive care unit by the nursing staff. The laboratory staff and nurses evaluated the use of the instrument as practical and user friendly.

Conclusion

In spite of the lack of standardisation of the ACT tests, the ACT Plus® seems a performant, reliable and practical instrument.

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TLR7/8-MATURED DENDRITIC CELLS FOR USE IN DC-BASED IMMUNOTHERAPY: SHEDDING LIGHT ON THEIR MIGRATORY POTENTIAL

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Introduction

Accurate migration towards the lymph nodes and high production of interleukin-12p70 are two absolute prerequisites for dendritic cells (DC) to be effective in immunotherapy. In this regard, it has been demonstrated that DC matured with R848, a Toll-like receptor 7/8 (TLR7/8) ligand, possess both characteristics. Recently, DC that are alternatively cultured in the presence of granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-15 (IL-15) have gained interest given their promising immunostimulatory potential. The aim of this study was to assess the effect of TLR7/8 treatment on these IL-15 DC with a main focus on their migratory capacity.

Materials & Methods

Differentiation of monocytes to DC was performed by addition of GM-CSF, either in combination with interleukin-4 (IL-4 DC) or IL-15 (IL-15 DC). Subsequent maturation was induced with R848, tumor necrosis factor- α and interferon- γ in the presence or absence of prostaglandin E₂ (PGE₂). Both short-term (2-3 days) and long-term (7 days) culture protocols were performed. Phenotypic maturation was analyzed using three-color flow cytometry. In order to study DC migration, we investigated cell surface expression of CCR7 and migratory potential towards CCL21, a well-known CCR7 ligand, in a standard Transwell™ chemotaxis assay.

Results and conclusions

Both IL-4 DC and IL-15 DC had a fully mature phenotype (CD40^{high}, CD80^{high}, CD86^{high}) upon treatment with R848. Moreover, maturation of both DC subsets was accompanied by increased surface expression of CCR7. The CCR7 expression profile was characterized by a slight but significant up-regulation in short-term DC and a progressive up-regulation in long-term DC. In the chemotaxis assay, long-term IL-4 DC exhibited the highest migratory capacity followed by short-term IL-4 and IL-15 DC. Long-term IL-15 DC showed defective migration towards CCL21. For all DC subsets tested, addition of PGE₂ to the maturation cocktail was found to be essential for the induction of potent DC migration.

P.60

NOVEL FUSION OF ABL1 WITH A PRE-MRNA PROCESSING GENE IN AN ACUTE BIPHENOTYPIC LEUKEMIA

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A 19-year old woman was diagnosed with acute biphenotypic leukemia. Cytogenetic analysis of the bone marrow detected a reciprocal translocation t(1;9)(p34-35;q34) in 10 out of 22 metaphases. Fluorescent in situ hybridization (FISH) analysis using bacterial artificial chromosome (BAC) clone RP11-83J21, which covers ABL1, proved the rearrangement of the ABL1 locus on 9q34, as this BAC probe was split between the der(9) and the der(1). The partner gene was identified with break-apart BAC clones (RP4-765A10 and RP11-244H3) as being SFPQ, a gene located on 1p34 that encodes a proline/glutamine rich polypyrimidine tract-binding protein-associated splicing factor.

The patient was treated with a classic acute myeloid leukemia (AML) induction regimen in association with imatinib. The ABL1-SFPQ rearrangement could no longer be detected thereafter, but the persistence of a medullar blast count at 8% associated with an acquired isolated monosomy 18 prompted an intensification of the treatment. Complete remission was obtained after this regimen, which was followed by a consolidation regimen and a non-familial allogeneic hematopoietic stem cell transplantation with a dose-reduced conditioning. The patient remains in complete remission about 10 months after the allograft.

Hidalgo-Curtis et al recently described SFPQ as a novel fusion partner of ABL1 in a B cell progenitor acute lymphoid leukemia. We here report the second case of a translocation fusing SFPQ to ABL1 in a biphenotypic leukemia that responded to the association of conventional chemotherapy, tyrosine kinase inhibitor and allograft.

SFPQ encodes for a protein belonging to a new functionally related group of fusion partners for tyrosine kinases involved in pre-mRNA processing. Although uncommon, these fusions are important to recognise because of their potential to be targeted effectively by tyrosine kinase inhibitors.

P.61 JAK2 V617F MUTATION SCREENING USING AUTOMATED DNA EXTRACTION, REAL-TIME PCR AND DNA-MELTING CURVE ANALYSIS: RESULTS IN 83 PATIENTS

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Background

JAK2 V617F mutation is frequently observed in patients with Vacquez disease and essential thrombocytemia, and can be encountered in patients with myelofibrosis. The objective of this study is to relate our experience regarding JAK2 V617F mutation detection using automated DNA extraction, real-time PCR and melting curve analysis.

Patients and methods

From November 2007 to October 2008, 83 patients (M/F: 43/40; Median age (range): 63.2 years (19.1y-88.3y)) suspected of myeloproliferative disease were screened for JAK2 V617F mutation. Samples consisted in bone marrow as well as peripheral blood specimens. Extraction was performed using MagNa Pure LC DNA Isolation Kit 1 (Roche Diagnostics, Basel, Switzerland) and real-time PCR was performed using LightCycler (Roche Diagnostics). Primers and probes were designed using LightCycler Probe Design Software 2.0 (Roche Diagnostics) and were produced by Eurogentec (Liège, Belgium). The melting curve analysis for detection of JAK2 1849G>T mutation was performed by LightCycler Software 4.1 (Roche Diagnostics). Demographic data, blood count, EPO values and bone marrow biopsy results were also reviewed. Statistical analysis was performed using SPSS 16.0.

Results

Twenty one patients were screened positive for JAK2 V617F mutation. Corresponding clinical diagnosis were Vacquez disease in 8 cases, essential thrombocytemia in 11 cases and myelofibrosis in one case. One patient refused the bone marrow harvesting procedure and thus his diagnostic could not be determined. In JAK2 V617F negative patients, bone marrow exam revealed 3 cases of JAK2 negative Vacquez disease, 5 cases of JAK2 negative essential thrombocytopenia and 3 cases of JAK2 negative myelofibrosis.

Conclusion

In our one year experience, JAK2 V617F mutation was encountered in 73 % of patients with Vacquez disease, in 69 % of patients with essential thrombocytemia and in 25 % of cases of myelofibrosis. Combination of automated extraction with real-time PCR allows a fast, simple and accurate detection of JAK2 V617F mutation that offer valuable information for patient care.

P.62

ENDOPLASMIC RETICULUM STRESS AND UNFOLDED PROTEIN RESPONSE IN B-LYMPHOID TUMOR CELL LINES

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¹UGent

In the endoplasmic reticulum (ER), newly synthesized proteins undergo folding and assembly. Disrupting these functions (ER stress) lead to initiation of the unfolded protein response (UPR), characterized by phosphorylation of eIF2alpha by PERK, cleavage of ATF6 and splicing of XBP1 mRNA. The ultimate goal of the UPR is to protect the cell by resolving protein overload, however, apoptosis can be induced if ER stress is persistent. Since the UPR plays a major role in immunoglobulin synthesis and B-cell differentiation, we investigated the UPR in four B-cell tumor lines representing different B-cell stages: Burkitt's lymphoma-derived cell line Raji, germinal centre (GC) type diffuse large B-cell lymphoma (DLBCL) cell line SUDHL6, non-GC DLBCL-derived activated cell line Ocily3 and myeloma cell line MM1.S before and after induction with pharmacological ER stress inducers. MM1.S showed a higher basal UPR activity than SUDHL6 and Ocily3, as was expected in plasma cells, given their high level of Ig synthesis. Surprisingly this was also the case for Raji. Treatment with ER stress inducers for 3-6 hours showed an upregulation of ATF4 in all tested cell lines, while splicing of XBP1 mRNA could only be observed in Ocily3 and MM1.S. Also, pro-apoptotic CHOP was upregulated remarkably earlier in the more differentiated cell lines Ocily3 and MM1.S. Finally, after 24 hours of treatment all cell lines showed a stimulated expression of ER chaperones (GRP's and Hsp's) and in Raji, SUDHL6 and Ocily3 the onset of apoptosis. LD50 measurements revealed that MM1.S was more resistant to ER stress inducers than Raji, SUDHL6 and Ocily3, probably because in myeloma basal UPR activation and chaperone synthesis is already submaximal and may therefore be protective. Oci-ly-3 in turn proved to be the most sensitive. In conclusion, application of ER stress inducers to stimulate the pro-apoptotic arm of the UPR might play a role in lymphoma treatment and deserves further investigation in this direction, particularly in non-GC type diffuse large cell lymphoma. In plasma cell malignancies however, their use seems limited due to the high basal protective UPR activity in these cells.

P.63

ASSESSMENT OF BCR-ABL PROTEIN BY FLOW CYTOMETRY AND COMPARISON WITH QRT-PCR IN CHRONIC MYELOGENOUS LEUKEMIA

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Introduction

The Philadelphia chromosome results in the formation of the BCR-ABL1 fusion gene that encodes a constitutively active tyrosine kinase BCR-ABL fusion protein p190, p210 or p230.

The study of this protein may provide additional information that could not be revealed with tests targeting the BCR-ABL DNA and mRNA.

The aims of this preliminary study were therefore to assess the new Cytometric Bead Array (CBA) for detection of the BCR-ABL fusion protein and to compare CBA with QRT-PCR.

Material and methods

Fresh EDTA samples from 4 CML patients (2 myeloid and one lymphoid blast crisis including one T315I mutation and one acceleration) were assessed by the new BD CBA immunoassay system (Becton Dickinson). They were tested in parallel with fresh samples from 4 healthy subjects and compared with QRT-PCR test from GeneXpert system (Cepheid).

Results

CBA requires 2,5 ml EDTA sample, 2 hours of technician time and results were obtained after 5 hours. Theoretically, this flow cytometry system detects p190, p210 and p230 fusion proteins. The GeneXpert system assay only requires 200µl EDTA whole blood and simple manual pipetting steps followed by fully automated nucleic acid purification and nested RT-PCR. Results are obtained after 2h30. However, this system is expensive and allows only the detection of the transcripts coding for the p210 fusion protein.

As shown in table 1, the fusion transcripts were detected by the GeneXpert whereas one false negative sample was obtained with CBA assay. This false negative could be explained by a technical problem or perhaps by a mutated protein not detected by the CBA assay.

However, the protein with the mutation T315I in the tyrosine kinase domain could be detected.

Conclusion

The detection of BCR-ABL fusion protein by flow cytometry is slower but cheaper than QRT-PCR. Both methods should be applicable in labs without molecular biology and detect the BCR-ABL protein/transcript with the T315I mutation. Sensitivity calculated on a limited number of p210 containing samples is 75% for CBA and 100% for QRT-PCR.

Patient	Pathology	Mutation analysis	Flow cytometry (Mediane Fluorescence intensity)			RT-PCR			
			Healthy Subject	Patient	Ratio (patient/healthy subject)	Result	Cycle Threshold	BCR-ABL/ABL*100	Result
1	Myeloid blast crisis	unknown	160	1013	6,3	Positive	13,4	47%	Positive
2	Myeloid blast crisis	unknown	132	122	0,9	Negative	15,2	29%	Positive
3	Lymphoid blast crisis	T315I	112	3856	34,4	Positive	12,4	invalid result ("too positive")	Positive
4	CML in acceleration	unknown	105	1064	10,1	Positive	12,4	invalid result ("too positive")	Positive

ABSTRACTS POSTERS LYMPHOPROLIFERATIVE DISORDERS
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P.64

SPLENOMEGALY AND DISSEMINATED LYMPHADENOPATHIES IN A NEWBORN: NEONATAL PRESENTATION OF AN AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME

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Autoimmune lymphoproliferative syndrome (ALPS) is an inherited disorder characterized by lymphadenopathy, hepatosplenomegaly, cytopenias, autoimmunity and increased risk of lymphoma. It is related to a defect in the Fas pathway involved in lymphocyte apoptosis. Most of the cases have been associated to mutations in the genes TNFRSF6 (type 1a), TNFSF6 (type 1b), CASP10 or CASP8 (type 2). Classically, clinical symptoms occur in early childhood (median age of 2 years), they are most pronounced before the age of 5 years and become less severe during adolescence and adulthood. We report here an unusual case of ALPS with neonatal onset. A male newborn presented with splenomegaly, bilateral axillary and inguinal lymphadenopathies with paradoxically good neonatal adaptation and general condition. The pregnancy and delivery histories were not contributive but the familial history was marked by spontaneously regressive splenomegaly and lymphadenopathies during childhood of the mother and by a hemolytic anemia of unknown origin in the maternal grandfather. Soon after birth, the child developed a thrombocytopenia and a severe hemolytic anemia that required intensive phototherapy and blood red cells transfusions. The biological workup was negative for the coombs test and for viral, bacterial and parasitic infection. However, it revealed an increase in ab-double negative T cells that is a required element in the diagnosis of ALPS. Moreover, the level of Fas-ligand was elevated in the serum of the newborn and his mother. A genetic analysis confirmed the diagnosis of ALPS with the presence of a heterozygous mutation D265G in exon 9 of the TNFRSF6 gene in both patients. Fas-ligand dosage and genetic analysis were normal in the father. The child was treated with corticosteroid started at day 15 of life (methylprednisolone 2mg/kg/day). He showed a progressive resolution of the cytopenia that allowed a reduction of the corticosteroid therapy despite the persistence of the splenomegaly. This case stressed that ALPS may occur in neonates and that its treatment needs to be adapted for this age.

P.65

CEREBELLAR METASTASIS OF HODGKIN'S LYMPHOMA IN AN HIV POSITIVE PATIENT: A CASE REPORT.

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Introduction

Hodgkin's lymphoma (HL) involving the central nervous system (CNS) is a rare event. Cerebellum's lesion has never been described in HIV+ patients with HL.

Case report

An HIV+ patient of 39 y presented a stage IV B nodular-sclerosis HL in complete remission post two courses of BEACOPP. Because of a septic shock, he continued with six courses of ABVD. During the last one, he developed an intracranial hypertension due to a cerebellar tumor. A total resection was performed, the Epstein-Barr virus (EBV) was identified in the tumor and the PET scanner showed a complete remission. Then, he was admitted in the ICU for lymphocytic meningitis. He received four IV administrations of Rituximab with disappearance of EBV in the CSF, achieved radiotherapy and returned at home after intensive rehabilitation. Immunohistochemistry and molecular biology were carried out in order to compare the two tumors. Neoplastic cells were CD 30+ and CD 15+. On the primary tumor, a rearrangement of heavy chain of Ig gene also retrieved in the cerebellar tumor confirmed the same clone. Rearrangement of light chain of monoclonal Ig kappa gene, presented on the brain only, suggests a sub-clonal population of the initial HL.

Discussion

Involvement of the CNS occurs in less than 0.5% HL. Whole brain irradiation and systemic chemotherapy +/- Rituximab remain the current treatment, but intrathecal administration has also been performed. EBV is strongly associated with the pathogenesis of HL in HIV+ patients and is correlated with a better survival. EBV is so a meaningful prognostic marker and may have impact on therapeutical decision.

Conclusion

This is the fourth published case report of involving CNS in HIV+ patient. The interest remains in the occurrence during the systemic chemotherapy, in an unusual site and also in the treatment by surgery leading to complete resection. The disappearance of EBV from CSF by administration of systemic Rituximab argues for good crossing the blood brain barrier and should be considered as a meningo prophylactic treatment in EBV+ HL especially in HIV+ patients.

P.66 EYELID SWELLING AND CLL

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CLL can infiltrate any organ. Nevertheless, involvement of ocular adnexa seems rare

Case report 1

A 54 year old man consulted because of a progressive swelling of both eyelids. The blood results revealed the diagnosis of CLL. Ophthalmologic examination confirmed the eyelid edema without masses in eyelids or lacrimal glands. CT imaging showed nodular infiltrative masses around the eye muscles with palpebral and periorbital soft tissue swelling. Subsequent treatment with fludarabine-cyclophosphamide, local irradiation (2x2Gy) and alemtuzumab did not ameliorate the palpebral oedema significantly. A temporary complete resolution of the eyelid swelling was only seen after treatment with dexamethasone.

Case report 2

A 69 year old man complained of palpebral swelling 15y after the diagnosis of CLL was made. Ophthalmologic examination confirmed the eyelid swelling caused by expansion of the lacrimal glands. Infiltration of the pars orbitalis and palpebralis was confirmed by CT imaging. Because the eyelid swelling was socially unacceptable involved field irradiation (2x2Gy to each eye) was given with a complete resolution of the swelling.

Case report 3

A 52 year old woman consulted the ophthalmologist because of epiphora and proptosis. After excluding hyperthyroidy a biopsy was taken from the soft tissue mass lying against the lateral orbita seen on CT imaging. Histology and immunohistochemistry revealed the diagnosis of a small lymphocytic lymphoma. Staging for CLL is ongoing.

Discussion

Ocular adnexal lymphoma is not frequently seen. In a large pathology study (353 cases) of ocular adnexal lymphoma (conjunctiva, eyelids, lacrimal gland and orbital soft tissue), extranodal marginal zone lymphoma constituted 52%, follicular lymphoma 23%, mantle cell lymphoma 5% and small cell lymphocytic/chronic lymphocytic leukemia only 4%. SLL/CLL in ocular adnexa can be diagnosed as part of a disseminated disease at diagnosis, at relapse but also as an isolated manifestation of the disease.

The last two years three patients were seen at our institution with CLL infiltration of ocular adnexa. In 2 of the 3 patients the orbital swelling led to the diagnosis of CLL.

P.67

EXPRESSION PROFILING OF MYC AND MYCN AND THEIR TARGET GENES IN T(11;14)-NEGATIVE MANTLE CELL LYMPHOMAS WITH MYCN TRANSLOCATION

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MYC (c-Myc) is the well known oncogene involved in the pathogenesis of B-cell malignancies. Chromosomal translocations involving 8q24/MYC are characteristic for Burkitt lymphoma and recurrent in DLBCL and myeloma. Another member of this transcription factor family, MYCN, is amplified in a subset of neuroblastomas (NB). Recently, we have identified a novel translocation involving MYCN, t(2;14)(p23;q32), in two cases of t(11;14)-negative mantle cell lymphoma. Further search detected the IGH/MYCN rearrangement in one case of CLL in transformation and one myeloma case. Real-time quantitative PCR applied in 3 of 4 cases with t(2;14) showed upregulation of MYCN compared to the expression levels measured in other lymphomas. The expression of MYCN and MYC are inversely correlated and seem to be stringently regulated in NB. Therefore, we profiled both genes in MCLs and CLLs with or without MYCN- or MYC-translocation, normal B-cells, lymph node cells, bone marrow cells, and NBs to compare gene expression levels. In contrast to their expression in NB, the expression levels of MYCN and MYC are both highly elevated in MCL and CLL with MYC-translocation. The cases with MYCN-translocation have only a higher expression of MYCN. MYC is more than tenfold upregulated in the MCL and CLL with MYC-translocation compared to the other cells.

To check whether MYCN targets the same downstream partners in lymphoma and NB, we analyzed the expression in a panel of MYCN targets. Since MYCN and MYC have a large number of target genes in common and basal MYC expression is relatively high in these cells, it is not always clear if the change of a target gene's expression status is caused by MYCN or MYC. DKK3, normally downregulated by MYCN, is slightly upregulated in the samples with MYCN-translocation but downregulated in the cases with MYC-translocation. The expression levels of genes (e.g. PTMA, S100A6, TGFBI) normally upregulated by MYCN in NB coincided more in the cases with MYC- or MYCN-translocation. In conclusion, MYCN-translocation causes upregulation of MYCN and subsequent activation of MYCN downstream genes in MCL and CLL. Further research is necessary to elucidate the precise impact of MYCN on these malignancies.

P.68

IMPROVED DETECTION OF CHROMOSOMAL ABNORMALITIES IN CLL BY CONVENTIONAL CYTOGENETICS USING CpG OLIGONUCLEOTIDE AND INTERLEUKIN-2 STIMULATION. A BELGIAN MULTICENTRIC STUDY

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CLL lymphocytes have a poor mitotic index, generating only 40-50% of abnormal karyotypes. Interphase FISH can increase the detection rate to 80%. Since unfrequent aberrations will escape FISH detection, there has been great interest in improving culture methods with an immunostimulatory CpG oligonucleotide (CpG). We performed a multicentric cytogenetic study to assess the impact of 2 different culture procedures on the detection of clonal abnormalities in 223 consecutive unselected cases with CLL referred to our centers for routine analysis (October 2007 to November 2008). Parallel 72 hour cultures of bone marrow or peripheral blood were set up with the addition of either a conventional B cell mitogen (TPA) or CpG and interleukin-2 (IL2). Cytogenetic analysis was performed on both cultures. FISH with 1 to 6 probes of a CLL probe panel set was performed in 207 cases. Clonal abnormalities were identified in 122 cases (55%). In 78 cases, the aberrant clone was detected in both cultures. Of these, the percentage aberrant metaphases was similar in both cultures in 17 cases, higher in the CpG culture in 45 cases and higher in the TPA culture in 16 cases. A clonal abnormality was not detected in either the CpG or the TPA culture in 7 and 37 cases, respectively. Thus, the percentage of abnormal karyotypes with CpG and TPA was 52 and 38%, respectively ($p=0.004$). The following aberrations were detected after CpG culture: del(13q)(n=30), +12(n=30), del(11q)(n=20), del(17p)(n=9), del(6q)(n=8), 14q32 aberrations(n=9) and translocations(n=55). After TPA culture, the following aberrations were detected del(13q)(n=20), +12(n=23), del(11q)(n=22), del(17p)(n=8), del(6q)(n=4), 14q32 aberrations(n=7) and translocations(n=32). The application of FISH allowed to detect cytogenetic abnormalities that were not picked up by cytogenetics in 77 cases: del(13q) (n=69), +12(n=1), del(11q)(n=5), del(17p)(n=1), and del(14q)(n=1). In conclusion, our results confirm that CpG/IL2 stimulation increases the detection yield of cytogenetic abnormalities in CLL. Interphase FISH can further increase the detection rate. However, neither cytogenetics nor FISH detected all aberrations, demonstrating the complementary nature of these techniques.

P.69

PRIMARY EFFUSION LYMPHOMA IN A NEWLY DIAGNOSED HIV PATIENT.

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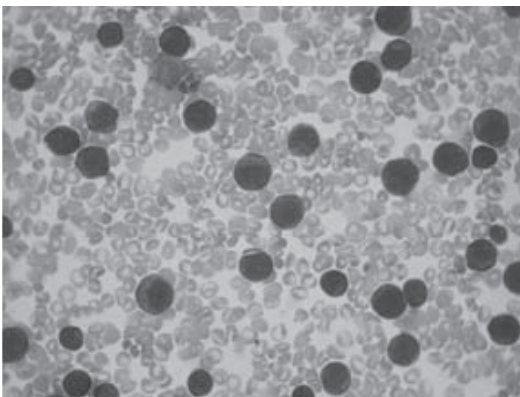
¹AZ Sint-Jan Brugge

Background

Primary effusion lymphoma (PEL) is a very rare lymphoma with an extremely unfavourable prognosis, defined by the WHO-classification as a neoplasm of large B-cells, usually presenting as serous effusions without detectable tumour masses. It is universally associated with human herpes virus 8 (HHV8/KSHV), most often occurring in the setting of immunodeficiency.

Case

Our patient, a 60-year old, cachectic male, with a history of alcoholism and suicidal tendencies presented infested with lice in scalp and body hair, crust like skin lesions, nail dystrophy, echymoses and motion disorders. Further investigations showed anaemia, highly inflammatory blood parameters, a newly diagnosed AIDS-infection with very low number CD4+ T-lymphocytes of Umol/l, latent syphilis infection and a fulminant haemorrhagic pericardial effusion. Morphological analysis of the pericardial fluid on a cytopspin preparation showed large mesothelial or immunoblastic to anaplastic cells with large, round nuclei often with nucleoli. The cytoplasm was moderate to abundant with the occasional presence of vacuoli. (figure 1). Flow cytometry showed large cells on forward scatter, CD45+ (common leukocyte antigen), CD38+ (activation marker) but lacking other B-cell markers, CD4 however was surprisingly positive. IgH/IgK gene rearrangement showed clonality by PCR. All these findings made us suspect the diagnosis of PEL and positive molecular analysis for HHV8/KSHV confirmed our diagnosis.



Hemorrhagic pericardial effusion with lymphoma cells

The patient did only receive anti-retroviral therapy, no chemotherapy and so far, four months in follow up PEL did not reoccur.

Conclusion

The distinction between PEL and other lymphomatous effusions such as ALCL is difficult on a cytologic and immunophenotypic basis alone. Therefore, detection of HHV-8 in the neoplastic cells is currently the only confirmatory test of PEL.

P.70

STRANGE VARIATIONS OF IGM LEVELS IN OPPOSITE PHASE WITH GAMMA-GLUTAMYLTRANSFERASE (GGT) LEVELS IN AN ALCOHOLIC PATIENT WITH WALDENSTRÖM'S DISEASE (WM)

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Waldenström's macroglobulinemia (WM) is a chronic B-cell lymphoproliferative disorder characterized by the abnormal slowly progressive production of monoclonal IgM. Splenomegaly, hepatomegaly, lymphadenopathy and bone marrow infiltration are typically present. In the majority of patients signs and symptoms are related to hyperviscosity.

We report the case of a 64y-old patient with WM in whom wide variations in IgM levels were observed over 3 years. He suffered also from chronic alcoholism with binge episodes. WM was diagnosed in Jul 2000 and 3 intermittent courses of chlorambucil were given up to Aug 2004, without any other treatment after that. Between Jan 2005 and Sep 2008, wide variations in IgM from 520 to 4090mg/dl and in GGT from 406 to 1618 U/l were observed, with peak and trough values in exact opposite phase. Indeed overtime, an inverse correlation was found between IgM and GGT ($r=-0.57$). The patient eventually developed a marked hepatosplenomegaly of mixed origin from steatocirrhosis and lymphoplasmacytic infiltration at liver biopsy. He never presented clinical or biological signs of hyperviscosity. Cryoglobulins were never detected. IgM were measured by a nephelometric assay.

To date we can only speculate about the nature of the inverse relationship between IgM and GGT.

In conclusion, IgM paraprotein may interfere with the GGT assay. Some examples of laboratory interferences have been reported (ref). Alternatively, a toxic effect of ethanol per se on the synthesis of IgM (J Burn Care Rehabil 16 :400, 1995 ; J Lab Clin Med 119 :32, 1992 ; Alcohol 24 :179, 2001) and the induction of GGT can be surmised

Keywords

Gammopathy, Waldenström's macroglobulinemia, interference, gamma-glutamyltransferase, nephelometric.

P.71 IDENTIFICATION OF A NEW LEUKEMIA/LYMPHOMA- ASSOCIATED BREAKPOINT CLUSTER INVOLVING PDL1 AND PDL2

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JAK2 located at 9p24, is recurrently targeted by genomic rearrangements in myeloid malignancies. To check whether this gene is also affected by recurrent 9pter chromosomal aberrations in B-cell malignancies, we analyzed 31 leukemia/lymphoma cases with various t(9p24) by FISH. Using a panel of BAC clones, the breakpoints were mapped in the following regions: (i) distal to JAK2 (13 cases), (ii) proximal to JAK2 (11 cases) and (iii) covered by JAK2 break apart probes (7 cases). Notably, in the latter cases the 9p24 breakpoints showed to be clustered in close vicinity to, but outside JAK2. Further analysis with fosmid probes narrowed down these breakpoints to the 300 kb region harboring PDL1 and PDL2, two genes coding for regulators of T-cell activation. The expression pattern of both PDL-genes was analyzed by QRT-PCR in five of the cases for which cDNA was available. This analysis showed overexpression of either PDL2, or both PDLs in two cases: (i) CLL in Richter transformation with IGL-associated t(9;22)(p24;q11) and (ii) splenic MALT lymphoma with complex karyotype including ins(9;1)(p24;p36-p?). The remaining three cases analyzed by QRT-PCR showed expression of both PDL genes similar to control samples.

In summary, we identified a new 9p24 breakpoint cluster of approximately 300 kb located in the region harboring PDL1/PDL2 genes. In at least two tumors, either PDL2 or both PDLs were significantly upregulated. So far, genomic aberrations of these genes have not been reported in hematological malignancies but aberrant expression of PDL2 has been found in approximately 50% of primary mediastinal B-cell lymphomas and Hodgkin lymphomas. Whether these genes are involved in development or progression of lymphoma remains unknown. Further analysis of the cases not targeting the PDL genes is ongoing.

P.72

HIGH RESOLUTION MAPPING OF THE CONSTANTLY LOST AND OVERREPRESENTED CHROMOSOME 7 REGIONS IN G/D HEPATOSPLENIC T-CELL LYMPHOMA

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Hepatosplenic T-cell lymphoma (HSTCL) is a rare peripheral T-cell lymphoma that derives from cytotoxic T-cells usually of g/d T-cell receptor type. This aggressive and incurable neoplasm is hallmarked by isochromosome 7q [i(7)(q10)] resulting in monosomy of 7p and trisomy of 7q. Which of these imbalances is critical for pathogenesis of this tumor is unknown. Recently, we identified ring chromosome 7 [r(7)] harboring multiple copies of 7q in 3 cases of g/d HSTCL. Genomic profiles of these tumors and 4 tumors with i(7)(q10) were analyzed using the Agilent oligonucleotide 244K platform. We found that r(7) was characterized by a constant loss of the 7pter-p14 and 7q34-qter regions, and amplification of the 7q21q33 sequences. Of note, the 7p14 and 7q34 breakpoints of r(7) occurred within the TCRG and TCRB genes clusters, respectively, suggesting that the r(7) is a by-product of an aberrant somatic recombination of the TCR loci. Moreover, all 3 cases showed a constant gain of the approximately 25 Mb region at 7q21q31 (2-6 copies) and an additional amplification of the smaller interval (5-10 Mb) at 7q21q22 (3-8 copies) containing CDK6. The cases with i(7)(q10) showed loss of the entire 7p arm and duplication of the 7q arm, as expected. Interestingly, one of these cases showed a homozygous deletion of the 1Mb interval at 7p22, heralding localization of a putative tumor suppressor gene implicated in a development of g/d HSTCL.

In summary, we showed that g/d HSTCLs are characterized by a constant loss of the 7pter sequences (38.2 Mb) and gain of the 7q21 region (5 Mb). We presume that these imbalances lead to inactivation of an unknown TSG at 7p and an aberrant activation of one or more oncogenes at 7q that play critical roles in a development and/or evolution of g/d HSTCL. To identify the targeted genes, we are currently performing mutational analysis of the 7p22 interval and a global transcriptomic analysis of available cases. Finally, we provided evidence that formation of r(7) in g/d HSTCL is underlied by an aberrant somatic recombination of the TCRG and TCRB loci, known to mediate numerous chromosomal translocations in T-cell malignancies.

ABSTRACTS POSTERS PLATELETS AND COAGULATION
P.73 – P.75

P.73

AN IMPROVED LABORATORY WORK-UP FOR THE DIAGNOSIS OF VON WILLEBRAND DISEASE

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Introduction

Laboratory diagnosis of von Willebrand disease (VWD) is based on the measurement of a combination of tests. The standard assessment of VWF functional activity is the ristocetin cofactor activity (VWF:RCo). In order to reduce the work-load of this labour-intensive aggregation method, we introduced the automated HemosIL VWF activity assay (IL, USA) as a screening test (confirmation of values below 60%). Another functional assay is the VWF collagen-binding (VWF:CB) assay, measuring the binding capacity of large VWF multimers to collagen.

Aim

To evaluate the performance of two VWF:CB ELISA tests and to demonstrate whether the VWF:CB can replace the VWF:RCo.

Materials and Methods

In this study we included the Collagen Binding Assay® (Life Diagnostics, USA) (CBA-LD) and the Asserachrom® vWF:CB (Diagnostica Stago, France) in the diagnostic work-up of 46 patients with VWD (35 VWD type I and 11 VWD type II) and 28 normal subjects.

Results

The CBA-LD was positive (cut-off < 50%) in 45 out of 46 VWD patients, whereas 2 non-VWD samples gave a value below 50%, resulting in a sensitivity and specificity of 98% and 92%, respectively. All 46 VWD cases were correctly diagnosed according to the Asserachrom vWF:CB, with only one incorrectly classified non-VWD sample as a VWD patient. Consequently, sensitivity and specificity was 100% and 96%, respectively. Remarkably, 2 patients have been misclassified as non-VWD by VWF:RCo (cut-off < 50%), with values of 51% and 66%, resulting in a sensitivity and specificity of 96% and 93%, respectively.

Re-evaluation of the cut-off values of the HemosIL VWF activity for confirmation testing, indicated a sensitivity of 100% with a cut-off of 65% compared to 96% with the 60% cut-off value. This higher cut-off resulted in a lower specificity with a positive predictive value of 94% (instead of 96%).

Conclusion

To reduce work-load, the HemosIL VWF activity with a cut-off of 65% is a reliable screening assay. However, a functional assay is still required to confirm the diagnosis of VWD. Our study shows that a VWF:CB assay can replace the VWF-RCo. The two evaluated VWF:CB assays show a comparable technical performance.

P.74 INTEREST OF THE QUANTIFICATION OF PLATELET GLYCOPROTEINS BY FLOW CYTOMETRY IN THE ESTABLISHMENT OF CONSTITUTIONAL MACROTHROMBOCYTOPENIAS

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Introduction

The mediterranean macrothrombocytopenia is a member of the family of hereditary thrombopathias with increased mean platelet volume.

This type of thrombopathias forms a seldom group avec very heterogeneous clinical expression and for which molecular mechanisms remain partially understood.

Discussion

The mediterranean macrothrombocytopenia is usually defined by a mild and chronic macrothrombocytopenia, an increased mean platelet volume, a normal thrombocytocrit, absence of important bleeding and a autosomal dominant transmission.

Several problems are encountered during the establishment of the diagnosis of Mediterranean macrothrombocytopenia.

First, haematological cell counters tend to not take into account macroplatelets in the measurement of thrombocytosis and mean platelet volume, leading to underestimation of those 2 parameters.

Then, several methods exist to calculate the thrombocytocrit.

Finally, molecular mechanisms are still in study, that's why the classification of constitutional macrothrombocytopenias will should be revised in the future allowing to maintain or not Mediterranean macrothrombocytopenia as a real entity in this group. However waiting for such progress, a careful examination of the blood smear ,the platelet aggregation (particularly with ristocetin) and above of all, application of a diagnostic algorithm and the quantification of platelet glycoproteins by flow cytometry can help us to make the diagnosis of Mediterranean macrothrombocytopenias.

Indeed, a lowering of the expression of Gplb was recently described for patients suffering from Mediterranean macrothrombocytopenia begging the question of a possible heterozygous Bernard-Soulier Syndrome.

Electronic microscopy, Western-blot and Molecular analysis of the genes encoding GPIIb and GPIX are ongoing.

Conclusion

The quantification of platelet glycoproteins by flow cytometry contributes to the diagnosis of hereditary macrothrombocytopenias and allows suspecting their classification.

P.75

DIAGNOSIS OF A PREVIOUSLY UNNOTICED FACTOR XIII DEFICIENCY AFTER OVARIAN HEMORRHAGE IN A 13-YEAR-OLD GIRL WITH A HISTORY OF EXTRADURAL HAEMATOMA AND DEFECTIVE WOUNDHEALING

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We report on a girl newly diagnosed with congenital factor XIII deficiency at the age of thirteen.

At the age of 3 she was admitted to the hospital with an extradural haematoma after minor trauma capitis. Laboratory analysis showed no abnormalities in screening coagulation test results (PT, APTT and platelet count). Levels of Von Willebrand (VW) factor antigen and activity were normal as well as platelet function tests. Later on there were several reports of extensive, but superficial bruising over the whole body, which even led to the suspicion of child abuse.

When she was 12 years old a botriomycoma was removed from her right flank with seriously impaired wound healing. Since no abnormalities in routine coagulation were detected, self mutilation was considered as a possible cause.

While still in wound care follow-up she presented at the emergency room with nausea and abdominal pain. Ultrasound and MRI showed an abdominal mass near the left ovary. An explorative laparotomy was performed and revealed a massive bleeding (most probably due to rupture of the follicle during first ovulation) and 1150 mL of blood was removed. Eventually a clot solubility test (CST) was performed because routine coagulation, platelet function tests, screening for VW disease and FVIII and FIX were normal. The qualitative CST turned out positive, indicating severe FXIII deficiency.

Therefore, we evaluated an automated quantitative assay for FXIII measurement to replace the qualitative CST. The Hexamate (MBL) is a latex immuno-assay (LIA) which can be implemented on a routine coagulation analyser.

The FXIII level detected in the patients plasma was below 4%. After administration of FFP postoperatively, FXIII was 22%. To stop bleeding 30 U/kg FXIII-concentrate was administered IV. 14 days after treatment her FXIII level was 12%. Today, she receives prophylactic administration of 10 U/kg F XIII every two to four weeks. Her wound is finally healing and she no longer presents easy bruising.

**ABSTRACTS POSTERS STEM CELL BIOLOGY
AND TRANSPLANTATION
P.76 – P.81**

P.76

RECONSTITUTION OF HUMAN B LYMPHOPOIESIS USING COCULTURE WITH HUMAN MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSC) support proliferation and differentiation of hematopoietic progenitors in Dexter-type cultures. The aim of the study was to analyse the B lymphopoiesis-supportive activity of MSC.

All MSC preparations used in this study were 100% pure by phenotype and were able to undergo adipogenic, osteogenic and chondrogenic differentiation. To assess MSC pro-hematopoietic activity, long term cultures were established with human cord blood CD34+ cells plated in contact with MSC harvested at passage (P)2, P4, P7 or P10, in the absence of exogenous cytokines. We analysed the outgrowth of CD10+, CD11b+, CD19+, CD33+ and CD34+ cells after 3 weeks. We observed that early passage MSC mediated expansion of CD34+ cells as well as differentiation toward both B lymphoid and myeloid lineages. Late passage MSC did not support outgrowth of CD34+ cells and differentiated myeloid cells but maintained a B lymphoid lineage supportive ability.

Next, the maturation stage of B cells generated was assessed by flow cytometric analysis and RT-qPCR analysis of B-specific transcripts. The cells tested positive for CD79 α , intracytoplasmic lambda or kappa light chains and μ heavy chains. Expression of surface μ heavy chains was also detected. Transcripts for λ 5, Pax-5, EBF, TdT, VpreB and RAG1 were clearly detected in outgrown B cells, but not in input CD34+ cells. Thus B cells generated in this coculture system recapitulate B cell ontogeny up to late pre-B cells. In conclusion, late passage MSC mostly display B lymphoid supportive ability while early passage MSC support myeloid differentiation. We describe a long term in vitro culture system initiated with human cord blood CD34+ cells and human MSC that supports B-lineage development in the absence of exogenous cytokines.

P.77

FACTORS AFFECTING THYMOPOIESIS AFTER NONMYELOABLATIVE CONDITIONING

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Background

Nonmyeloablative conditioning followed by allogeneic HCT is used in elderly patients with hematologic malignancies. It has been suspected that reconstitution of T-cell numbers would be impaired in elderly patients given nonmyeloablative conditioning because of age-related thymic atrophy. Here, we investigated long term lymphocyte reconstitution and thymic function in 64 patients given allogeneic peripheral blood stem cells (PBSC) after nonmyeloablative conditioning.

Patients and Methods

Median age at transplant was 56 years (range 10-69). Conditioning regimen consisted of 2 Gy total body irradiation with or without added fludarabine (n=57), or cyclophosphamide plus fludarabine (n=7). Thirty-nine patients received grafts from related and 25 from unrelated donors. GVHD prophylaxis consisted of mycophenolate mofetil and cyclosporine or tacrolimus. Immune recovery was assessed between 1 and 6.5 years after HCT by signal-joint T-cell receptor excision circle (sjTREC) quantification (211 samples), and flow cytometry. Further, in order to demonstrate a potential thymic recovery, sjTREC level changes from day 100 to day 365 were also assessed.

Results

There was a close correlation between sjTREC levels and naive CD4+ T-cells (defined as CD4+CD45RA+) counts ($P < 0.0001$). An inverse correlation was observed between the levels of sjTREC/ml and the recipient's age ($R = -0.37$, $p < 0.0001$). Further, sjTREC levels increased significantly from day 100 to 1 year after transplantation, this was more prominent in younger recipients ($p < 0.01$ for patients < 50 ; $p = 0.02$ for patients 50-60) and absent in patients > 60 . SjTREC levels still increased from 1 year to 2 years after HCT in patients < 50 years old ($p = 0.02$). In multivariate analyses, younger patient age ($P < 0.001$ and $P = 0.01$), and absence of extensive chronic GVHD ($P < 0.001$ and $P = 0.001$) were the main factors associated with high sjTREC levels and high number of naive CD4+ T-cells after nonmyeloablative conditioning.

Conclusions

Our data suggest that thymic neo-generation of T-cells occurred from day 100 in patients under 60. However, the levels of sjTREC remained low for patients above 60.

P.78

HEMATOPOIETIC RECONSTITUTION AFTER LONG TERM, UNCONTROLLED RATE FREEZING AND -80°C CRYOPRESERVATION OF HEMATOPOIETIC STEM CELLS

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The most widely used method for freezing and storing hematopoietic stem cells (HSC) is rate controlled freezing and storage in liquid nitrogen, either in liquid or in vapor phase. Some authors described a simple method of freezing and storage of HSC at -80°C without rate-controlled freezing using 5% DMSO and 5% HES. However, the ability of HSC, stored for more than two years at -80°C, to allow prompt hematopoietic reconstitution after myeloablative and non-myeloablative regimens has never been studied.

We retrospectively studied the hematopoietic reconstitution (HR) of neutrophils and platelets after autologous HSC transplantation after non controlled freezing and storage at -80°C. HR was defined by the number of days needed to obtain a neutrophil count above 500/ μ L and a platelet count above 20.000/ μ L for three consecutive days. Patients treated for an Acute leukemia (n=11) and patients for whom reconstitution data were not available (n=14) were not included in this preliminary study. The HR data of 174 autologous HSC transplantation performed in 147 patients (mean age 58 years) were analyzed. Patients were divided in three groups according to the duration of storage (Group I: < 1 year (n=139), Group II: 1-2 years (n=16) and Group III:>2 years (n=19)). The median time to obtain neutrophils and platelet reconstitution was 11 days. In univariate analysis, there was no statistically significant difference between the three groups for the time to neutrophils reconstitution. There was a statistically significant, but not clinically relevant, difference in time to platelet reconstitution between group I and III with a time to engraftment of 11 and 13 days respectively. There was no correlation between the length of storage and the number of days needed to achieve HR. A multivariate analysis performed on the entire population is ongoing.

Despite important limitations of this preliminary and univariate analysis, our series suggest that this simple and less expensive cryopreservation method for HSC allows for a reliable and prompt hematopoietic reconstitution after autologous HSC transplantation even when HSC stored for long periods are used.

P.79

REGULATORY T-CELLS AND CHRONIC GVHD AFTER NONMYELOABLATIVE CONDITIONING

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Purpose

We investigated the association between regulatory T-cell (Treg) levels and chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HCT) following nonmyeloablative conditioning.

Methods

Data from 74 patients given nonmyeloablative conditioning as treatment for hematological malignancies or renal cell carcinomas were analyzed. Conditioning regimens consisted of low-dose TBI with (n=45) or without (n=17) fludarabine, or cyclophosphamide plus fludarabine (n=12). T-reg (CD4+FoxP3+) levels on days 40, 100, 180 and 365 were determined by flow cytometry. Chimerism levels among total white blood cells, CD3+ T-cells and CD4+CD25+CD127dim/neg regulatory T-cells were determined by multiplex STR PCR or X-Y FISH. Thymic function was determined by assessing sjTREC levels.

Results

Mean Treg (+/-SD) levels in controls were 31 +/- 18 cells/ L. Mean (SD) Treg levels on day 100 were 20 +/- 24 cells / L (P< 0.01 in comparison to controls) in patients without grade II-IV acute GVHD before day 100, and 27 +/- 21 cells/ L (NS) in patients with an antecedent of grade II-IV acute GVHD. Mean donor CD3+ T-cells and Treg chimerism levels on day 100 were 75 +/- 29% and 81 +/- 18%, respectively (NS). Day 100 chimerism levels among CD3+ T-cells and Tregs were highly correlated (r=0.78, P< 0.01). The 1-year probability of moderate/severe NIH chronic GVHD in patients with day 100 Treg levels below or above median was 53% and 36%, respectively (P=0.13). SjTREC levels significantly increased from day 100 to day 365 after HCT (P< 0.01), demonstrating thymic recovery. Finally, Treg and sjTREC levels correlated on days 100 (r=0.48, P< 0.01) and 365 (r=0.47, P< 0.01) after HCT.

Conclusions

Our data did not show thus far a significant correlation between Treg levels and occurrence of chronic GVHD. The association between Treg and TREC levels on days 100 and 365 might suggest a role for the thymus in regulating Tregs levels after HCT, or that similar factors affect thymic function and Treg levels after nonmyeloablative conditioning. Data including higher number of patients will be presented.

P.80

INOLIMUMAB FOR THE TREATMENT OF STEROID-REFRACTORY GRAFT-VERSUS HOST DISEASE: A CASE STUDY

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Introduction

Treatment of GvHD remains a challenge, especially in case of refractoriness to steroids which is observed in 40% of patients. We here report our experience with inolimumab (an anti-CD25 monoclonal antibody, mAb) in severe steroid-refractory GvHD.

Case study

A 17 year old patient with a congenital bone marrow failure syndrome, was transplanted with an unrelated donor after becoming transfusion-dependent. After conditioning with fludarabine, busulphan and Thymoglobuline, she was transplanted with a PB graft consisting of 4.15×10^6 /kg CD34+ and 341×10^6 /kg CD3+ cells. She received cyclosporine A for GvHD-prevention. She developed acute GvHD at D+11 consisting of a generalized maculopapular rash, increased bilirubine (2.99mg /dL) and typical diarrhea. High-dose steroids (2mg/kg) was initiated with a good clinical response. At D+163, the GvHD flared whilst on steroids. CsA was reinitiated and despite of 250mg methylprednisolone for several days, the GvHD did not improve. We associated inolimumab (Leukotac R) per protocol: 0.3mg/kg for 8 days followed by 0.4mg/kg every other day. We determined lymphocyte activation markers (HLA-DR+, CD25+, CD57+, CD28-, CD62L-) before and during treatment with inolimumab. We observed a decline in CRP-levels and a gradual improvement of the GvHD, which allowed us to taper the dose of steroids. The number of CD25+ T cells dropped, whilst the other markers remained unchanged. The diarrhea worsened at day 12 of the inolimumab treatment, which urged us to re-administer inolimumab on a daily base. After 21 days the treatment was stopped. During the following weeks she developed sepsis and worsening of the GvHD. She succumbed at D+215.

Conclusion

Inolimumab (Leukotac R) is a murine anti-human mAb directed at the α -chain of the IL-2-receptor (IL-2R α , CD25) and has shown efficacy in steroid-refractory acute GvHD (Bay et al. Transplantation 2005). We present our experience with inolimumab in a patient with worsening of GvHD symptoms at D+163 and confirm activity of inolimumab in this setting. But, this effect was observed when it was given on a daily base.

P.81

RASSF8 OVEREXPRESSION EXPANDS KLS CELLS IN VITRO AND BLOCKS MURINE HEMATOPOIETIC STEM CELLS IN A PRIMITIVE PHENOTYPE

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A recent transcriptome analysis of human umbilical cord blood and bone marrow (BM) CD34⁺CD33⁻CD38⁻ckit⁺Rholo (Rholo) vs. CD34⁺CD33⁻CD38⁻ckit⁺Rhohi (Rhohi) cells identified the gene RASSF8, to be more highly expressed in the hematopoietic stem cell (HSC) rich compartment (Rholo) versus committed progenitor cells (Rhohi). In a subsequent screen, the knockdown of RASSF8 using morpholinos in zebrafish embryos resulted in decreased blood formation and reduced expression of early (scl and gata1) and late (hbae1 and lcp1) hematopoietic markers. To test the function of RASSF8 in mammalian hematopoiesis, we overexpressed it in lineage depleted murine bone marrow cells (Lin⁻) by retroviral transduction using the MSCV-RASSF8-IRES-GFP vector (rMIG-RASSF8). Sorted transduced GFP⁺ cells were cultured in serum-free medium (supplemented with SCF, TPO, Flt3L and Il-3) for three to five days. No significant differences were seen in overall cell expansion, cell death or cell proliferation between rMIG-RASSF8 transduced cells and cells transduced with the control vector (rMIG). However, we noted a significant accumulation of the stem cell enriched cKit⁺Lin⁻Sca1⁺ (KLS) population in rMIG-RASSF8 transduced cells (43±9%) compared to control cells transduced with rMIG (13±8%, p=0.003; n=4) after five days. The total number of colony forming cells (CFCs) produced by 750 rMIG-RASSF8 transduced Lin⁻ cells (24±7) was significantly lower than in rMIG transduced cells (54±8, p=0.008; n=3). Competitive repopulation assays using 25 000 rMIG-RASSF8 or rMIG transduced C57/Bl6 CD45.1 Lin⁻ cells versus 100 000 C57/Bl6 CD45.2 mononuclear BM cells were performed to evaluate HSC engraftment potential. Likewise, peripheral blood analysis up to three months post transplantation demonstrated nearly no reconstitution of rMIG-RASSF8 transduced CD45.1 cells (1±1%) vs 22±23% rMIG transduced CD45.1 cells (p<0.05, n=3). In conclusion, constitutive overexpression of RASSF8 increases the retention/expansion of KLS cells in vitro, blocks differentiation of progenitors giving rise to CFC and inhibits engraftment of HSC. This suggests that short term overexpression of RASSF8 might ultimately lead to new methods of HSC expansion.

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