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leukemia evolution occurred to 11 patients (5 RAEB1 and 6 non RAEB patients).

In non RAEB patients survival was significantly affected by IPSS score (median OS 45 months for intermediate1 plus intermediate2 patients vs 149 months for low risk patients,  $p=0.05$ , HR 2.47) and transfusion dependence ( $p 0.006$ , HR 2.93) as expected. These parameters did not significantly modify RAEB patients OS, possibly due to the low patient number.

Although the erythroid response did not correlate with known risk factors such as IPSS score, karyotype and transfusion requirement, it confirmed its positive prognostic role for survival in non RAEB patients ( $p 0.03$ , HR 2.14): median survival 71.5 months (range 12–156+) for responders, 30.6 months (range 5–149) for non responders.

A trend towards a better survival for responder was also observed among RAEB1 patients (median survival 17 months for responders, 10 months for non responders), however, due to the low numbers of patients in this group, the difference was not statistically significant, even if border line ( $p 0.072$ , HR 2.52).

**Conclusions:** In conclusion our long term follow-up confirmed the positive role of our combined treatment for response duration and survival in a group of non RAEB patients, most of them with unfavorable prognostic features, compared to literature data on EPO alone treatments.

#### 0843 ERYTHROPOIETIN PLUS DANAZOLE, PREDNISONE, B12 AND FOLATE IN REFRACTORY CYTOPENIA WITH MULTILINEAGE DYSPLASIA. MONOCENTRIC PROSPECTIVE STUDY

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**Background:** RCMD is a well defined entity in MDS. The overall median survival is about 30 months. The appropriate treatment of RCMD is still unclear.

**Aims:** To verify if immunosuppressive treatment with danazole and prednisone plus erythropoietins and vitamins is safe and feasible in RCMD treatment.

**Methods:** This is a monocentric, prospective, randomized study, regarding the period from July 2008 to December 2010.

30 patients with RCMD were randomized to receive erythropoietin alpha 40000 UI sc/weekly or erythropoietin beta 40000 UI sc/weekly, B12 400 mg/day and folate 5 mg/day (15 patients – group A) or erythropoietin alpha or beta plus B12 and folate at the same dose and danazole 400 mg/day for 1 year and prednisone 50 mg/day for 1 month, then gradually suspended in the following month (15 patients – group B). Median follow-up was 15 months (R 5–29 months).

In the group A median age was 67 years (R58–73). M/F was 9/6. 2 patients presented a karyotype with +8, and 1 with +9.

In the group B median age was 65 years (R55–70). M/F was 8/7. No patient had an abnormal karyotype.

In the group A all patients received erythropoietin beta. In the group B 8 patient received erythropoietin alpha and 7 beta.

In group A at diagnosis median Hb level was 9 g/dl (R7–10), PLT 90000 (R70000–98000), ANC 750 (R250–1000).

In group B at diagnosis median Hb level was 8 g/dl (R7–9), PLT 45000 (R30000–70000), ANC 450 (R250–900).

Every 6 months all patients received a liver ecography and blood liver test, to prevent danazole-related hepatocarcinoma, and only male patients received a transrectal prostatic ecography and PSA dosage, to prevent danazol related prostate carcinoma.

**Results:** In group A all patients after therapy achieved a better Hb level with a median increase of Hb of 1.5 g/dl after a median of 2.5 month (R1–5). No improvement was observed in neutrophil and platelets count. In group B all patients achieved a normal Hb (>10 g/dl), PLT (>100000/mcl) and absolute neutrophil count (ANC > 1000/mcl) after a median of 1 month of treatment (R1–3 month).

The 8 patients of group B treated with erythropoietin alpha achieved a normal level of Hb (>10 g/dl) with a median of 1 month sooner

than the 7 patients of group B and all patients of group A treated with erythropoietin beta (median: 1 month in group with epo alpha, 2 month in group with epo beta).

At December 2010 no patients died for all cause.

Patients in group A have a need of platelet transfusion of a median of 1 unit every 2 months and have a median number of hospitalization per year of 6 versus 1 for patients in group B.

No patient developed an hepatocarcinoma or a prostate carcinoma during follow-up period.

**Summary/Conclusion:** In RCMD treatment with erythropoietin, danazol and prednisone with B12 and folate support seems to be safe feasible and effective. In consideration of the small number of patients treated, these results need confirmation in a larger cohort of patients.

#### 0844 QUALITY ASSESSMENT OF INTERNET MORPHOLOGY EDUCATION PROJECT BASED ON VIRTUAL MICROSCOPY

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**Background:** Availability of new technologies has expanded the possibilities for education of microscopic morphology on internet. Among others virtual microscopy has enabled evaluation of whole microscopic smears from peripheral blood or marrow aspirate up to a significant magnification.

**Aims:** To assess the quality of virtual microscopy internet project used for pregraduate education of medical students. Access to the project is currently restricted only to students and members of project team at [www.e-hematologie.cz](http://www.e-hematologie.cz).

**Methods:** Seven experts from the Czech Republic, Finland, Germany and Italy have evaluated the pictures of single normal and pathologic cells and full scope scans of marrow or blood microscopic smears obtained by CellaVision DM 96 (Sysmex) and dotSlide system (Olympus).

**Results:** 94 categories of normal and pathologic cells on 1–5 pictures depicting single or few cells including special stainings and 58 virtual scans of normal peripheral blood, normal marrow aspirates and marrow aspirates in various hematologic conditions were evaluated. Technical quality of pictures including sharpness and magnification of cells, including lack of the possibility to further magnify the pictures was the most frequent finding (comments raised by 3 to 5 experts on proerythroblasts, promyelocytes, immature eosinophiles and basophiles; by 4 experts on quality of special staining). Experts agreed on the need to exchange the provided picture or scan for a new one because of poor selection in these cases of: mastocytes (6 of 7 experts); polymorphocytes (4/7); monoblasts, promonocytes, megakaryoblasts, micromegakaryocytes (3/7). A better selection of scanned smears was suggested by 3 experts in cases of APL, AML with inv16, MDS and acute monoblastic leukemia. Scans more than one case of HCL and chronic polymorphocytic leukemia should be provided.

**Conclusions:** A very good level of agreement between the authors of the project and external auditors was achieved. Modern tools enabling virtual microscopy have been confirmed as excellent or very good for education of morphology (agreement among all experts). Careful selection of the smears and appropriate use of technologies may avoid scans and pictures of inadequate quality. Implementation of unified morphology classification and links to other national and European projects are mandatory to further improve the quality of the project.

The use of these ICT tools is nowadays essential for a consensus morphological diagnosis, that is mandatory in those hematological malignancies where the identification and enumeration of abnormal cells still remain mandatory for the diagnosis.

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#### 0845 LONG-TERM OUTCOMES AFTER IMMUNOSUPPRESSIVE TREATMENT FOR MODERATE APLASTIC ANEMIA

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**Background:** The clinical course and current management of acquired moderate aplastic anemia (MAA) are variable and few data concerning long-term results of immunosuppressive therapy (IST) are available.

**Aims:** To evaluate of IST efficacy and long-term outcomes in patients with MAA.

**Methods:** We analyzed the long-term outcome of 59 patients with MAA (28 M and 31 F, age 6–65 years, median 25) treated with ATG and CsA (n=41, including repeated courses in 14 patients) or with CsA alone (n=18) in two centers between April 1994 and February 2011. MAA was defined as hypocellular bone marrow and at least bi-lineage cytopenia lasting more than 4 weeks without meeting the criteria for severe AA. This study includes both retrospective and prospective phases (before and after 2005 respectively). The hematological response was evaluated according to the strict response criteria (Camitta B., 2000). Adult patients or parents of children under 18 years of age signed informed consent.

**Results:** A total of 46 patients (78%) responded to IST but only 10 patients (17%) achieved complete response. Eleven patients (24%) relapsed and 9 responded again after retreatment with ATG or/and CsA. Late events included MDS/AML (n=5), rectal cancer (n=1) and hemolytic PNH (n=2). There were 1 early and 5 late deaths. With a median follow-up of 38 months (range 1–201), the probability of 5-, 10- and 15-year overall survival were 93.3±3.7%, 82.7±7.8% and 66.2±16.1% respectively. Due to high incidence of relapse and late events the failure-free survival was much lower: 52.2±8.2% and 32.3±8.6% at 5 and 10 year respectively with no apparent plateau in the curve.

**Conclusions:** These data demonstrate that despite encouraging short-term results the long-term prognosis of MAA after IST is rather poor and unpredictable. Longer follow-up in a larger cohort and further studies into biologic heterogeneity are warranted to determine optimal treatment strategy in MAA. Careful monitoring of hematologic response would be required in order to clarify the role and timing of allogeneic bone marrow transplantation.

### Myeloma and other monoclonal gammopathies – Biology 2

#### 0846 INSULIN GROWTH FACTOR BINDING PROTEIN-3 EXPRESSION AND ITS PROGNOSTIC SIGNIFICANCE IN MONOCLONAL GAMMOPATHIES

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**Background:** Multiple myeloma (MM) is a clonal malignancy of plasma cells characterized by several genetic and epigenetic aberrations.

IGFBP-3 gene is a member of the insulin-like growth factor binding protein (IGFBP) family and it can regulate cell growth and death by its ability to bind insulin-like growth factors (IGFs) as well as its IGF-independent effects involving binding to other molecules. Its role in carcinogenesis in other tumors is still debated. Previous studies demonstrated that in patients with MM the levels of IGFBP-3 protein in serum are decreased. We have recently shown that the gene expression of IGFBP3 in myeloma cell lines were decreased.

**Aim:** We analyzed the gene expression of IGFBP3 samples from patients with monoclonal gammopathies at diagnosis and we evaluated the correlation between IGFBP3 gene expression levels and overall survival of patients in order to determine the clinical relevance of this gene.

**Methods:** 128 samples of patients at the moment of diagnosis (14 MGUS and 114 with MM) were retrospectively evaluated. The diagnosis was based on standard criteria. IGFBP3 mRNA expression was measured in each samples by real-time PCR using TaqMan Gene Expression Assays and the 7900HT Real-Time PCR System (Applied Biosystems Foster City, CA).

**Results:** 128 patients; male 76; female 52; median age 68 years (range 40–89). ISS stage: stage I 41%; stage II 33%; stage III 26%. In 73/128 (57%) patients we found lower levels of IGFBP3 expression compared to the calibrator sample, the remaining 55/128 (43%) patients showed increased level of gene. We analyzed the correlation between overall survival and IGFBP3 levels and we surprisingly observed that patients with lower levels of the gene had a significantly better overall survival (p 0.0215)

**Conclusion:** These results suggest IGFBP3 down-regulation as a good prognostic factor. Further analysis of correlation of IGFBP3 gene expression with clinical and biological characteristics in these MM patients is ongoing. More studies are needed to better understand the role of IGFBP-3 in myeloma pathogenesis.

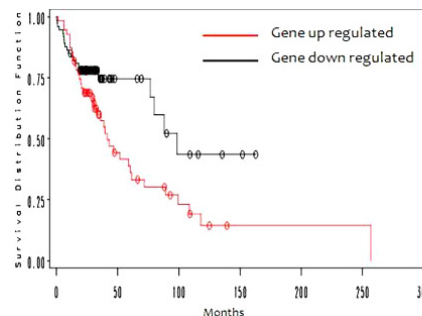


Figure Survival curves according to IGFBP3 levels. Kaplan-Meier overall survival curve shows the survival of patients stratified by the gene levels (p 0.0215 Log-Rank)

#### 0847 NEURAL STEM CELL MARKER NESTIN AS A POTENTIAL UNFAVORABLE FACTOR FOR MULTIPLE MYELOMA

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**Background:** Neural stem cell marker nestin is considered to be a characteristic marker of multipotent proliferative precursors with primitive and undifferentiated phenotype found in some embryonic and fetal tissues. Nestin expression has been also detected in many solid tumors and is proposed to be a suitable diagnostic and prognostic indicator of malignancy and a putative marker of cancer stem cells in solid tumors. Unexpectedly, our previous results confirmed nestin levels in mature CD138<sup>+</sup>38<sup>+</sup> plasma cells (PC) of multiple myeloma (MM) patients by flow cytometry; significant differences were found between nestin levels in MM and individuals without any hematological malignancy. One third of MM patients had more than 50% of nestin-positive PC. Nestin seems to be a specific marker only for CD138<sup>+</sup>PC. Expression of stem/progenitor cell marker nestin might be a novel prognostic factor for MM.