## Parallel Session 2: Hematopoietic Stem Cells

Chair: TBD

### 96228

#### AUTOMATED MULTICOLOR FLOW-FISH FOR TELOMERE LENGTH: A SENSITIVE AND RAPID TECHNIQUE TO IDENTIFY INDIVIDUALS WITH PRIMARY DEFECTS IN TELOMERE MAINTENANCE

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Adequate maintenance of telomere length is important for a functional 3-dimensional chromosome end structure which guarantees genomic stability and is involved in cell replication. Deficiencies or dysfunctions in molecules of the telomerase complex or in proteins associated with the telomere can lead to disturbed telomere length regulation. In the last years, mutations in molecules of the telomerase complex (dyskerin, RNA template of telomerase = hTERC) have been identified as the cause of dyskeratosis congenita (DC), a rare congenital hematological disorder so far being diagnosed based on typical clinical features (e.g. nail dystrophy, leukoplakia, aplastic anemia). Due to insufficient telomerase function telomeres can not be appropriately maintained and become short. In recent studies, 3-10% of patients diagnosed with idiopathic aplastic anemia or myelodysplastic syndrome have been found to carry a mutation in hTERC (= autosomal dominant DC) and there are reports on individuals with no obvious signs for DC but hTERC mutations. Identification of such patients is important; however, analysis for hTERC or dyskerin mutations is labor intensive. We developed automated multicolor flow-FISH for telomere length measurements, a highly sensitive and reproducible technique, and assessed normal age-related values for the percentiles of telomere length in diverse subsets of leukocytes based on telomere length measurements in leukocytes from over 400 healthy individuals aged 0-102 years. Very low telomere length values (below the 1st percentile) in all subsets of leukocytes clearly distinguished patients with bone marrow failure based on a primary defect in telomere maintenance from patients with bone marrow failure due to other causes. Furthermore, we identified phenotypically normal individuals with a molecular diagnosis of DC. We suggest that telomere length measurements by automated multicolor flow-FISH can be used to screen patients for a primary defect in telomere length regulation and that such analysis might even serve as surrogate test for the identification of patients with DC.

### 96525

#### MORPHOMETRIC AND GRANULOMETRIC FEATURES OF ERYTHROBLASTS AS A DIAGNOSTIC TOOL OF HEMATOLOGIC DISEASES

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Dyserythropoiesis is an important criterion for the diagnosis of myelodysplastic syndromes (MDS). Its evaluation is routinely done by subjective examination of May-Grünewald-Giemsa stained bone marrow (BM) smears. In order to objectivate this diagnosis we studied the utility of several morphometric and granulometric parameters. Bone marrow smears of 20 controls, 41 MDS patients, and 22 cases of megaloblastic anemia entered the study. Diagnosis of MDS was based on FAB criteria. Gray-scale transformed digitalized images of erythroblast nuclei were analyzed and the nuclear area, perimeter, longest chord and optical density were determined. Granulometry was applied with the gray level height of the basins as filter parameter. The number of residues and their mean area was registered for each gray level. Area, longest chord, perimeter, form factor, mean transversal diameter, as well as the mean optical density and its standard deviation discriminated well between erythroblasts of the groups. We could not distinguish erythroblasts of patients with refractory anemia (RA) from erythroblasts of patients with refractory anemia with ring sideroblasts (RARS), but both were different from refractory anemia with excess of blasts (RAEB) and megaloblastic anemia, the latter were similar. Applying granulometric features significant differences between the groups could be seen for nearly all mean area levels and for the lower levels of the number of residues. Granulometry was not able to differentiate between erythroblasts of RA and RARS patients. Standard morphometric variables could not distinguish between RAEB and megaloblastic anemia, but the area of granulometric residues (levels 20-40) distinguished clearly between both entities. In conclusion, the combination of usual morphometic parameters and granulometric features are very useful to distinguish normal BM, deficiency anemia and several types of MDS. Supported by FAPESP and CNPq.

### 96778

#### MULTI-COLOR FLOW CYTOMETRIC ANALYSIS FOR THE SELF-RENEWING POTENTIAL OF HEMATOPOIETIC STEM CELLS *IN VIVO*

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Stem cell self-renewal is critical for tissue regeneration or tumor formation in most mammalian systems. Cell cycle regulation may be one of the fundamental mechanisms controlling the process. We have recently demonstrated that deletion of an early G1-phase inhibitor, p18INK4C (p18), results in strikingly improved long-term engraftment. To correlate the engraftment data with multiple cellular parameters in a phenotypically defined stem cell population, we have extensively used the flow cytometric techniques to further examine the hematopoietic stem cell (HSC) pools in mice deficient in p18. We observed a 2-fold increase in frequency and 3-fold increase in absolute yield per harvest of the most primitive HSCs, which are positive for Sca-1and c-Kit but negative for Lineage markers and CD34 antigen, in p18 -/- mouse bone marrows compared with their p18+/+ controls. Further, we examine the following parameters possibly responsible for HSC expansion in the mutant mice. 1. Cell survival rate. As assessed with Annexin-V