**Background:** Previous studies in multiple myeloma (MM) showed clearly the suppressive role of CD4 T regulatory cells (Tregs). Recently, another regulatory cell expressing CD8 phenotype was identified and reported to be elevated and functionally active in solid tumor patients with prostate, colon and nasopharyngeal carcinoma (Kiniwa et al, 2007; Chaput et al, 2009; Li et al, 2011). These CD8 Tregs do suppress T cells through various mechanisms including release of regulatory and suppressive cytokines, interference with antigen-presenting cells and cell-cell contact. In this study, we examined number, functional activity and cytokine profile of CD8 Tregs from MM patients using flow cytometry. Further, we also studied the FoxP3 gene expression from CD8 Tregs.

**Methods:** Using multi-color flow cytometry we analyzed peripheral blood CD8 Tregs in 57 newly diagnosed MM patients. All patients and healthy volunteers (n=11, controls) gave informed consent according to Helsinki declaration. We analyzed CD8 Tregs and their associated markers with following flourochrome conjugated monoclonal antibodies: FITC-FoxP3, PE-CD45RO, CD28, CD62L, CD127, PE-Cy7-CD25 and APC-CD8. CD8 Tregs from 5 MM patients and controls were functionally evaluated by CFSE (carboxyfluorescein succinimidyl ester) based autologous mixed lymphocyte proliferation assay. From proliferation assay, culture supernatant was collected and profiled for IL-10 and IFN-γ using FACS Array.

**Results:** CD8 T cells expressing high level of CD25 was considered as CD8 Tregs (CD8+CD25hi+) and also these cells expressed FoxP3 (regulatory molecule), CD45RO, CD28, CD62L but not CD127. CD8 Tregs (median%: 5.91 vs. 0.43; P<0.001) and CD8 T cells co-expressing CD25 and FoxP3 (median%: 0.41 vs. 0.11; P<0.01) frequencies were significantly increased in MM patients compared to controls. Frequency of total lymphocytes (median%: 15.70 vs. 40.61; P<0.001) but not CD8 T cells were significantly reduced in MM patients compared to controls. In functional activity, CD8 Tregs isolated from MM patients and controls inhibited the proliferation of naive CD4 T cells in concentration dependent manner but CD8 Tregs from MM patients possessed more suppressive function than controls (P<0.05). Cytokine profiling showed that level of IFN-γ but not IL-10 was decreased in both MM and controls according to the number of CD8 Tregs added to the mixed lymphocyte reaction assay. Evaluation of FOXP3 gene expression by real time-PCR demonstrated that CD8 Tregs from MM patients had increased FoxP3 expression compared to controls which corroborated with flow cytometry finding. More interestingly, in comparison to CD4 Tregs, FoxP3 expression was decreased in CD8 Tregs.

**Conclusion:** Inline with previous findings from solid tumors, we were also able to demonstrate that CD8 Tregs are elevated and functional in MM. Our results suggest that regulatory cells with CD8 phenotype might enhance tumor evasion and disease progression in MM.

**245/B139**

## Identification and Characterization of Blood Microparticles from Light Scattering

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**Background:** Blood microparticles are phospholipid vesicles derived from blood cell membranes during stress conditions, including cell activation and apoptosis. These microparticles are relevant for many physiological processes, including intracellular crosstalk, transport, hemostasis, inflammation and coagulation, thus they are of scientific and clinical interest. Both optical and non-optical methods are used for determination of microparticles size, morphology, concentration, biochemical composition, and cell of origin. One of the most popular methods for microparticles analysis is a flow cytometric assay.

**Methods:** We used scanning flow cytometry, the method for measurement of angle-resolved intensity light scattering patterns (LSPs) of individual particles, and solution of the inverse light-scattering (ILS) problem. We measured LSPs with the Scanning Flow Cytometer fabricated by CytoNova Ltd. (Novosibirsk, Russia, http://cyto.kinetics.nsc.ru/). Since a homogeneous sphere is a good approximation to optical model of microparticles, the solution of ILS problem is based on fitting an experimental LSP by theoretical ones, calculated using the Mie theory. Using this solution we determine size and refractive index of individual microparticles including errors of these estimates. Thus we perform a complete morphological characterization of each measured microparticle. Finally, we determine the size and refractive index distributions of the whole population of blood microparticles, taking into account largely different reliability of individual measurements.

**Results:** Developed method was tested with a blood sample of a healthy donor, resulting in a good agreement with literature data. The only limitation of this approach is the size detection limit, which is currently about 0.5 μm for blood microparticles due to the used laser wavelength of 0.66 μm and may be decreased by using a shorter wavelength of 0.405 μm.

**Conclusions:**We present a new approach for accurate and statistically reliable measurement of microparticles size and refractive indexes using light-scattering flow cytometry. The problem of separation of spherical blood microparticles from non-spherical constituents of platelet-rich plasma, such as platelets and cell debris, was successfully solved employing the similarity of microparticles LSP to that of a sphere. This provides identification (detection) of microparticles directly from platelet-rich plasma using neither ultracentrifugation nor fluorescent labels. In particular, this approach allows us to detect microparticles larger than 1 μm, which is problematic for other methods.

**247/B141**

## Quantification of Microvesicles by Flow Cytometry in Whole Blood: Application to the Diagnosis of Hereditary Spherocytosis

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**Background:** Microvesicles (MVs) have high potential as diagnostic biomarkers. Standardization of their analysis by flow cytometry (FCM) is limited by preanalytical variables. One of the most important preanalytical variables is the centrifugation.

**Aims:** i) To develop and validate a new MV quantification method by FCM in whole blood (WB) to avoid the problems to the centrifugation process, and ii) to apply this method to healthy subjects and patients suffering from hereditary spherocytosis (HS).

**Methods:** The new quantification method in citrated 109mM WB was developed on a BD FACS ARIA I. Intraassay-, between- and interindividual variations were studied. Erythrocyte-derived MVs (EryMVs) and platelet-derived MVs (PMVs) were measured and compared in WB and derived platelet-free plasma (PFP) from healthy subjects (n=14), HS (n=6), and other haemolytic anaemias (n=14). The influence of the delay between sampling and analysis on PMV and EryMV concentrations, was also studied in PFP and WB (n=10).

**Results:** We developed and validated a new and stable quantification method in whole blood.