peripheral blood smear. After viral, infectious, rheumatologic tests were normal, we applied bone marrow aspiration and biopsy and it was normocellular. We determined PNH clon at the flaer test. Then, we started to eculizumab therapy. He has been treated with eculizumab for two years. And also he has been treated 1200 mg eculizumab every 12 days for twenty months. In the last hemogram wbc: 3300 mm^3, hgb: 6.2 g/dl, plt: 2000 mm^3. He had a lot of transfusions during two years. He has no donor for allogeneic stem cell transplantation. We are still looking for unrelated donor for him.

Conclusion: Eculizumab therapy has been shown to reduce the need for blood transfusion in patients with PNH.

PP-066

THERAPEUTIC POTENTIAL OF FISETIN, VITEXIN AND HESPERETIN ON CHRONIC MYELOID LEUKEMIA CELLS

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In Chronic Myeloid Leukemia (CML) treatment, despite therapeutic efficacy of tyrosine kinase inhibitors, resistance development and side effects cause problems. Fisetin, vitexin and hesperetin are plant-derived flavonoids. In this study, therapeutic potentials of fisetin, vitexin and hesperetin were determined in CML cells. Cytotoxic effects of flavonoids were determined by MTT assay while apoptotic effects were determined by changes in caspase-3 activity, loss of mitochondrial membrane potential (MMP) and Annexin V/PI double staining. Cytostatic effects of the flavonoids were evaluated by propidium iodide staining using flow cytometry. IC50 values of fisetin at 48 (163 μM) and 72 h (120 μM); hesperetin at 48h (179 μM) and 72h (162 μM); and vitexin at 48h (153 μM) and 72 h (147 μM) were calculated from cell proliferation plots. 50-, 100- and 200 μM fisetin caused 5.4-, 9.5- and 11.6 fold increases in percentage of apoptotic cells, respectively. There were 1.27- (50 μM), 1.8- (100 μM) and 4.3- (200 μM) fold increases in hesperetin-treated and 1.02- (50 μM), 1.5- (100 μM) and 3.6- (200 μM) fold increases in vitexin-treated apoptotic K562 cell populations. Changes in caspase-3 enzyme activities and loss of MMP results revealed that there were significant increases in caspase-3 enzyme activity and loss of MMP in response to fisetin, hesperetin and vitexin in a dose dependent manner. These results indicated that these flavanoids induced apoptosis through mitochondrial pathway and activation of caspase-3 enzyme. On the other hand, hesperetin and vitexin treatment arrested cell cycle at G0/G1 phase in a dose-dependent manner. In conclusion, fisetin could be evaluated as the most effective flavanoid and these flavanoids could have therapeutic potentials if supported with in vivo studies.

PP-067

EXPANSION OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS USING A GMP COMPLIANT CLOSED SYSTEM BIOREACTOR AND QUALITY ANALYSIS OF THE EXPANDED CELLS

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The aim of this study is to share the quality control results of bone marrow derived MSCs expanded using a bioreactor (Quantum Cell Expansion System, Teruma BCT, Colorado, USA).

Method: 25 milliliters of bone marrow aspirated from a normal healthy donor, 4 liters of media and 4 liters of PBS was loaded to the Quantum machine using the closed system set. The cells were fed by media according to the results of regular lactate measurements as recommended by the manufacturer. After 14 days of culture, the cells were removed from the system using trypsin and cultured for the second passage. The samples taken from the final product were analyzed for viability, cell count, microbial growth, endotoxins and telomerase enzyme activity. Flow cytometric studies, and lymphocyte proliferation inhibition tests were also performed. MSCs were differentiated to adipocytes and osteocytes.

Results: 160 million cells were obtained and cell viability was shown to be 99%. No microbial growth was observed and endotoxin analysis was negative. The cells were CD45 and CD34 negative but CD73, CD105 and CD90 positive. MSCs cultured with peripheral lymphocytes were shown to inhibit lymphocyte proliferation and the cells were shown to differentiate into adipocytes and osteocytes. Telomerase enzyme activity was determined to be below 1.5% RTA.

Discussion: Some studies have reported that immunomodulatory capacity of MSCs produced in different laboratories, although GMP compliant may not be standard. Moreover, culturing for too many passages and presence of hundreds of manipulations are important factors affecting the standardization of the cells. Bioreactors are advantageous as they provide a closed system (cells do not have any contact with the CO2 in the air), they require minimum air quality for production (GMP Class C) more cells are obtained in less time and the product quality is high. Production with bioreactors also help decreasing the cost and time.

PP-068

A NOVEL BIOMARKER FOR DRUG RESISTANCE IN CHRONIC MYELOID LEUKEMIA: MICRORNA-17

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miRNAs are single stranded small RNA molecules (20–22 nt), which do not have ability to code for proteins. These types of RNAs play significant roles on gene regulation through inhibition of their target genes. In animals, most of miRNAs show their translational inhibitor effect on target mRNAs by semi-complementation to 3'UTR sequences of mRNAs and deadenylation that cause degradation of these mRNAs. The importance of miRNAs is increasing in cancer diagnosis and treatment since they are one of the major regulators of genes such as oncogenes, tumor suppressor genes. miR-17 is an oncogenic miRNA that suppress the activation of tumor suppressor genes like CDKN1A, p21 and E2F1. Based on previous information, we aimed to determine the correlation between expression levels of miR-17 microRNA in newly diagnosed, tyrosine kinase inhibitors treated and drug resistant CML patients. To this aim, total RNAs were isolated from bone marrow samples of CML patients and expression levels of miR-17 were determined by Stem- Loop- RT-PCR, quantitatively. The results revealed that expression levels of miR-17 were significantly higher in newly diagnosed CML patients as compared to healthy samples. More importantly, there were significant downregulations in expression levels of miR-17 in patients treated with imatinib and nilotinib and showed minimum hematological response. It was reported with this study for the first time that miR-17 could be a promising biomarker/target for the treatment of chronic myeloid leukemia.

PP-069

ELTROMBOPAG IN REFRACTORY APLASTIC ANEMIA

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Objective: Aplastic anemia is a disease this causes a deficiency of all three blood cell types pancytopenia. First line treatment for aplastic anemia consists of immunosuppressive drugs, anti-thymocyteglobulin combined with corticosteroids and cyclosporine. Hematopoietic stem cell transplantation is also used for matched marrow donor. Eltrombopag is a small molecule agonist of the c-mpl (TpoR) receptor, which is the physiological target of thrombopoietin. It has been shown to produce a trilineage hematopoesis in some patients with aplastic anemia, resulting in increased platelet counts, along with red and white blood cell counts.

Case: A 32-year-old woman was admitted to our center because of fatigue, weakness and menorrhagia in May 2011. She had no splenomegaly and hepatomegaly. She had no chronic disease. There was no chronic illness in the family history. The test results were as follows: wbc: 1300 mm^3, hgb: 5.2 g/dl, plt: 4000 mm^3. It was matched to peripheral blood smear. After viral, infectious and rheumatologic tests were normal; we applied bone marrow aspiration and biopsy. It was aplastic anemia. She had no donor for allogeneic stem cell transplantation. We treated this patient first line by rapid anti-thymocyte globulin and cyclosporin. There was no response to