Methods: DNA is extracted from lymphocytes isolated from leukemia patient blood. MTT assay is used to assess cytotoxicity. Protein levels are assessed by Western blot analysis. Telomere length is assessed using qPCR. Telomerase activity is assessed using the Telomeric Repeat Amplification Protocol kit by Roche. DNA-PK autophosphorylation is determined by FACS analysis.
Results: We report that telomerase activity was present in 48% of lymphocyte samples from 24 CLL patients and that treatment with Imetelstat alone did not affect the survival of primary CLL lymphocytes *in vitro*. Nonetheless, Imetelsat increased the sensitivity of lymphocytes from CLL patients to fludarabine, independently of basal telomerase activity. Imetelstat inhibited fludarabine-induced DNA-PK autophosphorylation, a surrogate marker of DNA-PK activity, in CLL lymphocytes, to the same extent than the DNA-PK inhibitor NU7026. The effect of Imetelstat on fludarabine sensitivity was associated with a lower basal protein expression of the DNA binding subunit of DNA-PK, Ku80.
Conclusion: Our results suggest that Imetelstat can inhibit fludarabine-induced DNA-PK activity in primary CLL lymphocytes. We speculate that there may be a functional interaction between hTR and DNA-PK in primary CLL lymphocytes and conclude that Imetelstat in combination with fludarabine may be useful to decrease the tumour burden in CLL.

9205 ORAL

Mammalian Target of Rapamycin (mTOR) Activity Dependent Protein Expression and Rapamycin Sensitivity in Pediatric Acute Lymphoblastic Leukemias

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Deregulation of signal transduction pathways could be a key event in leukemogenesis. mTOR complexes with different rapamycin sensitivity are central mediators of several signaling pathways, regulate cell proliferation, survival and protein translation. The mTOR pathway has recently attracted a lot of attention as a potential target in oncological therapy, including hematological diseases. However, limited data exists about the activity of mTOR in lymphoid malignancies, especially in pediatric acute lymphoblastic leukemia (ALL), representing nearly one third of all pediatric cancers.
We characterized the expression of mTOR activity dependent phospho-proteins by ELISA (pmTOR, p4EBP1, pS6) in human leukemia/lymphoma cell lines, isolated peripheral mononuclear cells, T- and B-cells, and lymphoblasts from childhood ALL patients. Expression was measured before and during therapy and at relapses during a minimum of a 2-year follow-up of 22 patients. Phospho-protein levels and clinical data were statistically evaluated. The effect of rapamycin treatment on apoptosis and the amount of mTORC1/C2 complexes was measured by flow cytometry and immunocytochemistry in cell lines and short-term cultures.
Cell lines exhibited increased pS6 (2.4−8-fold) and p4EBP1 (62.5–72.5-fold) protein levels. Statistical analysis of more than 80 ALL samples and non-leukemic bone marrow/blood samples showed that p4EBP1 expression was significantly higher (20−58-fold) in ALL samples at diagnosis; the decrease of p4EBP1 expression followed the effectivity of chemotherapy in all patients. We also found that mTOR activity was significantly higher at diagnosis in the samples of patients with worse prognosis, both in B- and T-ALL; p4EBP1 expression was retained and increased above day 0 level at relapses. In vitro rapamycin treatment induced apoptosis in cell lines and in short-term ALL cultures only when p4EBP1 expression concomitantly decreased. In vitro results also suggest that Rictor/Raptor expression correlates with rapamycin resistance in lymphoma/leukemia cells.
Our results suggest that mTOR activity is elevated in ALL cells, which can be monitored by measuring p4EBP1 by ELISA. p4EBP1 may be an important marker for identifying patients with poor prognosis at diagnosis, following p4EBP1 expression could help in the earlier detection of relapse during the therapy; and it may also be useful for the selection of patients who may benefit from rapalog tratment.
Supported by OTKA K81624, K68341

Poster Presentations (Sun, 25 Sep, 14:00−16:30)
Haematological Malignancies and Myeloma

9206 POSTER

Redox-sensitive P73-related Pro-apoptotic Effect of the Polyphenolic-rich Aronia Melanocarpa Juice on Human Acute Lymphoblastic Leukemia Cells

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Background: Natural products derived from plants have received considerable attention as potential cancer chemopreventive and chemotherapeutic agents over few decades. On the basis of epidemiological and animal studies, it has been recurrently reported that diets rich in fruits and vegetables are associated with a reduced rate of cancer mortality. Natural products rich in polyphenols have been shown to have strong chemopreventive properties in different types of cancer cells. Moreover, the polyphenol-induced cytotoxic effect appears to target specifically cancer cells.
Aronia melanocarpa also known as black chokeberry is a shrub native from North America. *Aronia melanocarpa* juice (AMJ) is one of the richest sources of natural polyphenols. AMJ has been shown to have numerous health benefits, including cardioprotective, hepatoprotective and antidiabetic activities. Several *in vitro* and *in vivo* studies indicate that *Aronia melanocarpa* extracts have also antiproliferative effects against colon cancer cells. The aim of the present study was to determine whether AMJ inhibits proliferation of the human acute lymphoblastic leukaemia cells, and if so, to identify the underlying molecular mechanism in particular the role of reactive oxygen species (ROS).
Material and Methods: Human acute lymphoblastic leukemia Jurkat cell line, human primary lymphoblastic leukemia cells and normal human primary T-lymphocytes were used in the study. MTS assay, Cell cycle phase distribution and Apoptosis analysis were performed to study the effect of AMI on proliferation, cell cycle and apoptosis respectively. The formation of ROS was determined by staining with dihydroethidine (DHE). Western blot experiments were performed to detect p73, Cytochrome c, Cyclin B1, Caspase 3 and UHRF1 in Jurkat cells.
Results: We have found that AMJ inhibited cell proliferation and induced cell cycle arrest in G_2/M phase leading to apoptotis. These effects are associated with an upregulation of the tumour suppressor p73 and cleaved caspase 3, and a downregulation of cyclin B1 and UHRF1. AMJ significantly increased the formation of ROS associated with the release of cytochrome c into the cytoplasm. Treatment with intracellular ROS scavengers prevented AMJ-induced apoptosis and upregulation of p73 and active caspase 3 expression. Moreover, it was found that AMI selectively killed the primary lymphoblastic leukemia cells without effecting normal human primary T-lymphocytes.
Conclusion: These findings indicate that AMJ exhibits potent anticancer activity through a redox-sensitive mechanism in the p53-deficient Jurkat cells. In addition, AMJ exerted a strong pro-apoptotic effect in human primary lymphoblastic leukemia cells but not in human normal primary T-lymphocytes. In conclusion, these results suggest that AMJ has chemo-preventive and chemotherapeutic properties against acute lymphoblastic leukemia by selectively targeting lymphoblast-derived tumour cells.

9207 POSTER

Evaluation of Effect of Caffeic Acid Phenyl Ester on Acute T-Lymphoblastic Leukemia Cells by Mitochondria and Peroxisome

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Background: More than 70% of chemotherapeutic agents currently used for treatment of leukemia have been derived from natural sources. Leukemia mainly can be classified as acute and chronic and for the acute leukemia treated with current agents, 5 years survival rates have been around 40−50%. There have been new therapy models for specific targets in the last decade. New targeted drugs have been mostly combined with classical drugs. But, classical chemotherapy agents are still the main treatment for leukemia. Complications associated with classical drugs have brought forth new researches to develop new cancer treatment agents. Caffeic acid phenethyl ester (CAPE) is the active compound that has wide spectrum effects such as antioxidant, anti-inflammatory, anti-viral, carcinogenetic and anti-cancer. PPAR-gamma plays a key role in atherosclerosis, inflammation, obesity, diabetes, immune response and

pathophysiology of aging. In the recent years, PPAR-gamma, activated by J2 series cyclopentenol prostoglandin (cyPGs), was observed to have anti-proliferative, apoptotic, differentiation and anti-inflammatory effects on various cancer cells. The aim of this study is to investigate the cytotoxic and apoptotic effects of CAPE in CCRF-CEM cell line.
Material and Methods: The cytotoxicity of CAPE evaluated by Trypan blue dye exclusion test and XTT methods and apoptosis was examined with Acridine orange/Ethidium Bromide dye, Cell death detection ELISA and JC-1 mitochondrial membrane potential assay kit.
Results: CAPE had dose and time dependent cytotoxicity and had 10 μM IC_{50} in CCRF-CEM cell line. ELISA and Acridine Orange/Ethidium Bromide dye and JC-1 methods revealed that CAPE induced apoptosis in the cell line. The effect associated with PPAR-gamma and hemeoxygenase is evaluated with Western Blotting. According to the flow-cytometric analysis of JC-1 stain, cell viability is detected as 38.13% for control, 29.78% for 24^{th} hour, 26.76% for 48^{th} hour, 25.82% for 72^{nd} hour and 25.22% for 96^{th} hour. It was detected that 10μM concentration caused an increase till 24^{th} hour and in the following period it caused a reduction when the effect of CAPE on PPAR-gamma expression was analyzed.
Conclusion: Since CAPE is a chemotherapeutic and anti-tumoral agent with very less toxic effects on normal tissues, these results opened a new horizon for use of CAPE in the treatment of acute lymphoblastic leukemia.

9208 POSTER

Activity of CKD-581, Histone Deacetylase Inhibitor, in Cutaneous T-cell Lymphoma Models

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Cutaneous T-cell lymphomas (CTCLs) are characterized by accumulation of malignant T cells in the skin. Early disease resembles benign skin disorders but during disease progression cutaneous tumours develop, and eventually the malignant T cells can spread to lymph nodes and internal organs. Pan-histone deacetylase (HDAC) inhibitors (HDIs), including depsipeptide, vorinostat, and panobinostat, have demonstrated clinical efficacy in CTCL, and vorinostat has been approved and available as treatment for CTCL.
CKD-581, highly water-soluble HDI, developed in our institute has shown a strong cytotoxicity against several cancer cell lines including HCT-116, PC-3, A549 and H460 in previous study.
In this study we investigated the cellular and molecular effects of CKD-581 using both *in vitro* and *in vivo* models of CTCL.
Three cell lines (MJ, Hut78, and HH) were treated with CKD-581, LBH589 and SAHA, for 72 h and the cell viability was quantified using the MTT assay. In addition, western blotting analysis for ac-H3, ac-H4, ac-tubulin, p21 (WAF-1/CIP-1) and p-ERK and caspase 3/7 and 9 assays were performed to verify the associated molecular mechanisms involved in CKD-581 mediated cell death. After 72 h of incubation, IC_{50}s of CKD-581 on cell viability test were noted at 0.68 μM, 0.04 μM and 0.1 μM in MJ, Hut-78 and HH cells, respectively and showed a more potent inhibitory activity against human HDAC1, 2, 3, 6 and 8 enzymes than SAHA at single nMs. CKD-581 treatment caused an accumulation of acetylated histones (H3 and H4) and acetylated tubulin and increase of p21 WAF1 and phospho-ERK, and activation of caspase-3/7 and 9 in HH cell.
In a CTCL xenograft mouse tumour model, CKD-581 treatment resulted in a significant tumour regression compared with other HDI. Treatment of CKD-581 (50 mg/kg, b.i.wk, i.p.) caused 49% reduction in the mean tumour volume without a change of body weight while other HDIs showed weak antitumour activities. There was no change in neutrophil counts, but the number of platelets was slightly decreased in CKD-581 treated mice.
These data provide preclinical support that CKD-581 is a promising therapy for CTCL and its strong antitumour activity warrants further clinical investigations.

9209 POSTER

Imbalanced Frequency of Regulatory T Cells in Different Subsets of Chronic Lymphocytic Leukemia

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Background: Recent studies have shown the expansion of different subsets of regulatory T cells (Treg) in a variety of autoimmune and malignant diseases indicating their immune regulatory function. There is little data regarding the frequency and the role of Treg cells in hematopoietic malignancies, particularly chronic lymphocytic leukemia (CLL).
Materials and Methods: The frequency of $CD4^+CD25^+FoxP3^+$, $CD8^+CD25^+FoxP3^+$ and $CD8^+FoxP3^+$ cells was analyzed in peripheral T cells isolated from CLL patients and 8 normal subjects by flow cytometry. Patients were broadly classified into either progressive (n = 20) and indolent (n = 20), or immunoglobulin heavy chain variable region (IGHV) mutated (n = 24) and unmutated (n = 16) subsets.
Results: The frequency of $CD4^+CD25^+FoxP3^+$ cells was significantly higher in progressive (11±0.8% of total $CD4^+$ cells) compared to indolent CLL patients (5.75±0.7, $p < 0.001$) and normal subjects (2.4±0.5, $p < 0.001$). Other subsets of Treg, $CD8^+CD25^+FoxP3+$ and $CD8^+FoxP3^+$ cells were also significantly increased in progressive (4.3±0.44 and 7.67±0.65) as compared to indolent patients (1.65±0.26 and 4.6±0.81, $p < 0.001$ and $p < 0.001$) and normals (0.62±0.13 and 1.51±0.31, $p < 0.001$ and $p < 0.001$), respectively. This difference was also significant when analyzed between indolent patients and normal subjects ($p = 0.03$). No differences, however, were observed between IGHV mutated and unmutated samples in frequency of all subsets of Treg. Furthermore, the frequency of Treg showed no correlation with the prognostic markers CD38 and ZAP70.
Conclusion: Our results indicate that progression of CLL is associated with significant increase in circulating Treg cells implying the immune inhibitory function of these cells with subsequent expansion of leukemic cells and disease progression.

9210 POSTER

The Efficacy of Anticoagulant Treatment on the Evolution of Thrombotic Complications in Patients With Polycythemia Vera Syndrome

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Background: Polycythemia vera (PV) is a monoclonal myeloproliferative disorder due to the ability of PV erythroid progenitor cells to proliferate in the absence of erythropoietin. One of the most relevant problem of patients with PV is a haemostatic imbalance, resulting in increased risk for thrombotic events. These events have been attributed to quantitative and qualitative abnormalities of red blood cells and platelets arising from the clonal rearrangement of hematopoietic cells, to reduced levels of physiologic anticoagulants (antithrombin III, proteins C and S), and decreased fibrinolytic activity that in part may be secondary to increased plasma levels of plasminogen activator inhibitor 1 (PAI-1).
The objective of this study was to investigate the efficacy of anticoagulant treatment in the prevention of thrombotic events, in patients with PV syndrome with or without cardiovascular disease (CVD), by monitoring specific markers of the coagulation profile.
Material and Methods: The study comprises 40 patients divided in 2 groups: 20 patients with PV syndrome (PV) and 20 patients with PV with CVD associated (PV+CVD). The patients were tested by determining three factors of coagulation profile: von Willebrand factor, Protein C and PAI-1, before and after administration of anticoagulant therapy. Warfarin® was administrated as anticoagulant treatment, in doses that were adjusted according to the International Normalized Ratio (INR) values.
Results: The level of the three studied parameters were found significantly modified in both groups of patients (PV, PV+CVD) ($p < 0.05$). After the administration of anticoagulant treatment, in the first group (PV), it was observed a direct correlation between the treatment and the values of investigated parameters; in this group, the levels of studied parameters returned close to normal values. This correlation was less evident in the second group of patients (PV+CVD), as the monitored values, although lower than the levels at the begining of the treatement, remained within pathologic levels.
Conclusions: In PV syndrome the risk for thrombosis is due to endothelial dysfunction and changes in coagulation and fibrinolysis factors. The anticoagulant treatment prevents only partially the occurrence of thrombotic events, but does not completely stop it. In spite of the anticoagulant therapy, the patients with PV presents a high risk for developing thrombotic complications.