**9211** POSTER

**Tumour Infiltrating T-cells Predict Survival in Mantle Cell Lymphoma − an Immunohistochemical Study of 81 Patients**

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**Background:** The role of tumour infiltrating T-Cells in malignant B-Cell lymphomas is discussed controversial. There are only limited data on CD 8 and FOXP3 positive cells in mantle cell lymphoma.
**Material and Methods:** 81 biopsy specimens of patients (64 men and 17 women) with mantle cell lymphoma and a median age of 64 years (range: 41 to 86 years) were included in this study. The slides were stained immunohistochemically with CD3, CD8 and FOXP3. Positive T-cells of 10 High power fields (HPF) were counted and the average value was calculated.
**Results:** The CD 8 staining showed a range of 0 to 138 positive cells per HPF with a mean value of 19.4/HPF. A high account of CD 8 positive cells was associated with a significantly longer overall survival time (42 months) compared to MCL with a low account of CD 8 positive cells (28.8 months, $p = 0.029$). FOXP3 staining had a range of 0 to 104/HPF with a mean value of 28. Patients with MCL and a high number (>25/HPF) of FOXP3 positive cells had a median survival time of 38.2 months compared with the group with low account (<20/HPF) of FOXP3 positive cells (23 months). Kaplan Meier analysis revealed a significant difference ($P = 0.015$) in overall survival time.
**Conclusions:** High number of CD 8 and FOXP 3 T-Cells predicts a superior clinical outcome in patients with mantle cell lymphoma.

**9212** POSTER

**Account of Tumour Infiltrating Macrophages is a Prognostic Factor for Patients With Mantle Cell Lymphoma**

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**Background:** Mantle cell lymphoma (MCL) is a malignant lymphoma associated with a relatively aggressive clinical course and a median overall survival time of 3−4 years. Only limited data about tumour associated macrophages and their influence on survival in MCL exists.
**Material and Methods:** We analyzed the amount of CD68 macrophages in relation to the clinical outcome in patients with MCL. Lymph node biopsies of 77 untreated patients (17 women and 60 men) enrolled in two multicenter trials (1975–1985) with a median age of 66 years (range 41−86 years) were included in this study. Biopsy specimens were investigated immunohistochemically with monoclonal antibodies against CD68 (Ki-M1P). 10 High power fields (HPF) were evaluated by random.
**Results:** Patients with low account (less than 10/HPF) of CD 68 positive macrophages had a median overall survival time of 38.2 months, compared to 24.2 months for patients with high (more 10/HPF) CD 68 positive macrophages. The Kaplan–Meier analysis showed a significant difference in the overall survival time ($p = 0.0027$).
**Conclusions:** Patients with mantle cell lymphoma and a low number of CD 68 positive macrophages have a better prognosis and can predict outcome.

**9213** POSTER

**Apoptosis Regulating Proteins P53, Caspase 3, and Bcl2 Can Predict Survival in Mantle Cell Lymphoma**

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**Background:** The deregulation of apoptosis has been implicated in cancer, autoimmunity and degenerative disorders. At the molecular level an extrinsic death receptor pathway and the intrinsic (mitochondrial) pathway have been described. Only limited data exist on the expression of proteins involved in apoptotic pathways in mantle cell lymphoma.
**Material and Methods:** We investigated the expression of p53, the indicator of DNA damage of proteins involved in the regulation of the intrinsic mitochondrial pathway (BCL2, Bax) and of effector proteins of apoptosis (caspase 8, caspase 3) in 93 cases of mantle cell lymphoma and correlated the expression with the clinical outcome.
**Results:** Similar to previous studies, we found that p53 expression was associated with shorter overall survival. In contrast to diffuse large B-cell lymphomas, cases expressing the anti-apoptotic protein BCL2 had a favorable outcome. Interestingly, high levels of apoptosis in the tumour before treatment, as indicated by expression of active caspase 3, are a strong indicator of poor clinical outcome ($p < 0.001$).
**Conclusions:** These data indicate that the level of apoptosis itself is a strong prognostic marker in mantle cell lymphomas.

**9214** POSTER

**Effect of Methylation of P15INK4B Gene in Acute Lymphoblastic Leukemia and its Prognostic Value**

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**Background:** An early diagnosis is critical for the successful treatment of many types of cancer. The traditional methods of diagnosis are useful, but molecular markers can further subclassify the tumours. The methylation profile can distinguish tumour types and subtypes and perhaps the response to chemotherapeutic agents and survival. Methylation changes often precede apparent malignant changes and thus may be of use in early diagnosis of cancer. The aim of the present work was to study frequency of p15 silencing in childhood and adult ALL patients. Also, to evaluate the prognostic value of p15 methylation in ALL.
**Material and Methods:** This study was conducted on 36 newly diagnosed patients with Acute lymphoblastic Leukemia (ALL) (25 B-ALL, 11 T-ALL) attending the Hematology Unit of Ain Shams University Hospitals. A group of 15 apparently normal healthy children and adults of matched age and sex were also included. Methylation specific-PCR for assessment of methylation status of p15 in peripheral blood lymphocytes was done.
**Results:** The results of this study showed that p15 methylation was found in 83.3% of the studied patients. This result indicate that methylation of p15 is a common phenomenon in ALL. Also our results found that the mortality rate and relapse were higher among patients with p15 methylation while none of the unmethylated patients died or developed relapse.
**Conclusions:** This suggests that p15 methylation profile may have important prognostic implications for clinical monitoring and risk assessment of ALL patients. Prospective knowledge of pretreatment methylation may help determine candidate patients for demethylating therapies.

**9215** POSTER

**CD200 Expression Level on Chronic Lymphocytic Leukemia B Cells Correlates With Foxp3+ Regulatory T Cells Frequency in These Patients**

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**Background:** CD200 plays a key role in regulation of the immune system and has been shown to be up-regulated in different tumours including chronic lymphocytic leukemia (CLL). Despite some investigations of the CD200 expression in various tumours, little is known about its correlation with regulatory T cells. In current study, CD200 expression level was investigated in Iranian patients with CLL in comparison to normal B cells and its correlation was studied with foxp3+ regulatory T cells level in these patients.
**Material and Methods:** CD200 expression level was examined on peripheral blood leukemic B cells obtained from 21 CLL patients and peripheral blood B cells isolated from 8 age matched normal subjects by Flow cytometry. This technique was also used to determine frequency of foxp3+ regulatory T cells in the same CLL patients.
**Results:** Our results demonstrated significant up-regulation of CD200 in B-CLL in all patients compared to normal B cells ($p = 0.006$). Also CD200

mean florescence intensity (MFI) showed a significant over-expression in progressive (n = 8) versus indolent (n = 13) clinically subtypes ($p = 0.012$) of CLL patients. Moreover, we demonstrated that CD200 expression level is highly correlated with frequency of foxp3+ regulatory T cells ($r = 0.7$, $p = 0.007$) of CLL patients.
**Conclusions:** Our results indicate up-regulation of CD200 in CLL suggesting involvement of this molecule in low immune responsiveness in these patients and probably its association with disease progression.

**9216** POSTER

**Vascular Endothelial Growth Factor Receptor 1 (VEGFR1) Gene Expression Depends on Immunophenotype of Human Multiple Myeloma (MM) Cells**

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**Background:** Plasma cells of multiple myeloma (MM) patients express high levels of VEGF-A and VEGFR3 [1]. VEGFR1 expression was also found in MM cells providing the autocrine loop for MM cell proliferation. Earlier we have found that *VEGFR1* and *VEGFR3* gene expression disappears in mononuclear cell fraction of bone marrow aspirates from MM patients with high level (>60%) of plasma cells [2]. In our study, we characterized IM9, RPMI 1640 and RPMI 8226 MM cells by CD38, CD138, CD45, CD56 and CD19 differentiation markers expression and determined *VEGF-A, VEGF-C, VEGF-D* and their receptors *VEGFR1, VEGFR2, VEGFR3* gene expression in these cells. Resistance of these cell cultures to bortezomibe was also evaluated.
**Material and Methods:** Multiple myeloma cell cultures IM9, RPMI 1640 and RPMI 8226 were used. The expression of CD38, CD138, CD45, CD56 and CD19 markers in cell cultures was measured by flow cytometer. *VEGF-A, VEGF-C, VEGF-D, VEGFR1, VEGFR2* and *VEGFR3* gene expression was studied by RT-PCR technique. The sensitivity of MM cells to bortezomib was evaluated using MTT test.
**Results:** Multiple myeloma cell cultures IM9, RPMI 1640 and RPMI 8226 were positive for CD38/CD138 plasma cells specific markers and CD19-negative. CD45, but not CD56, was expressed in IM9 cells, and on the contrary, both RPMI cells were positive for CD56 and negative for CD45. MTT test showed that sensitivity of these 3 MM lines to bortezomibe was different: IM9 cells were the most resistant to this drug, and RPMI 8226 cells were more susceptible to bortezomib than RPMI 1640. *VEGF-A* and *VEGF-D*, but not *VEGF-C* genes were expressed in all MM cell lines. As concerns *VEGFR*s gene expression, RT-PCR revealed *VEGFR1* mRNA signal in IM9 cells only. No expression of *VEGFR2* or *VEGFR3* was found by means of RT-PCR in neither of cells studied. Thus, VEGF-A/VEGFR1-dependent signaling was active only in CD45+/CD56- IM9 cells.
**Conclusions:** As evaluated by the differentiation markers expression, IM9 cells had different immunophenotype as compared to RPMI 1640 and RPMI 8226 cells. Only IM9 (CD45+/CD56-) cells, but not RPMI 1640, RPMI 8226 (CD45-/CD56+) cells expressed *VEGFR1* mRNA; IM9 cells were the most resistant to bortezomib, as well. Our data suggest that immunophenotype of MM cells could be interconnected with *VEGFR1* gene expression.

**References**

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**9217** POSTER

**Epigallocatechin Gallate Inhibits Ribonucleotide Reductase in Human HL-60 Promyelocytic Leukemia Cells**

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**Background:** Epigallocatechin gallate (EGCG) is the major catechin found in green tea. This polyphenolic compound has been suggested to exhibit anti-inflammatory, anti-oxidant and immunosuppressive effects. The potential health benefits ascribed to EGCG include cancer chemoprevention, amelioration of cardiovascular health, and protection of the skin from damage caused by ionizing radiation.
Ribonucleotide reductase (RR; EC 1.17.4.1) is responsible for the *de novo* conversion of ribonucleoside diphosphates into deoxyribonucleoside diphosphates, which are essential for DNA replication. Harboring a tyrosyl radical, the enzyme can be inhibited by e.g. radical scavengers. RR is upregulated in tumour cells and therefore considered an excellent target for cancer chemotherapy.
**Materials and Methods:** The human HL-60 promyelocytic leukemia cell line was purchased from ATCC (American Type Culture Collection, Manassas, VA, USA). Cell cycle distribution was analyzed by FACS, deoxyribonucleoside triphosphate (dNTP) levels were measured by HPLC, ribonucleotide reductase *in situ* activity was quantified by incorporation of $^{14}$C-cytidine incorporation into nascent DNA of tumour cells, and protein levels of RR subunits (R1, R2, p53R2) were determined by western blotting.
**Results:** EGCG dose-dependently inhibited the growth of HL-60 leukemia cells, yielding $IC_{50}$ values of 30, 18, and 16μM after incubation of tumour cells for 24, 48, and 72 hours, respectively. Treatment of cells with EGCG resulted in an arrest in the G0/G1 phase of the cell cycle, increasing this cell population from 34.6% to 48.2%, whereas S phase cells decreased from 48.5% to 40.1%. Quantification of dNTP levels showed a significant reduction of the dATP pool, whereas the dCTP pool was significantly elevated. Regarding the dTTP pool, treatment with EGCG led to insignificant changes. Incorporation of $^{14}$C-cytidine incorporation into nascent DNA of tumour cells was significantly inhibited, being equivalent to an *in situ* inhibition of the enzyme. The expression of RR subunits (R1, R2, p53R2) remained unchanged during the whole time course, being consistent with the fact that the enzyme can be attenuated without influencing the protein levels.
**Conclusions:** Our data show that EGCG causes cell cycle arrest and inhibits ribonucleotide reductase activity in human HL-60 promyelocytic leukemia cells. EGCG therefore deserves further preclinical and *in vivo* testing.

**9218** POSTER

**Human Immunodeficiency Virus-Associated Plasmablastic Lymphoma**

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**Background:** Human immunodeficiency virus (HIV) infection has been associated with increased risk for development of lymphoproliferative disorders. The prevalence of HIV-related malignancies is expected to increase as HIV+ patients (pts) continue to live longer. Oral plasmablastic lymphoma (PBL) is not a frequent event among HIV+ individuals. Prognosis is usually poor regardless of the site of origin, with a mean overall survival of 15 months.
**Material and Methods:** We retrospectively reviewed the medical records of 4 cases of HIV-associated PBL that were undergoing radiotherapy (RT) in our department, two men and two women. Two patients have been submitted to chemotherapy and all were under highly active antiretroviral therapy.
**Results:** The mean age at presentation was 43 years (range: 39 to 63). Two pts underwent consolidation RT after complete response to chemotherapy with 40 Gy and two pts received RT with curative intent with 50 Gy. The mean follow-up after RT was 7 months (range: 4 to 15). To date, three pts achieved a complete response and the remaining relapsed, requiring reirradiation.
**Conclusions:** Our data are similar to international averages and shows that RT has a great importance in the treatment of PBL. We need to increase the length of follow-up to obtain more information. But, local RT proves successful in terms of local control. However, well defined guidelines for PBL are still lacking, which includes immune-chemotherapy, RT isolated or in combination.

**9219** POSTER

**Combined Modality Therapy for Stage I-II Diffuse Large B-cell Lymphoma Provides Excellent Local Control and Clinical Outcome in the Rituximab Era**

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**Background:** Standard therapy for stage I-II diffuse large B-cell lymphoma (DLBCL) is combined modality therapy (CMT): anthracycline-based chemotherapy with radiotherapy (RT). The addition of rituximab (R) to CMT has improved the outcomes in all patients with DLBCL. At the same time we witnessed a change in RT planning with computed tomography-planned RT based on targeting initial disease extent only. To assess the impact of these changes in practice on the pattern of failure, we examined the outcomes in recently treated cohort of patients with localized DLBCL, pre- and post-R era.