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.