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.

Material and Methods: Retrospective review of 295 stage I-II DLBCL patients (pts) treated with curative intent between 2002 and 2008. All pts had CMT and received RT at our hospital. Primary CNS lymphomas were excluded. Median age was 61, with M:F ratio 1.14. Ann Arbor stages were IA − 48%, IIA − 41%, I−IIB − 11%. Extranodal involvement was present in 66% (IE: 34%, IIE: 32%), and bulky disease ($\geqslant$ 10 cm) in 22%. Chemotherapy included: cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP, in 32%) and R-CHOP (65%), median 6 cycles (22% had 3 cycles). Median involved-field RT dose was 35 Gy, with 97% receiving $\geqslant$30 Gy. The median follow up was 4.2 yrs (6.8 yrs for CHOP-treated, 3.8 yrs for R-CHOP). In pts with disease progression or relapse, the site(s) of failure were documented to determine if it occurred in the RT field (local), at field margin or out-of-field (adjacent nodal region, or distant).
Results: Response was evaluable in 292 pts, with CR/CRu in 282 (96.6%) and $\leqslant$ PR in 10 (3.4%). To date, 30 patients relapsed. Failure sites for relapsed pts were: 2 local, 1 marginal, 23 distant, and 4 were both local and distant. No failures were seen in an adjacent nodal region. The cumulative 5-yr local disease failure rate was 6%, and was higher in the CHOP vs. R-CHOP patients − 12% and 2% respectively ($p = 0.001$). Of the 2 isolated local failures, 1 had suboptimal therapy (3 CVP follow by RT 28 Gy). The 5-yr distant disease failure rate was 12% (for CHOP: 14%, vs. R-CHOP: 10%, $p = 0.28$). To date, 40 deaths occurred (17 due to disease, 23 other causes) with actuarial 5-yr clinical outcomes: overall survival − 87% (95% CL, 82−91%), cause-specific survival − 94% (95% CL, 90−96%).
Conclusions: Modern CMT for stage I-II DLBCL has excellent clinical outcomes including very high local control rates. Adjacent nodal region failure is not a clinical problem for involved-field RT covering initial disease only.

9220 POSTER

The Role of Palliative Radiotherapy in Patients With Myeloma Bone Disease in the Era of the Novel Agents

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Background: The introduction of the novel agents Bortezomib, Thalidomide and Lenalidomide changed the philosophy of the treatment in multiple myeloma (MM) and the fate of the patients.
Aim: To analyze the application of palliative radiotherapy in incidence, outcome and time to first skeletal related event after radiation in patients with myeloma bone disease (MBD) receiving conventional chemotherapy (CC) or Bortezomib+Dexamethason (VD) regimen.
Patients and Methods: For the period 1995–2010, 341 patients with MM were studied, m/f ratio1.2/1, mean age 60.6 (32−83). The staging systems of Durie et Salmon and ISS were used. MBD was graded according to the Merlini scale. VD was applied in 27 patients. Biphosphonates were administered by the general rules. No significant difference was found in the distribution in sex, age, clinical stage, grade of MBD, and major parameters of the disease between the two groups. Statistical analyses were performed by variative, correlative, alternative analyses, independent samples T-test, one-way ANOVA and Kaplan Meier test (SPSS v15).
Results: In the group on CC in 136 (43.3%) MBD grade II and in 25(8%) MBD grade III was found, 47(15.0%) had soft tissue formations. In the VD group 11(40.7) had MBD grade II; 4(14.8%) had MBD grade III, plasmocytomas − 5(18.5%). 162 (51.6%) patients on CC and 16 (59.3%) on VD were irradiated. Pathological fractures were irradiated in 105 (77.8%) in the CC group and in 10 (90.9%) in VD, the severe skeletal destructions were irradiated in 24 (96.0%) vs 4 (100%), plasmocytomas in 37 (78.7%) vs 5 (100%) respectively. In 134 (82.7%) of CC patients pain alleviation was achieved, in 92 (87.6%) the fractures were stabilized, in 30 (81.1%) a reduction of the soft tissue formations occurred. In VD group pain was alleviated in 13 (81.3%), fracture stabilization occurred in 8 (80.0%), and palsmocytoma reduction in 5 (100.0%). Median time to reappearance of bone pain after radiotherapy in the CC group was 10 months (9−12) and to a new fracture 13 months (12−15). The median time to a skeletal related event for the VD group is not reached yet.
Conculsions: In the era of the new agents palliative radiotherapy is still an effective method in the treatment of MBD: it significantly alleviates pain, results in stabilization of the pathological fractures, reduces the size of the soft tissue formations and improves the quality of life of patients, not compromising further antitumour treatment.

9221 POSTER

Cardiac Effects After Low-dose Whole-heart Radiotherapy Following Doxorubicin-based Chemotherapy in Hodgkin's Lymphoma

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Hodgkin's lymphoma (HL) may present with extensive pericardial disease or cardiophrenic lymphadenopathy. Partial or poor response to first-line chemotherapy (CT) becomes a case for a whole cardiac radiotherapy (RT) with increased risk of cumulative CT-RT cardotoxicity.
Using echocardiography we assessed the left ventricular function before starting CT for Hodgkin's lymphoma and after the end of treatment in total 152 patients (pts). Since 2000, in a prospective MRRC study HL patients stages II to IV were treated with 4−6 courses of BEACOPP-21 or ABVD followed by 1−2 COPP in order to reduce doxorubicin toxicity before consolidation RT. All patients received ^{60}Cobalt mediastinal irradiation to 20−22 Gy. Fifty eight patients of Gr. 1 presented with pericardial effusion and therefore received low-dose (10−17 Gy, median 14 Gy) whole cardiac RT in the course of mediastinal (16 pts) or wide-field irradiation (42 pts) as one of the two daily fractions delivered in accelerated hyperfractionated regimen (AHFX)). In patients of the two control groups cardiac apex was shielded. The patients with residual mass after CT (Gr.2, $n = 45$) received mediastinal irradiation in AHFX regimen, those with complete response (Gr.3, $n = 49$) received RT in conventional fractionation once a day. Groups did not differ by doxorubicin dose (100–300 mg/m^2, median 150) and age at examination (20−57 years, median 32 years). Before CT, mean left ventricular ejection fraction (LVEF) was 64%; a systolic dysfunction (LVEF <60% or >72%) was more pronounced in Gr.1 (49% pts VS 27% pts in Gr.2 and Gr.3, $p = 0.03$).
After the combined-treatment program and after a median follow-up of 60 months, mean values of LVEF were, respectively, 62% (range, 50% to 75%), 63% (range, 54% to 70%) and 62% (range, 54% to 76%). LVEF below 55% was recorded in 7%, 4% and 13% pts, respectively, but none of the patients exhibited clinical signs of heart failure. Rest EF was correlated negatively with cumulative doxorubicin dose (Gr.1, $r = -0.83$, $P = 0.02$; Gr.2, $r = -0.97$, $P = 0.03$) and age (Gr.3, $r = -0.50$, $P = 0.017$). In patients aged $\geqslant$40 years, EF was correlated with the time from RT (Gr.1, $r = 0.56$, $P = 0.193$; Gr.3, $r = 0.75$, $P = 0.011$). Response to exercise testing with an increment of EF > 5% was observed in 50%, 60% and 62% pts, respectively.
Our study suggested that low-dose irradiation of whole heart for extensive pericardial disease did not impaired significantly cardiac function during first decade after doxorubicin-based chemotherapy as compared with partial irradiation of heart. Further observation is necessary.

9222 POSTER

Impact of DeVIC as Chemotherpeutic Agent for Concurrent Chemoradiotherapy for Nasal NK/T-cell Lymphoma

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Background: Extranodal natural killer (NK)/T-cell lymphoma (NKTCL), nasal type, is a rare aggressive lymphoma with poor prognosis. This is an Epstein-Barr virus-associated lymphoma and the lymphoma cells expressed P glycoprotein, resulting in tumour multidrug resistance (MDR). Reported 5-year overall survival for patients with localized nasal NKTCL treated with CHOP is lower than 50%. DeVIC (dexamethasone, etoposide, ifosfamide and carboplatin) chemotherapy was designed as a salvage chemotherapeutic regimen for aggressive lymphoma comprised of MDR-nonrelated agents and etoposide, which is thought to be effective against nasal NKTCL. We are now on the way to do an experimental chemoradiotherapy (CRT) using DeVIC as concurrent chemotherapeutic agents for nasal NKTCL. The aim of this study is to look at the initial outcome of this treatment to evaluate its effectiveness and feasibility.
Material and Methods: Six patients (range, 29 to 82; median, 68 years) were treated with CRT using DeVIC chemotherapy between April 2004 and February 2010. Median follow-up was 56 months (range, 11−80). Clinical features of these 6 patients were as follows: male:female = 4:2, 1E:2E=5:1, B symptom present = 0, elevated serum lactate dehydrogenase = 3, PS0:PS1 = 3:3, and IPI score low:low-intermediate = 3:3. All patients were given 3 to 6 cycles of full dose DeVIC regimen. The drug doses and administration schedule were as follows: dexamethsone, 40 mg/d on days 1 to 3, etoposide, 100 mg/m^2 on days 1 to 3, ifosfamide, 1.5 mg/m^2 on days 1 to 3, and carboplatin, 300 mg/m^2 on day 1. Basically the chemotherapy was given concurrently with radiotherapy (RT), and was