5 years in the three eras was 79% (95% CI, 68%-87%), 85% (95% CI, 77%-90%) and 93% (95% CI, 83%-97%), respectively. At 10 years, in the two earlier eras, it was 56% (95% CI, 43%-67%) and 75% (95% CI, 65%-83%), respectively. Despite the significant reduction of deaths due to the FL and its treatment, differences in overall survival among the three groups were not statistically significant. This may depend on the advanced age of most patients and on short follow-up for patients of the most recent era. A longer observation will be needed to clarify the issue.
Conclusions: The cause-specific survival of patients with FL treated at the IOSI has improved over the last 25 years. This improvement may be a result of the sequential application of more effective therapies and improved supportive care; however it has not yet translated in an improvement of the overall survival.

6006 ORAL

DNA repair gene ATM polymorphisms and risk of chronic lymphocytic leukaemia

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One of the most frequently altered DNA repair pathway in cancer cells is the one that corrects double-strand breaks (DSBs). The ATM gene encodes a protein kinase that plays a key role in the detection and repair of DNA DSBs. Once activated, the ATM protein triggers phosphorylation of CHEK2 which, in turn, phosphorylates p53, Cdc25 and BRCA1, promoting cell-cycle arrest and DNA repair. Through their effect in DNA damage check point regulation, ATM gene polymorphisms may modulate individual susceptibility to cancer. Chronic lymphocytic leukaemia (CLL) is one of the most common malignant lymphoid diseases in the western world. ATM alterations have been observed in CLL and are related with a poor prognostic. In particular, recent studies have suggested a role for ATM in disease progression in B-CLL. Furthermore, a recent study has found an association between non-synonymous SNPs in ATM and risk of CLL.
In addition to SNPs in the coding region, SNPs in no coding region (intronic, 3′UTR, 5þTR) have been found to be associated with some diseases. Therefore, in this study, we have conducted a large association study between ATM and CLL. A total of 19 SNPs were genotyped. SNPs were chosen to map the ATM gene by linkage disequilibrium (LD), including adjacent non-transcribed regions at both edges, based mainly on LD data from the CEU population taken from HapMap phaseI (The International HapMap Consortium 2004). The tagger algorithm, as implemented in Haploview 3.0, was used to select TAG-SNPs. The selection was based on the number of additional SNPs for which they can act as tags and SNPs generating the common amino acid substitutions were specifically forced into the tagging. Genotyping was performed by using the MassARRAY SNP genotyping system (Sequenom Inc., San Diego, CA). Haploview 3.0 (Barrett et al., 2005) was used to estimate LD and to search for any deviation of Hardy-Weinberg equilibrium in controls. For haplotype analysis, we used a sliding windows approach, considering window sizes of two or three consecutive SNPs. All analyses have been done in 740 patients and 748 matched controls.
The data obtained from this extensive analysis are expected to further contribute to our understanding of the relationship between ATM polymorphism and the risk of CLL.
This work was supported by RETICS G 3/179 and Gobierno Vasco SAIOTEK

6007 ORAL

MDM2 SNP309 is associated with poor outcome in B-cell chronic lymphocytic leukaemia but can be preferentially targeted by the MDM2 inhibitor Nutlin-3a

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Background: A single nucleotide polymorphism (SNP) at position 309 in the promoter region of MDM2 leading to increased expression of MDM2 and attenuated function of p53 tumor suppressor protein has been negatively associated with onset and incidence of solid tumors. Since inactivation of p53 by deletion and/or mutations also negatively impact on the clinical course of B-chronic lymphocytic leukemia (B-CLL), we analyzed the association of SNP309 with the clinical course and its interaction with the p53 status in B-CLL.
Patients and Methods: The frequency of SNP309 T/T, T/G or G/G genotypes was assessed by RT-PCR in a cohort of 140 B-CLL patients. In addition, the p53 status (wild-type vs mutated p53, deletion of p53) was assessed by single-stranded conformation polymorphism (SSCP) and fluorescence-in-situ-hybridisation (FISH) analysis, respectively. SNP309 genotype and p53 status were correlated with treatment-free and overall survival of patients. In addition, in vitro sensitivity of B-CLL cells to apoptosis induced by nutlin-3a, a specific inhibitor of the MDM2/p53 interaction was determined and correlated with their SNP309 genotype.
Results: A significant negative association of the SNP309 T/G and G/G genotypes with overall survival (T/G genotype: RR 3.7 95% CI 1.2−11.5, p = 0.02; G/G genotype: RR 9.1 95% CI 2.4−35.1, p = 0.001) but no correlation with incidence or onset of B-CLL was observed. Multivariate analysis of SNP309 genotype and p53 status identified both as independent negative prognostic markers. Nutlin-3a treatment reactivated the p53 pathway in B-CLL cells and led to significant induction of apoptosis. Interestingly, the clinically unfavorable SNP309 T/G or G/G genotypes rendered B-CLL cells more sensitive to apoptosis induced by nutlin-3a.
Conclusions: The MDM2 SNP309 genotype was identified as an additional independent risk factor in B-CLL. The higher sensitivity of tumor cells from T/G and G/G SNP309 carriers to nutlin-3a might be exploited therapeutically.

Poster presentations (Mon, 24 Sep, 14:00−17:00)
Leukaemia, lymphomas, transplantation (adults)

6008 POSTER

Selenite is a superior cytotoxic agent to human primary leukemia cells

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Background: The selenium compound, selenite, is rising as a promising cancer therapeutic agent in several experimental studies. However, the mechanism of selenium-induced cytotoxicity is poorly understood. AML is the most common leukemia in adults but the cure rate remains low. Commonly used drugs, as cytarabines and anthracyclines, often lead to drug resistance.
Materials and Methods: This study was conducted on an ex vivo model with acute myeloid leukemia (AML) patient material. The primary cells were treated in a drug panel with conventional cytotoxic drugs, and evaluated in comparison to selenite treatment (5 μM).
Results and Conclusions: We show that selenite is the most effective drug in the panel compared to commonly used drugs against AML in concentrations that could potentially be administered to patients. Equally important, all conventional drugs in the panel showed a correlation to each other by having an effect on the same group of patients. Selenite does not show this correlation indicating the ability to treat an, in part, unique group of patients. mRNA and protein levels of thioredoxin reductase and mRNA levels of the glutaredoxins were also measured While a strong upregulation of thioredoxin reductase mRNA levels were observed, the protein level decreased. This possible translational impairment may explain a part of selenites cytotoxicity. Both glutaredoxin 1 and 2 mRNA levels increased suggesting both mitochondrial and cytosolic oxidative stress caused by selenite treatment.

6009 POSTER

Risk factors for early mortality, relapse and overall survival in new cases of APL treated by arsenic trioxide

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Background: there are several known risk factors for APL treatment by all-*trans* retinoic acid (ATRA) and chemotherapy, but risk factors for new cases of APL treated by arsenic trioxide (ATO) are unknown.
Material and Methods: Between May 2000 and September 2006, we treated 141 new cases of APL (Median age 28±12.8 y/o min = 11, max = 71) by 2 hours iv infusion of 0.15 mg/kg ATO until complete remission. Trial approved by IRB and consent form obtained. Diagnosis was by clinical and morphologic characteristics and confirmed by cytogenetic and RT-PCR for detection of t(15,17) and presence of PML-RAR?. After complete remission patients received consolidation by 28 days infusion of ATO for one or four courses. Known risk factors for APL treatment outcome

(including PML-RAR?) isoforms, presence of MRD during follow up and WBC count at presentation analyzed for early mortality, relapse rate, DFS and OS.
Results: complete remission observed in 121 cases (85.8%) and early mortality rate was 14.9%. Short isoform of detected in 36% of patients and 18% presented by WBC more than 10000/?l. For early mortality, APL differentiation syndrome during treatment and WBC count more than 10000/?l were risk factors ($P < 0.001$ and $p = 0.011$ respectively).
For DFS only predicting factor for relapse was detection of MRD (by nested PCR or by real time PCR) during follow up ($P = 0.05$). For prediction of OS, again only risk factor was detection of MRD ($P < 0.0001$).
Conclusion: although WBC count before treatment and APL differentiation syndrome during treatment are risk factor for relapse, short isoform of APL is not a risk factor. After achieving CR only risk factor is detection of MRD.

6010 POSTER

Evaluation of the enhancement of the apoptotic efficacy of STI571 by PBOX-21 in human chronic myeloid leukaemia cell lines

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Chronic myeloid leukaemia (CML) is a haematological stem cell disorder that is characterised by the presence of a constitutively active tyrosine kinase, Bcr-Abl. The main treatment available today is a specific tyrosine kinase inhibitor, STI571 (Glivec®), but many patients are gaining resistance to this drug. Recently, some members of a novel set of compounds the PBOX's, have been shown to arrest astrocytoma cells in the G1 phase of the cell cycle. In this report we looked at the combination of STI571 with a representative PBOX compound, PBOX-21, in the induction of apoptosis in CML cell lines.
PBOX-21 was shown to transiently arrest Bcr-Abl-positive CML cells in the G1 phase of the cell cycle. This novel compound also synergistically enhanced the apoptotic effect of STI571 in a time and dose dependent manner as determined by calculation of the combination index values using the software Calcusyn. Co-treatment with PBOX-21/STI571 was equally potent in parental and STI571-resistant CML cell lines. Cleavage of the enzyme PARP, a classical hallmark of apoptosis also confirmed these results. PBOX-21 in combination with STI571 caused a down-regulation of the levels of the Bcr-Abl protein and its ability to phosphorylate substrates. Pre-treatment of the cells with a general caspase inhibitor and a serine protease inhibitor showed a significant reduction in the levels of apoptosis following PBOX-21/STI571 treatment implying a role for both these families in initiating apoptosis. Caspase-8, an upstream caspase was shown to be particularly important for the execution of apoptosis. Further analysis of part of the downstream PBOX-21/STI571-induced apoptotic signalling pathway by western blotting showed a decrease in the levels of the anti-apoptotic proteins Bcl-$_{XL}$ and Mcl-1, cleavage of Bcl-2 and an upregulation of the pro-apoptotic Bim protein.
We conclude that this novel combination may have the potential to be effective in the treatment of Bcr-Abl positive CML patients, including those that have become resistant to current treatments. Future work will involve testing the PBOX compounds alone and in combination with STI571 on a cohort of STI571-resistant patient samples and on a CML mouse model.

6011 POSTER

Karyotypic evolution during long-term IM treatment of CML patients

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The t(9;22)(q34;q11), generating Ph marker, is found in more than 90% of patients with chronic myeloid leukemia (CML). Some CML patients demonstrate additional abnormalities at diagnosis or during therapy. Ph variants are present in 5−10% of patients with BCR/ABL fusion translocations. There are abnormalities involving other chromosomes, beside 9 and 22, or cryptic rearrangements not detected by conventional cytogenetics. Moreover, submicroscopic deletions of sequences flanking the ABL and/or BCR genes breakpoints are noticed in about 15% CML cases.
We report a detailed cytogenetic data of CML patients before and during therapy with imatinib (IM). We observed karyotype changes in three groups of patients: 1st presenting t(9;22) as a sole anomaly, 2nd carrying t(9;22) with submicroscopic deletion, and 3rd with t(9;22) and additional aberrations, including variant Ph. The comparison of cytogenetic response, obtained during the time of observation (3 to 7 years for individual patients) showed some differences between mentioned groups. In all groups there were some patients that achieved complete cytogenetic remission (CR). There were: 12 out of 15 patients in first group, 4/9 patients in second group and only 2/7 in third group with additional aberrations to Ph. In the first group 3 out of 15 patients did not show complete cytogenetic response. One of them died after 52 months of treatment. Progression of changes after CR period (in the 60 and 66 month of therapy, resp.) was observed in 2 patients. Two other patients showed unrelated, aberrant clones during CR. In the second group 5/9 patients did not demonstrate complete cytogenetic remission in the time of observation. Three of them died after 47, 48 or 66 months of therapy. Two patients showed Ph clone with extra changes after CR period (detected in the 16 and 56 month, resp). Majority of patients in the third group did not achieve CR (5/7 cases). Two of them died after 24 and 34 months of treatment. Both patients showing CR during treatment, subsequently revealed other abnormalities (in the 42 and 60 month of treatment). Generally, in cases obtained complete cytogenetic response, subsequent progression of karyotypic changes was appearing: in 18% of patients from the 1st group, in 40% in the 2nd group and both patients from the 3rd group. Such progression of karyotypic changes (e.g. additional Ph copy, +8, −Y, mar) appeared between the 16 and 66 month of observation. Our data indicates that the result of IM treatment is connected to the type of karyotype changes, revealed before starting the treatment. The group of patients showed only typical t(9;22) as a sole abnormality has shown the best prognosis and they achieved relatively long disease free period.

6012 POSTER

The existence of humoral immunity to gliadin and cow's milk proteins in patients with lymphoma

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Background: Lymphomas are cancers that start in the lymphatic system. The aim of this work was to determine is there any IgA or IgG immune reactivity with cow's milk proteins, or with gliadin and to notice eventual disorders of oral mucosa in patients with lymphoma.
Patients, Material and Methods: In the aim to determine the immune reactivity of serum IgA and IgG with cow's milk proteins and gliadin, sera from 48 patients with various Non-Hodgkin's (NHL) lymphoma, before therapy or before therapy after the relapse, and group healthy people were analyzed by home made ELISA tests. Two kinds of antigens were used: skim cow milk pasteurized powder (ICN Biomedicals, Inc.) and crude gliadin (Sigma Chemicals). HRP labeled antibodies (Binding Site), were used as secondary antibodies. Blocker was 1% BSA. The absorbance of developed color by OPD was measured at 492 and 630 nm. Immunoreactivity to cow'milk proteins in arbitary units (AU/ml) was determined using positive control serum from our laboratory, while immunoreactivity to gliadin (IU/ml), was determined using five calibrators from Binding Site ELISA test. Dental check-up was conducted by a single dentist using daylight, dental mirror and dental probe.
Results: The enhanced immunoreactivity of serum IgA with cow'milk proteins higher than 8.036AU, was found in 6/48 patients with NHL and in 2/35 healthy people.
The enhanced immunoreactivity of serum IgG with cow'milk proteins higher than 44.48AU, was found in 7/48 patients with NHL and in 0/35 healthy people.
The enhanced immunoreactivity of serum IgG with gliadin higher than 10.19IU, was found in 4/48 patients with NHL and in 2/74 healthy people.The enhanced immunoreactivity of serum IgA with gliadin higher than 1.79IU, was found in 10/48 patients with NHL and in 4/74 healthy people.Disorders of oral mucosa were detected in 26/48 examined patients.
Conclusion: The data presented in this work indicate to the existence of the enhanced immunoreactivity of serum IgG and IgA to cow's milk proteins and to gliadin antigens in some patients with NHL, which was not so much pronounced in healthy controls. Data obtained indicate the need for the analysis of the real clinical importance of the observed immunoreactivity to food proteins in patients with lymphoma. Gingivitis, oral ulcers, anemic oral mucosa, pathological changes of tongue mucosa and sometimes purpura, were found in some of examined patients.