

(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 (Gleevec®), 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 Calcsyn. 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-x_L 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 if 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 arbitrary 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.