1005 POSTER

Indirubin-3'-monoxime, a novel cdk-inhibitor induces cdk-1 and survivin dependent growth arrest and apoptosis in bladder cancer cells.

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Background: In the traditional Chinese Medicine, the preparation Danggui Longhui Wan, has been used for years in treatment of chronic myelocytic leukaemia. The compound, Indirubin, has been shown to be the active constituent. The cell permeable Indirubin-3'-monoxime is a selective and potent inhibitor of cyclin-dependent kinases (CDK).

Material and Methods: In this study we investigated if Indirubin-3'-monoxime can induce apoptosis and tumor cell death in four different bladder cancer cell lines. The growth inhibitory properties were evaluated by EZ4U, a cytotoxic assay; apoptosis induction was determined by immunoblotting of cleaved PARP and flowcytometry of Annexin-V/PI staining during treatment. To evaluate further underlying molecular actions of indirubin-3'-monoxime on the cell cycle, cdk-1 and Survivin, a major apoptosis and mitotic spindle checkpoint-regulating protein were additionally determined by flowcytometry and immunoblotting. Further, we investigated a potential synergism of Indirubin with Paclitaxel, as this drug targets the mitotic spindle and cell cycle regulation, too.

Results: Our results show, that Indirubin-3'-monoxime induces reversible growth arrest in all four cell lines and an increase of apoptosis in two of them. A synergistic effect of a combination of Indirubin-3'-monoxime and Paclitaxel was shown in two cell lines. In the other two, Indirubin competed with Paclitaxel at cdk-1 and abrogated Paclitaxel's cytotoxic efficacy. We found different expressions of cdk-1 and Survivin in the cell lines. These may explain the different behaviour of the cell lines and may help in future to predict the response to the combined therapy.

Conclusions: In summary, Indirubin-3'-monoxime seems a promising candidate for molecular targeted cancer therapy. However, its interaction with other agents needs careful evaluation if given in combination.

Adult leukemia/Lymphoma

1006 POSTER

Prognostic value of bone marrow involvement by Hodgkin's disease.

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Background: It has been demonstrated by previous studies that in patients (pts) with Hodgkin's disease (HD) bone marrow (BM) scintigraphy (BMS) is much more sensitive than routine iliac crest biopsy. Unfortunately, prognostic value of scintigraphic data remain undetermined.

Material and method: Since 1992 BMS was performed in 319 pts with HD. Whole body BM visualisation started 45-90 min after i/v injection of 8-10 MBq/kg of 99mTc-colloids. Scintigraphic signs of BM invasion were classified as follows: localised lesions manifested by 1-2 focal defects, generalised involvement - by multifocal (3 and more) defects and diffusely diminished tracer uptake. BM invasion was confirmed by additional examinations (biopsy, MRI, bone scanning, X-ray). Pts with BM invasion underwent combined modality treatment: 6 and more cycles of chemotherapy and radiotherapy of various extent.

Results: BM invasion by HD was diagnosed in 56 cases: localised - in 39, generalised - in another 17 pts. For all 56 pts with BMI 5 year disease free survival was equal to 27% (15/56 pts). Only 2 of 17 pts with generalised BM survived 5 year without HD: DFS - 11%. Five year disease free survival for pts with locolised BM invasion (33%) by HD was 3 times higher than for pts with generalised BM (11%) involvement ($p<0.05$).

Conclusions: Scintigraphic pattern of BM invasion by HD has significant prognostic value: for localised BM invasion 5 year disease free survival is 33%, for generalised - only 11%.

1007 POSTER

Accuracy of end of treatment 18F-FDG PET for predicting relapse in patients with Hodgkin's disease (HD) and Non-Hodgkin's lymphoma (NHL)

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Whole-body positron emission tomography (PET) using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) offers the possibility of differentiating sites of vital tumor from necrotic residual masses. We have previously shown that PET has higher diagnostic and prognostic value than computed tomography in HD and NHL (Jerusalem et al, Blood, 1999; 94: 429-33). The aim of this study was to obtain further information about the accuracy of PET in the end of treatment evaluation of patients suffering from lymphoma. One hundred and eight patients (39 HD, 69 NHL) were recruited prospectively between 5/94 and 10/01. All patients underwent a whole-body PET 1 to 3 months after the end of chemotherapy and/or radiotherapy and had a further follow-up of at least 1 year. Twenty-six patients relapsed (NHL: 23, HD: 3). End of treatment PET was positive in 16/108 (15%) patients. In 12 patients (NHL: 11, HD: 1), PET had correctly identified residual disease (high lesion-to-background ratio: 7 patients, low lesion-to-background ratio: 5 patients) confirmed either by biopsy or by unequivocal conventional imaging studies after a median of 1 (range 0-20) month. In the other 4 patients (NHL: 2, HD: 2), this was a false positive PET. The lesion-to-background ratio was low in 3, suggesting an inflammatory rather than a tumoral lesion, and high in one patient who actually had developed rectal cancer. The 2 of 16 patients with increased ^{18}F-FDG uptake only outside of initial lymphoma involvement were both false positive (1 inflammatory lesion, 1 rectal cancer). Fourteen patients with a negative end of treatment PET relapsed 1-60 months (median: 13.5 months) later. Based on our data end of treatment PET had a sensitivity of 46% (12/26), a specificity of 95% (78/82), a positive predictive value (PPV) of 75% (12/16) a negative predictive value of 85% (78/92) and an overall accuracy of 83% (90/108). These patterns were not different between HD and NHL patients, except that because relapse was a rare event in HD, the impact of false positive PET on PPV value was much more important in HD (PPV: 33%) than in NHL (PPV: 85%). In conclusion, PET is very accurate in predicting short-term treatment failure. However, it cannot detect microscopic residual disease and thus its value is hampered by false negative results in patients later relapsing. On the other hand, a biopsy is always indicated before salvage therapy in order to exclude false positive PET results related to inflammatory lesions or to second primary tumors. All patients with increased ^{18}F-FDG uptake with a high lesion-to-background ratio have either residual tumor or a second primary tumor.

1008 POSTER

Randomized trial of accelerated hyperfractionated radiotherapy to 24 Gy versus standard 40 Gy in salvage programs for Hodgkin's disease

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Purpose: To evaluate whether or not low-dose accelerated hyperfractionated radiotherapy (AHFX) is sufficient for control of residual disease compared to standard 40 Gy after salvage chemotherapy (CT) for Hodgkin's disease (HD).

Material and methods: Since September 1999, conventional-dose CT was used in 168 consecutive patients with relapsed or primary refractory HD. Non-cross-resistant CT regimens were changed at each evidence of new progression until achieving systemic control of 6 months duration. If residual disease location was appropriate for full-dose radiotherapy (RT) the patient was randomly allocated either to receive involved-field RT to standard 40 Gy or to 24 Gy in AHFX regimen (1.3-1.5 Gy twice daily with 5 hours interval/24 Gy/8 days). Patients were stratified with regard to chemoresistant (>2 changes of CT regimens required), chemosensitive relapse or primary refractory HD.

Results: Total 92 HD patients were entered on this study. RT was given to 91 sites (79 nodal and 12 bone lesions) in the 66 patients with relapses (53 chemoresistant, 13 chemosensitive). Mediastinal adenopathy was irradiated in the 26 patients with refractory HD; only those with >12 months follow-up were included to this analysis. With a median follow-up of 21 months (range 1-42 months), there were alive and progression-free 26/53 (49%) patients after chemoresistant relapse, 10/13 (77%) patients after chemosensitive relapse and18/26 (69%) of refractory group. No differences were found between the two arms in favor of 40 Gy in any group.

In-field failures were registered after standard 40 Gy in 13.7% (7/51 patients, 95% confidence interval (CI): 4.2% to 23.1%) and after 24 Gy AHFX in 10.7% (7/66 patients, CI: 3.2% to 18.0%). Median time to in-field-failure was shorter in chemoresistant relapse: 4 months (range 1-11) versus 15 months (range 8-21) in chemosensitive relapse and refractory patients altogether. Out-of-field progression occurred in 51% (27/53 patients) and 28% (11/39 patients) cases, respectively. Median time to out-of-field progression was 4 months (range1-27) and 15 months (8-24), respectively. Deaths from progressive disease occurred in 10 relapsed patients; 2 patients died of treatment-related AML and AMML.

Conclusions: This randomized study suggests that the low-dose RT in AHFX regimen can provide the similar rate and duration of local control for post-CT residual disease as standard 40 Gy.

1009 POSTER

Phase II clinical experience with the novel proteasome inhibitor bortezomib (formerly PS-341) in patients with indolent and mantle cell lymphomas

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The ubiquitin proteasome pathway plays an essential role in the degradation of most short- and long lived intracellular proteins in eukaryotic cells. At the heart of this degradative pathway is the 26S proteasome, an ATP dependent, multicatalytic protease. The 26S proteasome plays a vital role is degrading regulatory proteins that govern cell cycle, transcription factor activation, apoptosis and cell trafficking. Some of the targets of ubiquitin proteasome mediated degradation include p53, p21, NF-κ B, Iκ B and *bcl*-2. Several lines of preclinical data have confirmed that inhibitors of the proteasome can act through multiple mechanisms to arrest tumor growth, tumor spread and angiogenesis. Phase I trials have confirmed tolerability of the drug and have suggested possible clinical activity in indolent lymphomas and myeloma. Correlative studies performed in the Phase I and II clinical trials have established a dose response relationship between dose and the extent of proteasome inhibition seen in peripheral blood mononuclear cells. To date, we have administered over 65 cycles of PS-341 (average 3.8 per patient) to 17 previously treated patients with relapsed or refractory indolent lymphomas (small lymphocytic lymphoma-CLL type (n=2); marginal zone lymphoma (n=1); follicular lymphoma (n=7) and mantle cell lymphoma (n=7). All patients were required to sign and informed consent and had to have adequate hepatic and renal function. Adequate hematologic counts including an ANC of > 1000 cells/μ l and a platelet count > 100,000/μ l were also required. All patients had received some form of treatment prior to receiving PS-341, including: CHOP; CVP; cyclophosphamide/fludarabine; rituximab; interferon, and one patient who had received two regimens of a complex combination chemotherapy program that included alkylating agents, tubulin inhibitors, anthracyclines and antimetabolites. Patients were treated at a dose of 1.5 mg/m2 twice weekly for two consecutive weeks with a one week rest period. Re-staging studies were routinely performed after two complete cycles of therapy. Both patients with small lymphocytic lymphoma were found to have stable disease after 2 and 4 cycles respectively. Of the 6 evaluable patients with follicular lymphoma, there was one CR, 5 PR (i.e > 50% reduction in tumor volume). Of 7 patients with mantle cell lymphoma (1 not evaluable for response yet), 3 patients had a PR, 3 had stable disease. One patient with MCL continues to maintain his PR (> 80% reduction in his disease) at 14 months since the completion of therapy. These preliminary data continue to support the biological activity of PS-341 in patients with indolent lymphomas, especially follicular and mantle cell lymphoma. Accrual to this trial continues.

1010 POSTER

Hairy cell leukemia: early immunological diagnosis and quantitative analysis of flow cytometry

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(1) Background. Immunophenotypic analysis of bone marrow (BM) and peripheral blood (PB) by flow cytometry is not widely used as a method for diagnosis HCL. The abnormal coexpression of the so-called 'HCL-restricted' markers - CD22+CD11c, CD25 and CD103 on monotypic, slightly large B-lymphocytes has been shown to be highly characteristic of HCL. The main aim of our study was to determine if patterns with low levels of neoplastic cells in BM or PB are valuable in the diagnosis and minimal residual disease (MRD) detection in HCL. Next we wished to determine if quantitative immunophenotyping given by molecules of equivalent soluble fluoresceine (MESF) could help to distinguish pathological B-lymphocytic pool. We investigated serially lymphocyte subsets after treatment with 2-Chlorodeoxyadenosine (CdA) to confirm CD4+ lymphopenia. (2) Material and methods. The abnormal immunophenotypes were studied in 18 patients with suspect HCL (all patients had other manifestations of HCL), or during follow-up of already treated patients. Flow cytometric measurement was performed on an EPICS ALTRA Flow Cytometer using double- or triple-staining and Expo 32 program for analysis. For evaluation of marker density expressed in flow cytometry by mean of fluorescence intensity, fluorescent calibration microbeads were used. (3) Results. In 12 HCL patients (67%) permanent complete remission was observed after treatment. In the rest of 6 patients (33%) we identified transient MRD+ phenotype but the clinical manifestation of relapse was followed in only three patients. The pathological cells in low levels were found in 4 patients at diagnosis (in the range of 7 to 18%) and in patients with MRD+ phenotype they were recognized repeatedly in the range of 2 to 8%. Furthermore, we observed in hairy cells significantly higher values of molecule numbers of B-cell markers, comparing to residual B-cells in nonleukemic lymphocyte gate of the same sample. We found profound and persistent CD4+ lymphopenia in majority of studied patients after CdA treatment. (4) Conclusions. Flow cytometric immunophenotyping is highly sensitive and specific method and is capable to detect low levels of malignant cells in HCL. Quantitative analysis of MESF values of pathological and normal residual B-cells seems to be a new marker of HCL, reliable detecting also small cell numbers in examined sample. A long-term decline of CD4+ T-cells correlated with the relatively low incidence of clinical progression of HCL.

1011 POSTER

Comparison of MOPP versus ABVD as Salvage Therapy in Patients Who Relapsed After Radiation Therapy Alone for Hodgkin's Disease

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Purpose: Randomized trials by cooperative groups have demonstrated the superiority of Adriamycin, bleomycin, vinblastine, dacarbazine (ABVD) over mechlorethamine, Oncovin, procarbazine, prednisone (MOPP) in the treatment of newly-diagnosed Hodgkin's disease (HD). We sought to compare the efficacy of the 2 regimens as salvage therapy in patients with relapsed HD after radiation therapy (RT) alone.

Methods: 100 patients with HD initially treated with RT alone between 1980 and 1997 subsequently experienced a relapse. 41 patients were salvaged with MOPP and 59 received an ABVD-containing regimen. Freedom from second relapse (FSR), defined as time from the end of salvage treatment to second relapse, death or end of follow-up, and overall survival (OS), defined as time from the end of initial treatment to death or end of follow-up, were estimated using the Kaplan-Meier method. Survival curves were compared using log-rank tests. Cox proportional regression models were used to evaluate potential predictive factors. Variables analyzed were: age at diagnosis, histology, number of initial sites, time to first relapse, relapse stage, extranodal disease at relapse and salvage chemotherapy regimen.

Results: The median follow-up time after first relapse was 12 years for all patients (range, 1-22 years), 17.3 years for MOPP patients (range, 7-22 years) and 8.3 years for ABVD patients (range, 1- 18 years). The 10-year FSR rates for all patients, MOPP patients and ABVD patients were 70%, 72% and 68%, respectively (MOPP vs. ABVD: p=0.62). The corresponding 10-year OS rates were 89%, 85% and 92%, respectively (MOPP vs. ABVD: p=0.64). On univariate analysis, age ≥50 at initial diagnosis significantly predicted for lower FSR (p=0.001) and OS (p=0.0001). On multivariate analysis, age ≥50 significantly predicted for inferior FSR [hazards ratio (HR)=9.1, p=0.0001] and OS (HR=8.5, p=0.001). No other factors were significant. Of the 41 MOPP patients, 12 (29.3%) developed a second malignancy (2 leukemia, 1 non-Hodgkin's lymphoma and 9 solid tumors). Of the 59 ABVD patients, 11 (18.6%) developed a second malignancy (1 leukemia, 2 non-Hodgkin's lymphoma and 8 solid tumors).

Conclusions: Patients who relapse after RT alone for HD have a high salvage rate. Older age at diagnosis is the only significant predictor for poorer salvage outcome. In contrast to initial HD therapy, MOPP and ABVD showed no significant differences in efficacy as salvage therapy for RT failure. Potential explanations for the lack of differences could be a greater likelihood in a single-institutional setting to push for full doses