(p = 0.001) were of prognostic value for CR achievement. Karyotype (p = 0.01) and weight loss (p = 0.02) remained of prognostic value for DFS, while age (p = 0.02), CD34 expression (p = 0.001), and biological abnormalities (p < 0.0001) remained of prognostic value for OS.

HD-AraC combined with amsacrine appears as a useful salvage regimen in refractory AML. We are proposing a prognostic model in order to identify patients in whom such regimen could be useful and those who must be oriented to new drug trials.

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Identification of a synthetic 6 base phosphodiester oligonucleotide with the capacity to alter the expression of cell cycle components

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Purpose: We have developed a series of synthetic 6 base non-antisense phosphodiester oligonucleotide with the ability to inhibit the proliferation of a wide range of cancer cells. BT-99-25, an oligonucleotide with a GpT dinucleotides motif within a specific sequence context, has been found to be a potent inhibitor of Jurkat T cells division. In this study, we have determined the effect of BT 99-25 on the cell cycle progression of Jurkat T cells and identified participating cell cycle components.

Methods: Jurkat T cells, an acute lymphocytic leukemia cells, were incubated for 24 and 48 h with different concentrations (0.5 to 50 μM) of BT-99-25. Cell cycle progression of Jurkat T cells was analyzed by flow cytometry. Changes in cyclin D1,2,3, cyclin E, cyclin A, p27 and pRb phosphorylation status were studied by Western blot analysis.

Results: We found that BT-99-25 caused a time- and concentration-dependent inhibition of division of Jurkat T cell that was associated with an arrest in cell cycle progression. BT 99-25 blocked cell cycle in the G0/G1/S phase. At 50 μM, this arrest persisted during the 48 h of treatment. Cell cycle arrest was associated with a decrease in cyclin D1,2,3 content, an increase in p27 protein level and an increase in the underphosphorylated form of pRb. BT 99-25 caused an increase in the level of cyclin E after 24 h of treatment. Surprisingly, the level of cyclin E returned to that seen in the untreated Jurkat T cells after 48 h of treatment. No marked changes were observed in the level of cyclin A.

Conclusion: We have identified a synthetic 6 base non-antisense phosphodiester oligonucleotide with the ability to induce cell cycle arrest of Jurkat T cells at the G0/G1/S phase. The ability of this oligonucleotide to arrest leukemic cell division offers considerable promise for the treatment of leukemia or to potentiate the activity of conventional anticancer drugs.

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Induction of apoptosis in leukemia cells by synthetic 6 base phosphodiester oligonucleotide

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Purpose: BT-99-25, a synthetic 6 base non-antisense phosphodiester oligonucleotide with a GpT dinucleotides motif within a specific sequence context, has been found to be a potent inhibitor of Jurkat T cells division. In this study, we have evaluated whether BT 99-25 is capable of directly inducing apoptosis in Jurkat T cells.

Methods: Jurkat T cells, an acute lymphocytic leukemia cells, were incubated for 24, 48 and 72 h with different concentrations (0.5 to 50μM) of BT-99-25. Apoptosis was determined by measurement of phosphatidylserine translocation (Annexin V-FITC binding), detection of fragmented DNA by TdT-mediated dUTP-biotin nick end labeling (TUNEL) and by the release of soluble nuclear mitotic apparatus protein (NuMA). The presence of caspase-7 was evaluated by Western blot analysis. Active Caspase-3, cleaved poly(ADPpolyribose)polymerase (PARP) and mitochondrial membrane potential were analyzed by flow cytometry.

Results: We found that BT-99-25 directly induced apoptosis in Jurkat T cells in both a time- and concentration-dependent manner as measured by the translocation of phosphatidylserine at the cell surface and the release of NuMA. Although apoptotic cells were detectable at 24 h post-treatment, maximum apoptosis occurred at 72 h. After 24 h treatment, the cells showed a slight change in the mitochondrial membrane potential although a significant mitochondrial hyperpolarization was found after 48 h. BT-99-25-induced apoptosis was accompanied by the proteolytic activation of caspase-3 and -7 and by the degradation of PARP. The induction of apoptosis by BT-99-25 was significantly reduced by pretreatment of Jurkat T cells with the protein synthesis inhibitor cycloheximide.

Conclusion: We have identified a synthetic 6 base non-antisense that has the ability to inhibit cells division and to directly induce apoptosis of Jurkat T cells. The chemotherapeutic potential of this oligonucleotide is currently under investigation.

Adult non-Hodgkin's lymphoma

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A phase I/II trial of liposomal doxorubicin (tlc-d99, myocet) in combination with cyclophosphamide, vincristine, and prednisone (comp) for newly diagnosed intermediate and high grade non-Hodgkin's lymphoma (nhl)

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Background: CHOP chemotherapy is standard front-line treatment for patients (pts) with intermediate and high grade NHL, but the complete remission (CR) rate is less than 50% and better treatment is needed. TLC D-99 (Myocet) has an improved pharmacokinetic profile over free doxorubicin with decreased toxicity. We substituted TLC D-99 for free doxorubicin in the CHOP regimen to determine the toxicity profile and obtain efficacy data.

Methods: Initially, 3 dose levels of TLC D-99 were planned: (1): 40 mg/m2; (2): 60 mg/m2; (3): 80 mg/m2 all given IV over 1 hour. Doses of cyclophosphamide (750 IV mg/m2), vincristine (2.0 mg IV) and prednisone (100 mg PO x 5) were not varied. When no dose limiting toxicity (DLT) was seen by level 3 and high efficacy was seen at all levels, the dose of TLC D-99 was reduced to 50 mg/m2 to compare efficacy/toxicity of COMP to standard CHOP. Cycles were repeated every 21 days.

Results: 27 pts have been accrued to date. Median age: 52 years (range 25-84); histologies: diffuse large cell lymphoma in 19; follicular large cell in 5; Burkitt-like in 1; and high grade (not specified)in 2. 17 pts (63%) had stage III or IV disease; elevated serum LDH: 14 (52%); 14 (52%) had intermediate or high risk international prognostic index scores. Toxicities have been primarily hematologic: transient grade 3 or 4 neutropenia in 23/25 toxicity-evaluable pts. No G-CSF was given with cycle 1. 1 grade 3 anemia and 1 grade 4 thrombocytopenia was reported, with most non-hematologic toxicities grade 1 or 2. No DLT (grade 3 or 4 non-hematologic, or prolonged hematologic toxicity) has been observed. Thus far, a median of 6 chemotherapy cycles (range 1-8) have been administered. 2 pts progressed on study; all others who received up to 8 cycles of study therapy (18 pts) have had CRs. 7 pts are ongoing; 2 have had confirmed PRs to date and are continuing; the remaining 5 are not yet evaluable for response. Overall CR rate is 82%; median CR duration: 6.5+ mos (range 1.7-18.3+ mos), with follow-up ongoing. CRs were observed at all dose levels.

Conclusions: The COMP regimen is extremely well tolerated, and is highly active with a complete remission rate of 82%. Accrual is ongoing at Level 4.

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Aberrant methylation of gene promoters in gastric lymphoma

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Purpose: Stomach is the commonest site of extranodal B cell non-Hodgkin's lymphoma (NHL). The two most usual lesions are diffuse large B-cell (DLBC) lymphoma and Mucosa Associated Lymphoid Tissue lymphoma (MALToma). Hypermethylation of CpG regions in promoters leading to gene silencing is frequent in cancers, but is not well defined in lymphomas. Distinct pathways of lymphomagenesis may exist in different types of lymphoma, and could be reflected by different patterns of gene promoter methylation.

Method: DNA from 11 patients with MALToma, 19 patients with DLBC and 30 patients with nodal lymphomas were studied. Genomic DNA was extracted from the sample and was then bisulfite-modified. The modified DNA was amplified by methylation specific polymerase chain reaction (MS-PCR) for the detection of CpG methylation. Eight genes were investigated: p15, p16, E-cadherin, p73, VHL, Caspase 8 and hMLH1.

Results: MALToma had higher frequencies of p15, p16, E-cad and p73 methylation than gastric DLBC lymphoma (72% vs 37%, 81% vs 68%, 63% vs 53% and 45% vs 37%). On the other hand, gastric DLBC lymphoma