

($p = 0.001$) 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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POSTER

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 μ M) 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 μ M, 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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POSTER

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 50 μ M) 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(ADP-ribose)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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POSTER

A phase I/II trial of liposomal doxorubicin (tfc-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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POSTER

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

had comparable frequencies of p15, p16, E-cad and p73 gene promoters methylation as nodal lymphoma. None of the lymphomas investigated showed methylation on VHL, Caspase 8 and hMLH1 gene promoters.

Conclusion: In gastric lymphoma, p15, p16, E-cad and p73 methylation may be important in the pathogenesis of MALToma. Also, MALToma and DLBC lymphoma may evolve through different genetic pathways. In these three types lymphoma, methylation of VHL, Caspase 8 and hMLH1 may not be of pathogenetic significance.

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POSTER

The value of thallium-201-chloride scintigraphy in staging and monitoring radiotherapy response in low-grade non-Hodgkin's Lymphoma

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Purpose: To study the value of thallium-201-chloride scintigraphy in staging and monitoring radiotherapy response in low-grade non-Hodgkin's lymphoma (LG-NHL).

Patients and Methodes: Thirty-six patients with LG-NHL referred for radiotherapy were conventionally staged by physical examination, CT-scan, chest radiograph, ultrasound, biopsy and fine-needle aspiration cytology. The combined information of these modalities was considered the "golden standard" (GS) and compared to thallium-201-chloride (TI-201) scintigraphy, which was based on planar total body imaging and SPECT of neck and thorax. SPECT of other regions was performed at indication only. All treated localizations were restaged after radiotherapy by GS modalities and TI-201.

Results: In a total of 288 regions studied (36 patients, 8 regions per patients; see the first 8 columns of the table), 115 were positive for lymphoma, 173 negative. Of these 115 true positive localizations 111 were found based on GS modalities and an additional 4 were found on TI-201 and subsequently confirmed by GS (2 axilla's, 1 parailiac and 1 femoral). All 115 localizations were irradiated and the clinical response (CR, PR, SD, PD) was evaluated by GS and TI-201 modalities 3-4 weeks later. For all localizations and time-points the sensitivity, specificity, positive predictive value, and negative predictive value were evaluated (see Table).

	Neck	Axilla	Mediast	Spleen	Para aortic	Para iliac	Femoral	Extra nodal	All except upper abdomen	All
GS (-)	16	19	26	26	22	22	16	26	125	173
GS (+)	20	17	10	10	14	14	20	10	91	115
TI-201 (+)	17	12	7	4	6	7	14	9	66	76
Sens. (%)	85	59	70	40	43	50	70	70	68	63
Spec. (%)	100	89	100	100	100	100	100	92	97	98
PPV (%)	100	83	100	100	100	100	100	78	94	95
NPV (%)	84	71	90	81	73	76	73	89	81	80
PPV after RT (%)	81	100	71	100	100	100	100	67	88	90

(Sens = sensitivity, Spec = specificity, PPV = positive predictive value, NPV = negative predictive value)

Conclusion: Due to accumulation of activity in liver, kidneys and intestine TI-201 is less adequate in upper abdominal localizations (average sensitivity 42%). A positive TI-201 accurately predicts lymphoma activity (PPV: 95%), but a negative scan misses about 20% of all true positive lymphoma localizations. If the TI-201 was positive prior to irradiation then repeat scans will accurately predict treatment response (PPV: 90%) and therefore might be useful in follow-up. Apart from upper abdominal localizations TI-201 scintigraphy is helpful in diagnosing and monitoring radiotherapy response in LG-NHL.

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POSTER

Low dose (2 x 2 Gy) involved field radiotherapy in recurrent indolent lymphomas induces rapid and lasting remissions without significant toxicity; results of the HORA-1 phase II study

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Purpose: To study the efficacy of 2 x 2 Gy involved field radiotherapy (IF-RT) in recurrent indolent lymphoma patients (pts).

Patients and Methods: IF-RT to a dose of 2 x 2 Gy (interval 48 hours) was given to all known lymphoma sites in 79 evaluable pts with recurrent indolent lymphomas, stage IV (involved bone marrow). The patient population consisted of 42 females and 37 males, median age 59 years (range 35-86 years). Median time since primary diagnosis was 44 months (range 3-358 months). Patients were pretreated by an average of 4 chemo- and/or radiotherapy regimens (range 1-11). Bulky disease (>5cm) was present in 45 patients. Histological classification and subtyping revealed follicular lymphoma in 59 patients (29 grade I, 28 grade II and 2 grade III), B-CLL/SLL in 12, extranodal marginal zone lymphoma, MALT-type, in 3, mantle cell lymphoma in 3 and lymphoplasmacytic lymphoma in 2 cases. Endpoint of the study was in-field lymphoma control.

Results: IF-RT resulted in 49% CR (n=39), 39% PR (n=31), overall response rate (RR) 88%, 8% SD (n=6), 3% PD (n=2) and a spontaneous remission in 1 patient. As expected, toxicity was very mild. Only 2 pts showed a transient grade II leukopenia. RR was independent of sex, age (< or > 59 years), intensity of prior treatment (< or > 4 regimens), time since diagnosis (< or > 44 months), histology or tumour size (< or > 5 cm). Of the 31 patients in PR, 15 developed in-field recurrence after a median time of 4 months (range 3-15 months), of whom all except 3 had bulky disease. Of the 39 patients in CR, 5 progressed in-field or out-field, after a median time of 10 months (range 3-15 months). All except 1 had bulky disease. Of the 70 responding pts 44 are ongoing upto 33 months.

Conclusion and future plans: IF-RT (2 x 2 Gy) in recurrent indolent lymphomas induces excellent RR, without significant toxicity even in a heavily pretreated patient population. This regimen is now being compared with Chlorambucil in a prospective phase III trial in previously untreated lymphoma patients (HOVON - EORTC).

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POSTER

Combined chemotherapy and radiation versus radiation alone in the management of localized angiocentric lymphoma of the head and neck

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Purpose: To clarify the clinical benefit derived from the combined use of chemotherapy (CT) and involved-field radiotherapy (RT) in the management of stage I and II angiocentric lymphomas of the head and neck.

Methods: Of the 143 patients with angiocentric lymphoma of the head and neck who were treated at the Yonsei Cancer Center between 1976 and 1995, 104 patients (RT group) received involved-field RT alone with a median dose of 50 Gy (range: 20-70 Gy), while 39 patients (CT+RT group) received a median 3 cycles (range: 1-6 cycles) of CT before starting involved-field RT. The response rate, patterns of failure, complications, and survival data of the RT group were compared with those of the CT+RT group.

Results: Despite a higher response rate, local failure was the most common pattern of failure in patients of the both groups. The patterns of failure, including the systemic relapse rate were not influenced by the addition of combination chemotherapy. Although both modalities were well tolerated by the majority of patients, aberrant immunologic disorders or medical illnesses, such as a hemophagocytic syndrome, sepsis, intractable hemorrhage, or the evolution of second primary malignancies were more frequently observed in patients of the CT+RT group. The prognosis of patients in the RT group was relatively poor, with a 5-year overall actuarial survival rate of 38.3% and disease-free survival rate of 32.4%, respectively. However, their clinical outcome was not altered by the addition of systemic chemotherapy. Achieving complete remission was the most important prognostic factor on univariate and multivariate analyses, but treatment modality was not found to be a prognostic variable influencing survival.

Conclusions: Involved-field RT alone for angiocentric lymphoma of the head and neck was insufficient to achieve an improved survival rate, but the combination of chemotherapy and involved-field RT failed to demonstrate any therapeutic advantage over involved-field RT alone.