ESHD patients (pts); b)the optimum extension of RT fields (IF vs STN); c)treatment releated toxicity.

Methods: From February 1990 to July 1996, 140 consecutive pts with no laparotomy proven HD, staged I bulky and/or B, IIA, IIA bulky and IIEA, were randomized to receive either 4 cycles ABVD plus IFRT or 4 cycles ABVD plus STNI. ABVD dose intensity was 0,84. RT doses were 30 Gy to uninvolved sites, 36 Gy to involved sites and 40 Gy respectively to partial responders sites.

Results: After a median follow-up of 87 months, 136 pts (median aged 29; range 17-64) are evaluable. Treatment outcomes are as follow: ABVD+STNI (66 pts): complete remission (CR)=100%; FFP=97%; OS=93%; ABVD+IFRT (70pts): CR=97%; FFP=94%; OS=94%. 30% pts achieved CR after the third ABVD cycle; 88% after the fourth. 14/15 partial responders achieved CR with RT. One pt developed acute leukemia in the STNI arm. Acute and late toxicities were mild.

Conclusion: 4 ABVD courses plus IFRT are an effective treatment in ESHD with mild toxicity.

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Adult leukemia

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Molecular biology of acute promyelocytic leukemia (APL) in peruvian patients: PML/RAR alfa isoforms distribution in latino patients

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Background: APL is a distinctive form of AML genetically defined for presence of PML/RAR alpha fusion gene in most of the cases, with a distribution of 54-57% of long isoform (bcr1), 8-10% of variable(bcr2) and 37-40% of short isoform(bcr3)(bcr1/bcr3 ratio 1.4:1) An unexplained higher than expected frequency of APL (>20% of all AML) has been previously reported in "latino" populations as ours; however no differences in molecular characteristics have been determined for these populations.

Methods: We evaluated 24 peruvian pts. diagnosed of APL between March 1998 to December 2000 to determine molecular and clinical characteristics in this "latino" population. All pts. had morphological, cytochemical and conventional cytogenetics studies and RT-PCR for RNA analysis for PML/RAR alpha isoforms.

Results: 22/24 pts. with evaluable molecular results are included in this report. Ethnicity of all pts. were mestizo ("latinos"). Median age was 18.6 years old, F/M ratio: 1.2/1.0; FAB morphology was hypergranular in 17/22 (77.2%) and variant in 5/22 (22.7%). Cytogenetics showed t(15,17) in 65% of pts., however all pts. had confirmed molecular diagnosis of APL by PCR. Distribution of PML/RAR alfa isoforms was:long (bcr1) in 16/22 (72.7%), variable (bcr2) in 1/22 (4.5%) and short (bcr3) in 5/22(22.7%). All pts. were treated with IV Liposomal Atra followed by chemotherapy. Characteristics of pts. according to molecular isoforms are: a)Long Isoform (n:17): median age 17, with 31% of high risk pts.(Sanz Index),25% of ATRA syndrome and 86.7%of pts. achieving complete response(CR).

b) Short Isoform(n:5):median age 30, with 20% of HR pts.,40% of ATRA syndrome and only 50% of CR pts.

c) Variable Isoform: only one pediatric high risk pt (7 years old),who failed to achieve CR.

Our data shows a higher than expected frequency of long isoform in latino or mestizo population in Peru with a higher bcr1:bcr3 ratio(3.1:1) compared with prior reported series. We also observed a tendency of older age, higher ATRA syndrome frequency and lower ATRA sensibility associated with short isoform.

In conclusion our serie shows a distinctive molecular expression of APL-specific PML/RAR alpha gene in latino (mestizo) population in Peru, different from reported in other ethnicities. Further molecular analysis of APL in "latino" populations will allow us to understand the biological and clinical significance of these findings.

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Inhibition of ribonucleotide reductase by trimidox potentiates the cytotoxic and apoptotic effects of Ara-C in HL-60 human promyelocytic leukaemia cells

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The enzyme ribonucleotide reductase (RR) catalyzes the rate limiting step of the de novo synthesis of deoxynucleosidtriphosphates (dNTPs). Its significantly increased activity in malignant tumor cells makes this enzyme an excellent target for cancer chemotherapy. Trimidox (3,4,5 trihydroxybenzamidoxime) was proven a potent inhibitor of RR causing a significant depletion of dCTP pools in HL-60 human promyelocytic leukaemia cells. In the present investigation we analyzed the effects of a combination treatment regimen using trimidox and Ara-C, a well established chemotherapeutical agent for the treatment of leukaemia. Deoxycitidinekinase, the enzyme which activates Ara-C by phosphorilation underlies a negative feedback mechanism by dCTP, therefore a decrease in dCTP levels results in an increase of Ara-C metabolism and the incorporation of Ara-C into DNA.

We investigated the effects of a treatment with trimidox on the incorporation of radiolabelled Ara-C into DNA and found that trimidox synergistically enhanced the incorporation of Ara-C. Preincubation of HL-60 cells with 75 and 100 μM trimidox caused an increase in Ara-CTP pools by 90 and 150% compared to control values, respectively, which resulted in a 1.51 fold (with 75μM trimidox) and 1.89-fold (with 100μM trimidox) increase in Ara-C incorporation into DNA. Synergistic cytotoxic effects of combination treatment using Ara-C and trimidox were also confirmed by colony formation and growth inhibition assays. In growth inhibition assay, a synergistic combination index of >1 was yielded by treating the cells with 15 μM trimidox combined with 5 and 10 nM Ara-C. In soft agar colony formation assay the combination of 0.5 and 0.75 μM trimidox with 0.5-3 nM Ara-C showed significant synergism. We also found that the combination of 5 and 10 nM Ara-C with 10 and 15 μM trimidox resulted in the potentiation of the apoptotic effects of Ara-C. We conclude that trimidox is able to synergistically enhance the cytotoxic and apoptotic effects of Ara-C and therefore might be considered a valuable alternative for the combination chemotherapy of leukaemia.

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Salvage therapy combining high-dose cytarabine with amsacrine in refractory acute myeloid leukemia (AML): analysis of prognostic factors

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Refractory AML have a very poor prognosis. High-dose cytarabine (HD-AraC) has been proposed as salvage therapy in combination with amsacrine. The aim of this study was to assess toxicity and efficacy of this combination. Prognostic factors were also assess in order to determine patients susceptible to benefit of such a therapy. 91 patients referred to our hospital have been treated by HD-AraC (3 g/m2/12 hours for 4 days) combined with amsacrine (90 mg/m2/d for 3 days). 69 and 22 patients failed to one course of chemotherapy according respectively to the LYLAM85 or the LAM90 protocol. 45/91 patients (50%, 95% CI: 39-60%) achieved CR. 35 patients were refractory to the salvage therapy and 11 died from toxicity. Median DFS was 12 months. 26 patients received consolidation therapy. 19 patients with an HLA-identical sibling donor underwent allogeneic transplant. 27/45 patients (60%) who achieved CR have relapsed. Median OS was 10 months. There was 12 long survivors (13%). Karyotype was the main prognostic factor for CR achievement ($p = 0.001$), DFS ($p = 0.01$) and OS ($p = 0.0009$). In univariate analysis, CR achievement was also related to WHO performance status < 2 ($p = 0.007$), LDH level ($p = 0.02$), CD34 expression ($p = 0.03$) at diagnosis; platelet > 80 G/l ($p = 0.0001$), and the absence of circulating blasts ($p = 0.001$) and biological abnormalities ($p = 0.009$) before salvage therapy. DFS was negatively influenced by weight loss ($p = 0.03$), and WBC count > 10 G/l ($p = 0.03$) at diagnosis; and biological abnormalities before the salvage regimen ($p = 0.007$). Age ($p = 0.002$), toxic exposure ($p = 0.01$), CD34 expression ($p = 0.02$), weight loss ($p = 0.006$), and performance status > 2 ($p = 0.01$) at diagnosis; platelet < 80 G/l ($p = 0.02$), and biological abnormalities ($p = 0.0003$) were associated with shorter OS. In multivariate analysis, CD34 expression ($p = 0.001$), LDH level ($p = 0.02$), biological abnormalities ($p = 0.007$), and circulating blasts

(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