C10

INHIBITION OF P38MAPK-DEPENDENT PHOSPHORYLATION AND DEGRADATION OF SRC-3/AIB1 MODULATES RARα-MEDIATED TRANSCRIPTION AND FAVOURS GRANULOCYTIC MATURATION OF ACUTE PROMYELOCYTIC LEUKEMIA CELL LINES

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The transcriptional activity of RARs and RXRs is modulated by phosphorylation processes involving the receptors and specific co-regulator proteins. Among the large number of co-activators that have been identified, the SRC/p160 family and p300/CBP stand out. These co-activators are phosphorylated in response to several signaling pathways. Phosphorylation was shown to regulate co-activator activity through the control of their interaction with nuclear receptors or other co-regulators. In addition, like RARs, co-activators are targets for the ubiquitin-proteasome pathway. Transactivation experiments performed on COS cells show, that during ligand-dependent activation of RARα, the p160 co-activator SRC-3 is phosphorylated by p38MAPK and subsequently degraded by the proteasome. The co-transfection of a dominant-negative p38MAPK construct demonstrates that this biphasic process is correlated to an attenuation of RARα-mediated transcription. In fact, inhibition of p38MAPK enhances the interaction RARα-SRC-3 and this correlates with increased RARα-mediated transcription. Moreover, since inhibition of p38MAPK blocks also the degradation of SRC-3, the phosphorylation of this protein is also a permissive signal for proteasome-mediated degradation. In COS cells, RA-induced SRC-3 phosphorylation and degradation occur only within the context of RARα and possibly PML-RARα complexes, suggesting RAR isotype specificity. Myeloid leukemia HL60 and NB4 express SRC-3 and the protein is degraded in the presence of RA treatment. Degradation of SRC-3 is blocked by the p38 inhibitor, PD169316. Combined treatment of NB4 or HL60 with PD169316 and RA increases granulocytic maturation of these cells and increases the expression of several markers associated with granulocytic maturation (cEBP-β, STAT1). PD169316 exerts its effects essentially on SRC-3 since a specific siRNA directed against SRC-3 not only decreased SRC-3 expression but also attenuates NBT reducing activity and depresses RA target gene activation. Since RA is used in the treatment of acute promyelocytic leukaemia (APL) and several other malignancies, the combined use of pharmacological inhibitors of p38MAPK may have clinical potential. In this context, it is worth underscoring that inhibition of p38MAPK can also relieve the RA resistance of NB4-LR2 and NB4-007/6 cell lines.

POSTER SESSION I
BIOLOGY AND CHARACTERIZATION STUDIES

P01

THE PROTECTIVE EFFECT OF GANGLIOSIDE ON HUMAN CORTICAL NEURON TREATED BY ARSENIC TRIOXIDE

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Background. In the present paper, we report on the results obtained about the tolerable difference to arsenic trioxide between leukemia cells and normal axoneuron. Looking for the specific protective agent of nerve cell might provide a safe and feasible warrant of As_2O_3 in central nervous system leukemia treatment, and might offer a method of arsenic poisoning relevant nervous lesion.

Objective. Study on the protective effect and the possible mechanisms of ganglioside on cortical neuron treated by arsenic trioxide.

Methods. Five groups of human cortical neuron incubated *in vitro* were enrolled in this trial, the control, the 5 μ-mol/L As_2O_3 group, and the 5 μ-mol/L As_2O_3+ganglioside 50,100,200 μg/L groups. Cytosolic calcium$[Ca^{2+}]^i$ of cortical neurons was labeled by fluorescent probe Fluo-3/AM, the changes of $[Ca^{2+}]^i$ were monitored by laser confocal microscopy in real time, the activation of protein kinase C on these changes were assayed by Phosphorus radioisotope assay, the percentage of apoptosis was analyzed by flow cytometry.

Results. The $[Ca^{2+}]^i$ of cortical neurons began to rising at the 3th min after 5 μ-mol/L As_2O_3 treatment, and in $As_2O_3^+$ ganglioside group, which was began to rising at the 9th min after incubated *in vitro*, and the $[Ca^{2+}]^i$ increasing degree was not remarkable than that in the As_2O_3 group. After incubated *in vitro* in 27 min, the $[Ca^{2+}]^i$ of cortical neurons in As_2O_3 group reached 301±27 nmol/ L, while, in As_2O_3 ganglioside group, the $[Ca^{2+}]^i$ was only 188±20 nmol/ L. The PKC activities of cortical neurons, after incubated *in vitro* for 24h, were 435.5±18.1 on cell membrane and 339.5±11.2 in cell plasma nmol/min protein in As_2O_3 group and 159±20.4 on cell membrane and 19±21.5 in cell plasma nmol/min protein in As_2O_3+gangliosied 200 μg/L group. The apoptosis percentages of cortical neurons were 78.5% in $As_2O_3$3 group, and 13.5% in As_2O_3+gangliosied 200 μg/L group.

Conclusions. Ganglioside inhibited the increase of $[Ca^{2+}]^i$, the PKC activity, and the apoptosis of cortical neurons treated by arsenic trioxide concentration-dependently, and the target preventive points of ganglioside was the cell membrane of cortical neuron which the As_2O_3 interfered initially.

P02

STUDY ON THE TOLERANT DIFFERENCE BETWEEN HUMAN CORTICAL NEURONS AND ACUTE PROMYELOCYTIC LEUKEMIA LEUKEMIC CELLS TO ARSENIC TRIOXIDE

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Background. Arsenic trioxide (As_2O_3) is a very effective therapeutic agent for acute promyelocytic leukemia (APL), especially in cases resistant to conventional chemotherapy. It is also effective in other leukemia subtypes, malignant

lymphomas and solid carcinomas. However, generally when administrated by intravenous infusion, the blood-brain-barrier (BBB) inhibits its diffusion into the central nervous system (CNS) which limits its use in the prevention and treatment of central nervous system leukemia (CNSL). We have investigated a non-traumatic method that facilitates its entry into the CNS when it is given in routine clinical dosage, however, hitherto the safety range of As_2O_3 concentrations in the CNS, which are effective for killing leukemic cells but harmless to human cortical neurons, is not known. It is recognized that protein tyrosine kinase (PTK), protein tyrosine phosphatase (PTPs), protein kinase C (PKC) and cytoplasmic calcium ($[Ca^{2+}]^i$) are closely related both to apoptosis of cortical neurons and leukemic cells. In this study, we have made a preliminary observation about the roles of PTK, PTPs and PKC in apoptosis and observed the changes of $[Ca^{2+}]^i$ in human cortical neurons and leukemic cells treated with As_2O_3 in different concentrations *in vitro*.

Methods. Human leukemic cells and cortical neurons were treated with Fluo-3/AM(a Ca^{2+} probe); changes of $[Ca^{2+}]^i$ was measured by laser confocal microscopy in real-time. After the addition of As_2O_3 in different concentrations, the changes in PTK and PTPs function and the activation of PKC were detected by confocal microscopy and phosphorus radioisotope assay, and the assay for DNA ladders was done in leukemic cells and cortical neurons.

Results. As_2O_3 at 1 μ-mol/L increased the $[Ca^{2+}]^i$ of NB4 cells remarkably but showed no effect on neurons. Vanadate, a PTPs inhibitor, dose-dependently promoted the increase of $[Ca^{2+}]^i$ caused by 2,5,10 μ-mol/L As_2O_3. The mean increase rates in 280 seconds after exposure to different concentrations of As_2O_3 were: 6.5±2.3% , 21.7±2.1%, 49.9±2.5% in NB4 cells, and 6.7±2.1%19.4±2.5%, 52.3±2.7% in cortical neurons. Genistein, a PTK inhibitor, dose-dependently decreased the rise of $[Ca^{2+}]^i$ caused by 2,5,10 μ-mol/L As_2O_3. The mean inhibition rates in 280 seconds after addition of different concentrations of As_2O_3 were 6.7±2.9%, 25.6±2.5%, 52.2±3.5% in NB4 cells, and 7.8±3.1%, 8.1±2.8%, 51.3±3.3% in cortical neurons. PKC was activated when exposed to 1 μ-mol/L As_2O_3 for 3 hours and its activity kept increasing in NB4 cells; DNA ladders appeared after exposure to 1 μ-mol/L As_2O_3 for 24 hours; however at this concentration, no such appearance was found in human cortical neurons. At 2 μ-mol/L As_2O_3, activation of PKC and DNA ladders appeared in cortical neurons.

Conclusions. The phosphorylation and dephosphorylation of PTK and PTPs participated in non-specific apoptosis of both leukemic cells and cortical neurons treated with As_2O_3, and the apoptotic process was accompanied with PKC activation. The $[Ca^{2+}]^i$ elevation was closely associated with increasing PKC activation. The tolerances of leukemic cells and cortical neurons to As_2O_3 were different. At 1 μ-mol/L, As_2O_3 could effectively kill leukaemic cells but was harmless to human cortical neurons. If the level of As_2O_3 that entered the central nervous system could be kept in the range of 0.5 μ-mol/L, As_2O_3 might practically be used to treat and prevent central nervous system leukemia.

P03

EFFECTS OF ADMINISTRATION STYLES OF ARSENIC TRIOXIDE ON THE INTRACELLULAR ARSENIC CONCENTRATION AND THE EFFICIENCIES OF DIFFERENTIATION AND APOPTOSIS

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Objective. Study on the experimental evidences of constantly slow intravenous As_2O_3 infusion regimen on relieving leukocytosis of acute promyelocytic leukemia (APL).

Design and Methods. Leukemia cells were incubated in two kinds of As_2O_3 media respectively for 24 hours *in vitro*, the 2 μmol/L constant As_2O_3 concentration culture media and the varying As_2O_3 concentration culture media. Patients were enrolled into two groups randomly, in trial group, received continuously slow intravenous As_2O_3 infusion regimen, in control group, received routine regimen, the dosage and diluted criterion of As_2O_3 in the two groups were the same. The intracellular arsenic concentrations, the apoptosis rates, and the expression ratios of differentiation phenotype, $CD33^-/CD11b^+$,on cell surface were assayed by atomic fluorescence and flow cytometer.

Results. The intracellular arsenic concentrations in constant arsenic concentration culture medium and the trial group were higher than that in changing culture medium and control group, but the expression rates of $CD33^-/CD11b^+$, the differentiation phenotype, were inversed. The apoptosis rates were higher in trial group than that in control.

Conclusions. Compared with routine As_2O_3 regimen, the continuously slow intravenous As_2O_3 infusion increased intracellular arsenic concentration, improved the efficiency of apoptosis and relieved differentiation, which might be the mechanisms on relieving leukocytosis and obtaining maximal therapeutic benefit.

P04

SYNTHETIC RETINOIDS MODULATE BOTH PROCOAGULANT ACTIVITY AND CELLULAR DIFFERENTIATION OF BLAST CELLS FRESHLY ISOLATED FROM ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS

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All-trans- retinoic acid (ATRA) induces complete remission in up to 90% of APL patients with a rapid resolution of the coagulopathy typical of this disease. Previous studies indicated that the expression of the two main tumor cellular procoagulants, i.e. tissue factor (TF) and cancer procoagulant (CP), is downregulated in bone marrow blasts from APL patients given ATRA therapy. Furthermore, in the APL cell line NB4, the synthetic retinoids selective for the retinoic acid receptors (RAR) α, β and γ, differently downregulated the APL cell procoagulant activity. Aim of this study was to evaluate whether the same retinoids may affect the cellular procoagulants of blast cells freshly isolated from APL patients, and whether this modulation is associated with cellular differentiation by retinoids. The following retinoids were used: ATRA (a pan-RAR agonist), Am580 (selective RAR-α agonist), CD2019 (selective RAR-β agonist) and CD437 (selective RAR-γ agonist). Promyelocytic blasts, isolated from bone marrow specimens of 8 consecutive patients diagnosed with APL, were treated for 24h with increasing

concentrations of each retinoid (0.01 to 1 μmol/L). TF and CP expression were then characterized and quantified in cell sample preparations by chromogenic and immunological assay. The effect on differentiation of blasts into neutrophils was evaluated by fluorometric analysis of the CD11b surface-antigen expression. The results show that ATRA treatment significantly reduced the expression of both TF (44±18% reduction; $p<0.005$) and CP (31±15% reduction; $p<0.05$) activities. These results were confirmed by the antigenic assays. Cell differentiation analysis showed that ATRA significantly increased CD11b expression (control vs ATRA-treated cells: 12.5±3.5% vs 28.5±4.3% positive cells; $p<0.01$). Experiments with the three synthetic retinoids indicated that the RAR-α agonist significantly reduced both TF (27±17%; $p<0.001$) and CP expression (24±20%; $p<0.001$). This modulation occurred simultaneously with cell differentiation (CD11b: control cells vs Am580: 12.5 ± 3.5% vs 39±3.9% positive cells; $p<0.01$). The RAR-β agonist was ineffective in modulating both the procoagulant activities and cyto-differentiation. Finally, the RAR-γ agonist did not affect TF, but significantly reduced CP expression (20±7%; $p<0.001$). This effect was not associated to cell differentiation. In summary, our data indicate that in freshly isolated APL cells, ATRA down-regulates TF and CP expression, as previously observed *in vitro* in the APL NB4 cell line and *in vivo*, in APL patients during ATRA therapy. Similarly to the NB4 cells, the modulation of the two procoagulants appears to be mainly mediated by RAR-α and occurs together with signs of cellular differentiation. An additional role for RAR-γ in CP modulation is suggested in fresh APL cells. This study on freshly isolated blast cells is of potential clinical interest, as these compounds might offer a model for testing *in vitro* the cell sensitivity to retinoids more selective than ATRA (with less side effects) for the control of cellular procoagulant activities.

P05

UP-REGULATION OF C-MYC IS RELATED TO TRANSIENT GROWTH OF NB4 CELL AFTER EXPOSURE TO ATRA

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Myc/Max/Mad often play pivotal role in the proliferation, apoptosis, differentiation and cell cycle progress of leukemia cells. Usually, C-myc expression is implicated in cell growth and proliferation. In contrast, expression of Mad mRNA and Mad protein appears to be induced by various differentiation inducing agents in different cell lines. Therefore, Myc and Mad represent the rate limiting components in the Myc/Max/Mad network. As to the PML-RARα positive leukemia cell line NB4 cells, it could be induced by all-trans retinoic acid(ATRA) and other differentiation inducers. But there is a special transient up-regulation of NB4 growth after exposure to ATRA *in vitro*, and the mechanism on it has not been elucidated clearly. In the present study, we detected the hypothesis that transient up-regulation of c-Myc expression was contributed to the transient growth of NB4 cells by way of activation hTert and CAD on its target gene. To detect this hypothesis, NB4 cells was exposed to ATRA at different time points, the results showed that c-Myc was up-regulated with activation of hTert and CAD on c-Myc target gene firstly, and then down-regulated. Our results support the hypothesis that c-Myc expression and activation of hTert and CAD on c-Myc target gene play critical role in transient growth of NB4 cells after exposure to ATRA.

P06

DUAL EFFECT OF ARSENIC TRIOXIDE ON HEMOPOIESIS: INHIBITION OF ERYTHROPOIESIS AND STIMULATION OF MEGAKARYOCYTOPOIESIS

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Although the arsenic compounds are now widely utilized in clinics in the treatment of various tumors, their effects on normal hematopoiesis do not have been explored. In the present study we provide evidence that arsenic trioxide (As_2O_3) exerts *in vitro* a potent inhibitory effect on normal erythropoiesis and a stimulatory action on megakaryocytic differentiation. The inhibitory effect of As_2O_3 on erythropoiesis is related to: a) the inhibition of Stat5 activation with consequent reduced expression of the target genes Bcl-XL and Glycophorin-A; b) the activation of an apoptotic mechanism that leads to the cleavage of the erythroid transcription factors Tal-1 and GATA-1, whose integrity is required for erythroid cell survival and differentiation; c) the reduced expression of heat shock protein 70, required for GATA-1 integrity. The stimulatory effect of As_2O_3 on normal megakaryocytopoiesis is seemingly related to an upmodulation of GATA-2 expression and to stimulation of MAPK activity.

P07

STAT3 BUT NOT STAT1 IS IMPLICATED IN THE EFFECT OF G-CSF ON PROLIFERATION OF PRIMARY ACUTE PROMYELOCYTIC LEUKEMIA CELLS INDUCED BY ATRA

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Objective. To detect the signal pathway of G-CSF on proliferation of primary APL cells after treatment with ATRA.
Methods. Directly cell count was taken to detect the proliferation of fresh APL cells. Serum G-CSF and G-CSF in the supernatant of cultured leukemia cell was estimated by ELISA method, and its mRNA expression was measured by RT-PCR. Western blot was used to measure level of STAT1α or STAT3.
Results. The results indicated that APL cells showed a significant up-regulation of average serum G-CSF and G-CSF secretion as compared with that of APL cells without exposure to all trans retinoic acid. The proliferation ratio of APL cells was statistically correlated to the number of peripheral white blood cells. The cases with G-CSF secretion were further demonstrated by their mRNA and protein expression. As the important factor of signaling pathway, the level of STAT1α protein was up-regulated as long as being exposed to ATRA *in vitro* with or without G-CSF secretion. But STAT3 was up-regulated only in cases with G-CSF secretion.
Conclusions. G-CSF secretion plays an important possible role in the proliferation of APL cells after exposure to ATRA by way of up-regulating STAT3 expression.
Key words. G-CSF , STAT1α, STAT3.

P08

SWITCH FROM MYC/MAX TO MAD1/MAX ON TARGET GENE RELATES TO DIFFERENTIATION OF HL-60 CELLS

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Objective. To investigate the role of Myc family and its target gene in proliferation and differentiation of HL-60 cells.

Methods. All-trans retinoic acid(ATRA) and low concentration of arsenic trioxide (As_2O_3)were used to induce model of terminal differentiation and partial differentiation of HL60 cells. WST1 assay and NBT reduction assay were used for the detection of cell proliferation and differentiation, flow cytometry for detection of cell cycle. Western blot assay and RT-PCR were used to measure the protein and mRNA expression of c-Myc and Mad1, ChIP assay was taken to detect Myc/Max/Mad1 on their target gene.

Results. Proliferation of these cells of terminal differentiation induced by ATRA was inhibited, and cell cycle arrested in G0/G1 phase. Meanwhile, the expression of c-Myc protein and c-Myc mRNA obviously decreased, accompanied with a switch from c-Myc to Mad1 on target gene hTERT. Whereas HL60 cells with partial differentiation induced by low concentration of As_2O_3 still expressed high level of c-Myc , and c-Myc still combined with the promoters of hTERT gene, without obvious variation of cell proliferation and cell cycle, with a little Mad1 mRNA expression.

Conclusions. c-Myc expression and targeting on hTERT was contributed to blocking HL60 from partial differentiation to terminal differentiation.

P09

A NOVEL HISTONE DEACETYLASE INHIBITOR BML-210: IN VITRO ACTIVITIES AGAINST HUMAN LEUKEMIA CELL LINES

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Effects of a new class of chemotherapeutic agents, a histone deacetylase inhibitor BML-210 alone and in combination with retinoic acid (RA) have been examined on growth, differentiation and apoptosis of the human leukemia cell lines (NB4, HL60, THP-1 and K562). BML-210 alone (5-30 microM) markedly inhibits the proliferation of all cell lines and induces apoptosis in a dose-dependent manner. Treatment of the cells with BML-210 caused cell cycle arrest at the G1 phase. BML-210 alone induces HL-60 and K562 cell differentiation to granulocytes and erythrocytes, respectively (up to 30%), and markedly accelerates and enhances both HL-60 and NB4 cell granulocytic and K562 cell erythroid differentiation mediated by differentiation agents - retinoic acid and hemin, respectively. BML-210 alone or in combination with RA caused induction of histone acetylation after 2 h of treatment and affects the transcription factors binding activity to the promoters of genes regulating cell cycle (p21) or apoptosis (FasL) and influences expression of Sp1, NF-kappaB, p21 and FasL. These findings let us to suggest that BML-210 may be a promising antileukemic agent to induce apoptosis and modulate differentiation through the modulation of histone acetylation and gene expression.

P10

OXYGEN RADICALS MODULATE GRANULOCYTIC HL-60 CELL DIFFERENTIATION AND APOPTOSIS IN A STAGE SPECIFIC AND DIFFERENTIATION INDUCER-DEPENDENT MANNER VIA INVOLVEMENT OF PKC AND NF-KAPPAB

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The causal relationship between intracellular reactive oxygen intermediates (ROI) production and retinoid acid (RA) or dbcAMP-mediated granulocytic differentiation and apoptosis of human acute promyelocytic leukemia HL60 cells have been studied. The modulation of intracellular redox status by D, L-buthionine-(S, R) sulfoximide (BSO) and N-acetyl-L-cysteine (NAC) exerts a time-, inducer-dependent and stage-specific effects on HL-60 cell differentiation and subsequent apoptosis. Treatment with BSO during commitment stage enhances dbcAMP-, but suppresses RA-mediated differentiation, while NAC inhibits both. Prolonged treatment with BSO alone or in combination with dbcAMP only results in a dose-dependent increase in apoptosis and prevention it by NAC. The modulation of ROI level or PKC activity by calphostin C before induction and during commitment stage of differentiation causes inducer-dependent changes in cell differentiation or apoptosis via involvement of NF-kappaB activity. These observations suggest that intracellular ROI may play a role in regulating of HL-60 cell differentiation and apoptosis and provide a link between oxidative signalling and intracellular signalling network via the involvement of PKC and NF-kappaB.

P11

TH1 AND TH2 CYTOKINE PRODUCTION BY T-CELL IN 6 ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS: STUDY OF THE ACUTE MYELOID LEUKEMIA PATIENTS POPULATION

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$CD4^+$ T-helper cells are an integral part of the effective immune response against various malignancies; however in tumor-bearing patients they are frequently functionally unresponsive. Several sets of data indicate on the important role of T-helper 1 subset in the control of tumor growth. In order to assess the influence of T-cell-response polarization on the course of the disease intracellular cytokines (IFN-γ, IL-4) were investigated in T-cells in AML patients.

Methods. Th1 and Th2 cytokine production was detected in $CD3^+CD8^-$ (considered as CD4) and $CD3^+CD8^+$ lymphocytes from 15 AML patients (among of them were 6 APL patients, 3 had molecular relapse during treatment) by the flow cytometry analysis. Lymphocytes were isolated from the whole blood by Ficoll-Hypaque density centrifugation, activated with PMA (phorbol2-myristate-13-acetate, 50ng/ml) and calcium ionophore (250 ng/mL) for 12 h in the presence of Golgi Plug (brefeldin A) and stained for surface CD3, CD8 antigens and intracellular IFN γ, IL-4. Samples from AML patients were collected at diagnosis (n=10), duration of remission (n=14, samples=30), and at relapse (n=4, samples=6). Nine healthy donors constituted the control group. Statistical data were computing by program Statistics for Windows 5.5.

Results. There were $CD3^+CD8^+$IFN-γ 37,6±12,2%, $CD3^+CD8^-$IFN-γ 14±3%, $CD3^+CD8^+$IL-4 5,4±4,4%,

$CD3^+CD8^-$IL-45,4±1,8% in 9 healthy donors and there were 37,9±22%, 31,9±18,5%, 5,1±3,6%, 3,5±1,7% in 10 AML (6 APL) patients at diagnosis respectively. There were 42,7±16,3%, 27,5±9,3%, 5,9±4,5%, 6,4±3,4% in 14 AML (6 APL at molecular remission) patients at remission respectively and there were 38,1±18,7%, 18,8±5,1%, 11,9±6%, 13,6±2,2% in 4 AML (3 APL prior to molecular relapse) at relapse respectively.

The percentages of IFN-γ producing $CD3^+CD8^+$ cells did not differ much in AML patients and in donors. The percentages of IFN-γ-producing $CD3^+CD8^-$ T cells in AML patients were similar at diagnosis and in remission and exceeded such counts in healthy donors (p<0,02, p<0,0001). The amount of IFN-γ-producing $CD3^+CD8^-$cells decreased at relapse. Simultaneously prior to relapse and at relapse the increase of IL-4 producing cells, both $CD3^+CD8^+$ and $CD3^+CD8^-$, was observed (p=0,05, p<0,0001). Additionally, it was registered, that IL-4 producing $CD3^+CD8^-$ cells were increased prior to molecular relapse (PML-RAR-α^+) in APL patients.

Conclusions. The activation of pro-inflammatory cytokine response (Th1) was detected in AML patients at diagnosis and during remission. Increase of Th2 cytokine IL-4 was registered before AML relapse and molecular APL relapse. The results provide the evidence of an altered cytokine secretion by T-cell subsets in AML patients at different time points of acute leukemia treatment.

P12

TELOMERE LENGTH IN PATIENT WITH ACUTE PROMYELOCYTIC LEUKEMIA REFLECTS RESPONSE TO TREATMENT WITH ARSENIC

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Introduction. The telomeric DNA together with its associated proteins protects the chromosome ends from degradation or aberrant recombination. The length of telomere in cancer cells depends on a balance between the telomere shortening at each cell cycle and the telomere elongation resulting from telomerase activity. In leukemias and in some solid tumors, a correlation between decreasing telomere length and an increasing severity of disease has been described. Telomere reduction was previously demonstrated in acute and chronic leukemia. Acute promyelocytic leukemia (APL) characterized by a specific chromosomal translocation t(15;17) that form a PML-RARÎ± fusion gene. Arsenic trioxide (As_2O_3) is able to induce complete remission in t(15;17)-positive APLs. Arsenic trioxide treatment promotes telomere shortening and apoptosis.

Methods. 300 peripheral blood samples were taken from 30 APL patients before, during and after therapy with Arsenic Trioxide. Leukemic blasts were isolated by ficoll-gradient, and then genomic DNA extracted by salting out protocol from those samples, and NB4 cells. Genomic DNA was digested with Rsa1 and Hinf1 restriction enzymes; electrophoresis was performed in 0.8% agarose gels. Finally telomere length was determined by southern analysis.

Results. We studied telomeric DNA in APL leukemic cells from patients as well as NB4 cell line as a human APL model. Marked differences were observed in the sizes of the telomeric repeats in the normal blood cells and APL leukemic cells. The leukemic cells of 30 patients with APL showed a variable reduction in the length of telomeric DNA, ranging from 2.0 to 7.0 kb, while the telomere length in PB mononuclear cells obtained from the same patients during complete remission was 9.0 to 10 kb.

Conclusions. Arsenic therapy leads to telomere shortening, growth arrest, and leukemic cell death (by apoptosis). Longer telomeres were found in APL patients after induction by arseninc treatment compared with those found in diagnostic specimens. Most likely this was due to the loss of the leukemic clone (with shorter telomeres) and the emergence of normal hematopoietic cells (with longer telomeres) after induction therapy. These data indicate that telomere length shortening in APL patient treated with arsenic can be used as a marker to monitor disease condition and response to therapy.

P13

ASSESSMENT OF ARSENIC TRIOXIDE (AS_2O_3) EFFECT ON TELOMERASE ACTIVITY IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS AND NB4 CELL LINE

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Introduction. Telomerase the patching up enzyme that extends shortened telomeric repeats of chromosomal ends is differentially expressed in cancer cells and in normal cells. Telomerase is activated in more than 85% of malignant tumors; therefore it might be as an important target for cancer therapy strategies. Telomerase inactivation is considered as a key reason of apoptotic and anticancer effects of As_2O_3 in Acute promyelocytic leukemia (APL) patients. To investigate anti telomerase effect of As_2O_3, we evaluated APL patients and NB4 cell line as a model of APL pre- and post-As_2O_3 treatment.

Methods. Arsenic was used with dosage of 0.15 mg/kg/day as a routine treatment for APL patients at shariati hospital. Sequential peripheral blood samples were collected pre- and post- As_2O_3 treatment from 25 patients. The human NB4 cell line was cultured in presence of different concentration of As_2O_3 M). Telomerase activity was assayed using 0, 0.25, 0.5, 0.75, 1.0 and 2.0 TRAP-ELISA procedure and gel electrophoresis in both APL patient and NB4 samples.

Results and Conclusions. Establishment of quantitative assays for telomerase activity in APL patients had shown more than 80% elevated levels of telomerase activity in comparison with normal individuals and decreased after remission to normal level. Telomerase activity was shown down regulating in NB4 cell line after 24-48h as a result of As_2O_3 therapy. Decreasing in telomerase activity in time was intensively correlated with increasing in As_2O_3treatment doses in NB4 model. These data indicate that telomerase can be a potent target for cancer therapy in APL treatment with As_2O_3 and to monitoring disease condition.

P14

FISH ANALYSIS SHOWING THAT GENOMIC DELETIONS ARE NOT ASSOCIATED WITH T(15;17) IN ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL) is characterized by the reciprocal translocation t(15;17)(q22;q21), disrupting the

PML and RARα genes, which are localized on chromosomes 15q22 and 17q21, respectively. The t(15;17) generates two chimeric genes: PML-RARα arises on der(15), whereas the reciprocal RARα/PML fusion is located on the der(17). Microdeletions on the derivative chromosome carrying the reciprocal fusion gene have been recently reported in some myeloid leukemia translocations. Microdeletions in APL cases have been investigated by Kolomietz *et al.,* and by Bacher *et al.,* who utilized the Vysis LSI PML-RARα translocation probe in Fluorescence In Situ Hybridization (FISH) studies. In the former study, FISH experiments were performed with dual color translocation probe that is not able to detect microdeletions on der(17) chromosome. The latter investigation was performed by using dual color, dual fusion translocation probe but, although this probe is teorically able to reveal genomic sequences loss, the analysis was conducted on interphase nuclei; this kind of approach cannot discriminate between insertions and deletion events. Therefore, in these two studies the analysis on microdeletion in APL has not correctly carried out. We used appropriate FISH probes, specifically designed to detect deletion in der(17). Forty-seven APL patients were tested by conventional cytogenetic and RT-PCR at diagnosis. Cohybridization FISH experiments were performed by using a mixture of two probes (BAC RP11-247C2 and PAC RP5-1112G21, from de Jong libraries), including the PML gene on chromosome 15 and RARα gene on chromosome 17, respectively. Their precise position was derived from the University of Santa Cruz database (*http://genome.ucsc.edu*). On metaphases of APL patients these probes generated two clear fusion signals on der(15) and on der(17) chromosomes in addition to single signals on normal 15 and 17 chromosomes. Twenty metaphases were evaluated for each patient. The analysis did not reveal any microdeletion, since an evident fusion signal on der(17) was observed in all analyzed metaphases.

In our series there was 1 (2.1%) case with cryptic PML-RARα fusion gene created by insertion event, in agreement with the frequency of this abnormality in APL cases reported in literature. FISH experiment showed an abnormal hybridization pattern: RP5-1112G21 gave the classical splitting while RP11-247C2 showed signals of equal intensity on normal chromosome 15 and on der(15). No RP11-247C2 signal was detected on der(17). The present study suggests that t(15;17) is not accompained by deletion on der(17). On the other hand, it may be conceivable that the 3% of APL cases with microdeletion reported by Bacher *et al.,* really could represent insertion events. In conclusion, to date there are not experimental evidence of microdeletions in APL. However, very small deletions (few kb), escaping FISH detection, cannot be excluded.

P15

UNUSUAL CLINICAL AND MORPHOLOGICAL FEATURES OF ACUTE PROMYELOCYTIC LEUKEMIA WITH A ZINC FINGER GENE REARRANGEMENT

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Approximately 98 percent of patients with acute promyelocytic leukemia (APL) have a balanced translocation, t(15;17) (q22;q21), involving the *retinoic acid receptor*-α (RARα) gene on chromosome 17 and the PML gene on chromosome 15. A variant translocation t (11;17) (q23;q21) has been described involving the RARα gene and the promyelocytic leukemia zinc finger (PLZF) gene. Only about 12 of these cases have been previously reported and it has been recognized that they have different morphological features than seen in classical APL. In particular these cells have a more regular nucleus and a lack of Auer rods. Some can be hypogranulated but the majority are hypergranulated. A classification system has been suggested which can differentiate PLZF-RARα and PML-RARα. We describe a 73-year-old male with this rare variant translocation who did not concur with this classification system and had atypical morphological features not described previously. No Auer rods or Faggot cells were present and the majority of cells were hypogranular, not immediately recognizable as promyelocytes. They appeared to have a late maturation block. Haemophagocytosis was also a feature. Immunophenotyping showed the cells to be promyelocytes (CD33, CD9, and CD13 positive. HLA-DR$^{+/-}$, and CD34 and CD56 negative). Reverse transcription PCR for the PML-RARα fusion product was, however, negative. Cytogenetic analysis, performed on 24 hour cell cultures, showed a t(11; 17) (q23; 21) in 18 of 19 mitoses examined. Fluorescence in situ hibridization, using a break apart rearrangement probe, confirmed a RARα gene rearrangement in about 75% of the cells. The presentation in this patient was also unusual with episodes of recurrent bone pain for about one year before his eventual demise. There was no evidence of DIC initially and the clinical course suggested a smoldering leukemic process, which has not previously been described in APL.

P16

AUTOFLUORESENCE - A USEFUL DIAGNOSTIC FEATURE OF THE IMMUNOPHENOTYPE OF ACUTE PROMYELOCYTIC LEUKAEMIA

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Background. The diagnosis of acute promyelocytic leukaemia (APML) is currently made on a combination of morphology, immunophenotyping and an immunofluoresence-based assay for the cellular pattern of PML protein expression. Autofluoresence is the term used to describe the light emitted naturally by an unstained, illuminated cell. While autofluorescence in APML is recognised as a phenomenon by those working in flow cytometry, little attention has been paid to its potential diagnostic utility.

Aims. To quantitate the degree of autofluoresence in 25 consecutive cases of APML and to compare the results with 25 cases of non-APML AML.

Methods. Twenty-five consecutive cases of cytogenetically confirmed APML presenting to a single centre from 1993 to 2004 were included in this study. Twenty-five consecutive cases of non-APML AML from 2003 to 2004 served as controls. Immunophenotyping was performed at diagnosis on erythrocyte-lysed whole bone marrow (BM) samples stained with monoclonal antibodies directly conjugated with fluorochromes. For this study, fluorescein isothiocyanate (FITC) - and phycoerythrein (PE) -conjugated isotype controls were used to allow for comparison of the degree of autofluoresence. For staining, 100 microlitres of RPMI/heparin-diluted BM samples, containing approximately 2×10^6 nucleated cells, were placed in each tube and incubated with the appropriate combination of monoclonal antibodies as per the manufacturers instructions. Data acquisition was performed on a FACScan, or later a FAC-

SCalibur, flow cytometer (Becton Dickinson). Instrument calibration and fluorescence compensation was performed weekly using CALIBRATE beads (Becton Dickinson). The CELLQUEST PRO software (Becton Dickinson) was used for data analysis.

Results. In all 25 APML cases, the FITC-conjugated and PE-conjugated isotypic controls were shifted to the second log decade due to autofluoresence. The fluorescent intensity of normal negative controls was in the first log decade. To quantitate the degree of autofluoresence, comparison was made between the relative fluorescent intensity for both the FITC-conjugated and PE-conjugated isotype controls for the 25 APML cases and 25 consecutive cases of non-APML AML (Figures 1 and 2). For both fluorochromes, autofluoresence was higher in APML cases than in any of the non-APML AML controls.

Summary. The presence of autofluoresence in leukaemic blasts appears to be strongly suggestive of APML. This can be distinguished from the non-specific Fc binding of monocytic leukaemia by observation of equivalent binding of all control antibodies and an identical fluorescence pattern in both unstained cells and controls. It may therefore represent a helpful adjunctive diagnostic marker in APML.

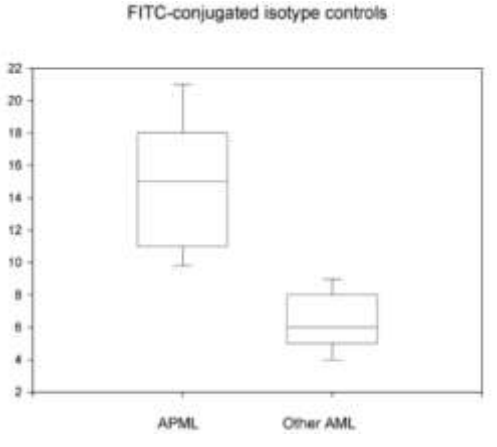

Figure 1.

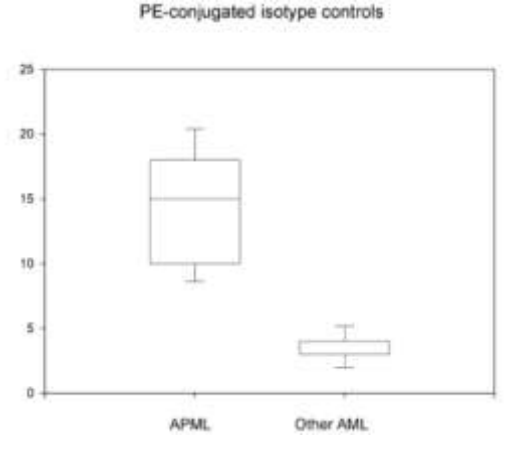

Figure 2.

P17

FOUR ACUTE PROMYELOCYTIC LEUKEMIAS PRESENTING WITH ATYPICAL DIAGNOSTIC PATTERN

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Acute promyelocytic leukaemia (APL) is a disease characterized by the presence of the t (15;17) translocation leading to the formation of PML-RARα fusion gene; its presence is the marker for the response of the disease to retinoid therapy. Almost all cases with t (15;17) showed a specific morphological and cytofluorimetrical pattern that permitted the recognition of 2 distinct entities: the hypergranular APL (M3) and the hypogranular or microgranular variant form (M3v). Approximately 10% of APL lacks the t (15;17) and is associated with morphologic variant and different response to retinoid. We report 4 cases of acute leukaemia observed in our Haematological Department from July 2003 to June 2005 that showed disagreed features between morphology and cytometry. Two patients presented atypical promyelocytes in absence of characteristic phenotype; the other 2 showed immunophenotype consisting with the diagnosis of APL, but morphologic appearance of blast cells other than that of atypical promyelocytes. Only two patients demonstrated RARα rearrangement. During the same period 7 cases of hypergranular APL t (15;17) positive were diagnosed. Three females and 1 male (mean age 53 years, 39-66) were referred to our hospital because of fatigue and skin haemorrhage; one patient presented ECG and laboratory signs of myocardial ischemia. All patients were anaemic (mean haemoglobin value 8,7 g/dL, 8, 1-9, 3) and thrombocytopenic (mean platelet count 44×10^9/L, 12-83). Haemostatic parameters were slightly abnormal: hypofibrinogenemia (190 mg/dL, 160-210) and increased D-dimer level (3837 ng/dL, 2507-6142) were present. All but one patients showed hyperleukocytosis (mean WBC count $62,7\times10^9$/L, 36,7-99); the latter patient had normal WBC count ($4,9\times10^9$/L). A mean percentage of 86% (67-96) of blast cells positive to myeloperoxydase reaction was present. The nucleus was bilobated as in M3v in 2 cases and more regular in the other. Auer rods were found in all cases (no faggot cells were recognized). The bone marrow smears demonstrated hypercellularity in all cases with a mean of 94% (88-99) of blast cells with a strong positivity to myeloperoxydase reaction; Auer rods were found in 3 out of 4 patients (in 2 cases faggot cells were recognized, included one case with only M3v blasts in PB).

RT-PCR and FISH demonstrated PML-RARα in 1 case and RARα rearrangement in another. Final diagnosis was transitional M2/M3 acute myeloid leukaemia.

The 2 patients presenting RARα rearrangement were treated with ATRA at standard dose and chemotherapy (low-dose Aracytin) or immunotherapy (antiCD33) but, despite no dramatic changes in haemostatic parameters and adequate transfusional support, they died because of CNS haemorrhage, respectively, 5 and 9 days after diagnosis. One patient did not respond to 2 lines of induction chemotherapy and died 9 months after diagnosis. The latter patient, suffering from myocardial ischemia and treated with antiCD33, was too early to be evaluated (diagnosis May 2005).

Our experience suggests that M2/M3 variant form of AML is loaded by a poor prognosis also in cases showing RARα rearrangement. More deepened molecular studies are warranted in this small group of patients in order to identify the mechanism involved in ATRA resistance.

P18

NOT PUBLISHED

P19

ANALYSIS OF δN-P73 EXPRESSION IN ACUTE PROMYELOCYTIC LEUKEMIA

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The p53 paralog, p73, is a nuclear protein whose ectopic expression, in $p53^{+/+}$ and $p53^{-/-}$ cells, recapitulates the most well-characterized p53 effects such as growth arrest, apoptosis and differentiation. p73 gives rise to multiple functionally distinct protein isoforms attributable to alternative promoter utilization generating NH2-terminally deleted dominant-negative proteins (δN-p73) and alternative mRNA splicing of the COOH-terminal exons (α, β, γ, δ, ε). It is likely that the fine tuning of p73 contributes specifically to regulate cell growth, cell death, differentiation and development. Unlike p53, p73 is rarely mutated in human cancer and many studies have explored alternative mechanisms for its inactivation. Hypermethylation is the predominant mechanism of p73 inactivation in lymphoid leukemogenesis while other hematological malignancies (AML, non-Hodgkin lymphoma and CLL) have not been found to be hypermethylated. The absence of p73 methylation in *de novo* AMLs and the low rate of p53 mutations suggest the existence of alternative mechanisms of p73 inactivation in the pathogenesis of AMLs. We have recently shown that the lack of δN-p73 is a frequent feature of acute promyelocytic leukemia (APL) (Rizzo *et al*,. Leukemia, 2004). Here we report the analysis of δN-p73 expression in 33 APL patient samples at diagnosis and after conventional treatments. In agreement with our previous findings we detected lack of δN-p73 expression in APL patient samples at diagnosis. Of note, detectable expression of δN-p73, comparable to that observed in normal promyelocytes, was found in the APL patient samples analysed after conventional treatments. To investigate the molecular mechanisms underlying the modulation of δN-p73 expression before and after conventional treatments used for APL (i.e. retinoic acid plus chemotherapy combination), we are currently analyzing the effects of PML-RARα on the expression of δN-p73. Our ongoing experiments show that PML-RARα significantly down-regulates the transcriptional activity of the P2-p73 promoter (δN-p73). Of note, retinoic acid up-regulates the P2-p73 promoter only in the presence of PML-RARα. Our results suggest that the peculiar modulation of δN-p73 in APL might be involved in APL leukemogenesis and further supports the notion that acute promyelocytic leukaemia is a biologically different subset of AMLs.

P20

DIAGNOSTIC CHARACTERIZATION OF ACUTE PROMYELOCYTIC LEUKEMIA BLASTS AND PROMYELOCYTES BY FLOW CYTOMETRY IMMUNOPHENOTYPING

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Introduction-Aim. Prompt diagnosis of Acute Promyelocytic Leukemia (APL) is essential for institution of appropriate treatment. Whereas the hypergranular form of APL (M3) can be easily recognized by morphology, the microgranular variant (M3v) may be confused with other subtypes of AML, in particular monocytic leukemias. Several studies have shown that immunophenotyping by Flow Cytometry (FC) can be helpful in the diagnosis of APL. Other studies have suggested differences in immunophenotype between M3 and M3v in addition to distinct FC distribution patterns (*hypergranular* for M3 and *hypogranular* for M3v). The aim of this study was to assess the value of FC in the diagnosis of APL.

Patients-Methods. Our study included 31 consecutive adult patients with APL. The diagnosis of APL was based on standard morphological criteria and cytochemical stains, and was confirmed by detection of the PML-RARα gene rearrangement by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The APL leukemic cell population (blasts and promyelocytes) was analyzed by FC. Forward and right angle scatter (FW-SC/RT-SC) patterns were obtained. Staining with monoclonal antibodies against surface cell antigens (HLA-DR, CD45, CD34, CD33, CD13, CD11b, CD15, CD11c, CD14, CD1a, CD2, CD3, CD4, CD7, CD8, CD56, CD19, CD10, CD20, CD22, CD61, Glycophorin-A) and nuclear / cytoplasmic cell antigens (cCD3, cCD79a, MPO, Tdt) was performed to determine the phenotype of APL leukemic cells.

Results. Twenty-three out of the 31 patients were classified as M3 and the remaining eight as M3v according to morphology. The mentioned above, *hypergranular* and *hypogranular* FW-SC / RT-SC distribution patterns of APL leukemic cells were recognized. The FW-SC / RT-SC data correlated with morphology in 20 out of 23 patients with M3, and 7 out of 8 patients with M3v. In all 31 cases, the leukemic cells were positive for CD33, CD13 and MPO. No one case with expression of HLA-DR was encountered. CD34 was positive in 3 of the 8 M3v cases, and in 3 of the 23 M3 cases. CD2 positivity was detected in 3 of the 8 M3v cases and in 2 of the 23 M3 cases.

Conclusions. Our data suggest that APL can be reliably distinguished from other AML subtypes on the basis of FC distribution pattern in combination with expression of certain antigenic markers (CD33, CD13, MPO). However, M3v cannot be clearly differentiated from M3 by FW-SC / RT-SC analysis and immunophenotyping data.

P21

ACUTE PROMYELOCYTIC LEUKEMIA: TWO CASES WITH CRYPTIC ASPECTS IN MORPHOLOGICAL DIAGNOSIS

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The APL represents 10-15% of adult Acute Myeloid Leukemia (AML), in the past ten years this disease focused the attention on the presence of hybrid PML-RARα rearrangement in the blast cells, leading a new therapeutical approach based on the use of Retinoic Acid (ATRA). Two main morphological subtypes of APL are recognised: hypergranular or classic and hypogranular or variant subtypes. Recently a few of atypical APL cases, characterized by hyperbasophilic microgranular blasts with cytoplasmic budding (mimicks micromegakaryocytes), has been described (A. Aventin,1998). In these cryptic forms citogenetics and molecular assays are required to confirm the APL diagnosis. We report two cases (two females, 54 and 50 years old respectively) of atypical morphology of APL, admitted to haematology dipartment on February 2005. At light microscopy both bone marrow and peripheral blood smears, hyperbasophilic microgranular blast cells with cytoplasmic budding, mimicking mycromegakaryocytes were predominant while hypergranular blast cells were less than 10%. As immunophenotype the atypical cells were HLA-DR$^+$, CD34$^+$, CD13$^+$,CD33$^+$, CD2$^+$, CD9$^+$. Cytogenetics and molecular assays showed, in both cases, the presence of t(15,17)(q22;q21) and PML/ RARα rearrangement (bcr3), confirming the APL diagnosis. The patients were enrolled in APL 0903x-101128 GIMEMA protocol for the induction treatment (risk assessment: INTERMEDIATE). Complete Remission (CR) was achieved in both cases on day 45, after induction therapy. To date both patients are in CR, one of them achieved molecular remission after 1st consolidation therapy. These two cases prove heterogeneity APL blasts morphology, thus cytogenetics and molecular biology play a relevant role for a correct diagnosis. Furthermore the morphological evaluation of the Complete Remission as well as relapse should be made in order to detect this cryptic blast cells to avoid a possible misdiagnosis.

P22

UNCERTAIN PROGNOSTIC SINIFICANCE OF DEL 18 IN ACUTE PROMYELOCITIC LEUKEMIA

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Acute promyelocitic leukaemia (APL) is a biological and clinically sub-type of acute nonlynfocytic characterised by translocation t(15-17) with molecular expression of PML RAR-α. Recently myelodisplastic syndrome (MDS) has been reported after treatment for APL. Its rare that MDS precedes APL. We describe a patient with APL and del 18p. Within the last 6 months before the APL diagnosis the patient received α interferon plus ribavirin because of HCV infection. At the diagnosis he presented leukopenia and thrombocytopenia; marrow blasts immunological characterization showed: CD9 63%; CD117 55%; HLA-DR 15%; CD45 90%; CYMPO 85%. The t(15-17) was detectable by FISH in about 50% of blasts. Deletion of 18p was present in 30% while the somatic cells didn't show any deletion . The patient started chemotherapy according to protocol AIDA 2000 GIMEMA, intermediate risk, and obtained complete remission (CR) 35 days after induction therapy. After consolidation therapy t(15-17), PML-RAR-α and del 18 were negative. During the maintenance therapy with ATRA PLM RAR-α remained negative while the clone with del18 increased up to into the 60% of cells while there wasn't a evidence of APL relaps into the bone marrow. The del 18 is an unusual finding and it is not previously described in haematological disorder including MDS. On the controversy in linfoproliferative disorders HCV associated there is chromosome 18 impairment as only t(14,18). In these cases antiviral therapy may induce regression of t(14,18) bearing B-cell clones. However in mouse models ribavirin is mutagenic in bone marrow cells. We cannot explain the role of association of APL with del18p since the patient remains in CR without MDS evolution or linfoproliferative disorder.

P23

QUANTITATIVE ANALYSIS OF WT1 GENE FOR MINIMAL RESIDUAL DISEASE DETECTION IN LEUKEMIC PATIENTS

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Introduction. WT1 gene encodes a transcription factor which is involved in differentiation and proliferation of Hemtopoeitic precursor cells as well as some other tissues like kidney, ovary, heart etc. It is also expressed in 80% of Acute Leukemia cases (AML, ALL) and in plastic crisis of CML as determined by various qualitative and quantitative RT-PCR methods. It is proposed to be a useful marker in minimal residual disease (MRD) detection and leukemia management.

Methods. To assess the relevance of this gene, sequential peripheral blood samples from 60 APL who have been treated with arsenic trioxide (As_2O_3) were analyzed for the expression level of WT1 mRNA, using Real-Time Quantitative RT-PCR. Samples from patients were obtained at the time of diagnosis, during treatment (follow-up), in complete remission (CR), before relapse and relapse.

Results. Samples of diagnosis and relapse showed significantly higher WT1 expression levels (90%), compared to samples from patients in CR or healthy volunteers. Our study revealed that rising of WT1 expression predicts a forthcoming relapse 1-6 months before overt clinical relapse. A linear correlation between quantities of WT1 and PML-RARα fusion transcripts could be seen in APL patients treated with arsenic trioxide.

Conclusions. There was a strong correlation between WT1 and specific fusion gene expression in APL patients, showing the significant potential of WT1 as a non-specific leukemia marker (NSLM) for monitoring of MRD and treatment approaches in APL.

Keywords. WT1, MRD, Real-time, Arsenic trioxide.

P24

DIAGNOSTIC CHARACTERIZATION OF ACUTE PROMYELOCYTIC LEUKEMIA

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This type of leukemia is seen in 10% to 15% of adult AML, and is characterized either by abnormal promyelocytes with distinctive large granuales and multiple Auer rods (faggots or sultan bodies cells) or, less commonly, by atypical hypogranular or microgranular cells with bilobed or multilobed nuclei.These cells contain procoagulant material which, when released into the circulation, causes disseminated intravascular coagulation (DIC). Exessive bleeding due to DIC is common. The microgranular variant of APL may be mistake with Acute monocytic leukemia (AMOL). In the microgranular variant M3, only occansional cells have granules visible by light microscopy, but despite this there is strong cytoplasmic positivity with Sudan black B (SBB), myeloperoxidase and chloro-acetate esterase (CAE). The translocation t(15;17) (q22;q21) or t(15;17) (q22,q11-12) is the genetic hallmark of APL, resulting in the PML-RARα fusion protein. PML-RARα Recuits histon deacetylases (HDAC) and represses target genes of wildtype RARα leading to a block of myeloid differentation. Typical immunophenotipic findings in APL: CD34. HLA-DR and Tdt usually is negative and CD2 is expression in a minority, $CD13^{+}$ $CD33^{+++}$.

P25

GENETIC CHARACTERIZATION AND FOLLOW-UP OF MRD IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS FROM ARGENTINA AND URUGUAY

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Background. Acute promyelocytic leukemia (APL) is characterized by the t(15;17) translocation and promyelocytic leukemia *retinoic acid receptor* (PML-RARα) transcript. Three molecular isoforms of PML-RARα transcript can be produced depending on the break-fusion site in PML gene, linked to a common RARα, exon 3 segment: short (S)-form type (PML exon 3), long (L) form type (PML exon 6) and variable (V) form type (variably deleted PML exon 6). Accurate and rapid diagnosis of these hallmarks is essential for specific therapy choice, prognostic assessment and MRD monitoring in APL. Recently, alterations in FLT3 gene (Internal tandem duplications-ITD and D835 mutations) are detected by PCR in 30% of APL patients (pts) and would be associated with aggressive disease. This study reports the molecular characterization of 65 APL pts diagnosed as having APL (AML-M3) according FAB classification from Argentina and Uruguay, mostly of Spanish-Italian origin, studied between 1996 to 2005.

Design and Methods. Forty nine pts were adults (median age 42 yrs, range 16-80; 26 were males and 23 females) and 16 children (median age: 8 yrs, range. 3-15 yrs, 10 were males and 6 females) .With the exception of 2 patients who suffered secondary APL (after leukemia and breast cancer treatment) all cases had *de novo* APL. Sixty one from 65 pts were studied by immunophenotyping cytogenetics and nested RT-PCR at presentation, in the remaining 4 cases there were not material available for genetic studies, diagnosis was established by cytomorphology and clinical evaluation. The patients came from different institutions which used different therapeutic strategies but all included ATRA. Follow-up was performed in 45 pts by molecular techniques for an average of 42 months (range: 3 to 108 months).

Results and Interpretation. The PML-RARα and or t(15,17), were identified in 57 from 61 pts studied at time of presentation: 33 (62%) pts were L-isoform (three of them with AML1/ETO co-expression), 17(32%) S-isoform and 3 (6%) V-isoform. Three cases had no molecular data and the t(15,17) was established by conventional cytogenetics, and the remaining 1 detected by aberrant FISH pattern. Only three cases with succesful karyotype analysis lacked the t(15,17) but were PCR+. After diagnosis, 3 pts died early without treatment and 6 during induction. Minimal residual disease monitoring was performed in 45 PML-RARα^{+} pts by nested PCR. After induction 42 pts (93.3%) achieved CCR and 3 (6.7%) showed persistence of leukemic clons. One (2%) died during consolidation and 44 pts (98%) remained with molecular remission after completing consolidation. Actually, 36 pts (80%) are alive with no evidence of MRD. The remaining 8 cases died by hematologic relapse preceeded by PML-RARα postive test. Two of which coexpressed AML1-ETO fusion gene and another one was a secondary APL. FLT3 gene status was established in 42 APL pts at presentation. Twelve (28.6%) pts showed FLT3 mutations: 8 (19%) were ITD positive and 4 (9,5%) showed D835 mutation. Ten from 12 FLT3^{+} pts (83%) showed S-isoform and 2 pts (17%) were L-isoform.

Conclusions. Our findings in these cohort of patients show: 1) PML-RAR isoforms distribution is similar to APL European pts; 2) S-isoform was found mostly among children (44% vs 27% adults); 3) FLT3 mutations were associated with S-isoform (p<0,00032); 4) RT-PCR positive convertion after consolidation predicts relapse.These results remark the importance of the molecular characterization of APL pts at diagnosis: PML-RARα transcript isotype, FLT3 status and the prognostic value of RT-PCR. Further follow up with larger number of patients is required to fullly adress the association of FLT3 mutated status with poor clinical outcome This colaborative study encourages us to continue to work on APL characterization of molecularly based prognostic factors and their impact in the clinical management .

P26

PROGNOSTIC VALUE OF ADDITIONAL CHROMOSOME ABNORMALITIES IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS: AN UPDATE OF THE PETHEMA TRIALS

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Acute promyelocytic leukemia (APL) is genetically characterized by a reciprocal translocation between the long arms of chromosomes 15 and 17, the t(15;17)(q22;q21), which results in the fusion gene PML-RARα. Conventional karyotyping on banded methaphases shows that about one third of APL patients carry additional cytogenetics changes in leukemic cells. The aim of the present study was to compare the clinical and biological characteristics of APL patients with or without chromosomal changes in addition to the t(15;17) and their potential impact on outcome. Between

November 1996 and December 2004, a total of 700 *de novo* genetically confirmed APL patients were enrolled in two consecutive PETHEMA trials (APL96 and APL99). Cytogenetic analysis was available in 484 cases (69%) who disclosed the following features: 248 M/236 F; median age 41 yrs. (range, 2-83); median WBC count 2.1×10^9/L (range, 0.3-210); median platelet count 21×10^9/L (range, 1-183); 389 M3 typical/90 M3 variant; relapse risk group (Sanz *et al.,* Blood 2000): 90 (19%) low-risk, 278 (57%) intermediate-risk and 114 (24%) high-risk. Treatment consisted of all-trans-retinoic acid (ATRA) and anthracycline monochemotherapy for induction, followed by three consolidation courses of anthracycline monochemotherapy with or without ATRA, and maintenance therapy with intermittent ATRA and low dose chemotherapy (methotrexate and 6-mercaptopurine). Additional chromosome aberrations were observed in 116 cases (24%). The most frequent secondary changes were + 8 (n=44), either alone (n=34) or associated with other aberrations (n=10), followed by abn(7q) (n=6), abn(3q) (n=5), i(17) (n=4), abn(1p) (n=4), del(9q) (n=4), abn(20q) (n=4), abn(8q) (n=3), abn(11q) (n=3) and +21 (n=3). No clinical, biological, morphological, immunophenotypic or molecular differences were observed between the group of patients with t(15;17) alone and the group of patients with additional changes. Moreover, no statistical differences were found in terms of complete remission rate, 8-year disease-free and relapse-free survival (90% vs 91%, 87% vs 86% and 11% vs 14% for patients with t(15;17) alone and patients with additional changes, respectively). In conclusion, patients with APL and additional cytogenetic abnormalities do not show distinct features as compared with patients with t(15;17) alone. Moreover, additional chromosomal changes have no influence in outcome of APL patients receiving state-of-the-art therapeutic protocols.

P27

MICROHETEROGENEITY IN ACUTE PROMYELOCYTIC LEUKEMIA MOLECULAR SIGNATURE

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Background. Several gene profiling studies using microarray technology have revealed a specific expression signature for acute promyelocytic leukemia (APL).

Aims. To identify the possible association between specific molecular signatures and relevant biological and clinical features in APL including risk group, white blood cell (WBC) count at presentation, M3 or M3v subtype and the presence of flt3 mutations (flt3-ITD and/or flt3-D835). *Patients and Methods.* We analyzed gene expression profile of 53 newly diagnosed APL patients enrolled in PETHEMA trials [27M/26F; median age: 41 yr. (range: 9-80); median WBC count $\times10^9$/L:2.67 (range: 0.5-128); median platelet count $\times10^9$/L:19 (range: 4.4-135); FAB subtype: 39 M3/14 M3v] using a cDNA microarray (OncoChip®) designed for analyzing genes involved in cancer. Supervised analyses were performed by paired t-test and ANOVA test with 100,000-fold permutations. Significant genes were selected based on the lowest false discovery rates (FDR). GeneCluster and TreeView programs were used for clustering analysis, and class prediction was performed with Support Vector Machines (SVM).

Results. Supervised analysis comparing the gene expression profiles of patients stratified by WBC count at presentation, presence of flt3 mutations, FAB subtype and risk group revealed annexin-II ligand (S100A10) expression as a common significant factor for all variables analyzed. In fact, patients with a high expression for this gene had > 10×10^9/L WBC count (59% vs. 4%; $p<0.001$), higher frequency of ITD- or D835-flt3 mutations (72% vs. 14%; $p<0.001$) and M3v subtype (52% vs. 0%; $p<0.001$). Flt3 mutations and FAB subtype cause specific expression patterns with an accuracy of 85% and 89%, respectively.

Conclusions. Our results suggest: (a) a specific gene expression profile in APL could be linked to flt3 mutations and specific morphologic subtypes; and (b) S100A10 high expression level might be associated with adverse clinical and biological features.

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P28

A TRANSLOCATION T(17;20)(Q21;Q12) MASKING A CRYPTIC T(15;17)(Q22;Q21) IN A PATIENT WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL) is genetically characterized by a gene fusion transcript involving the gene for *retinoic acid receptor*α (RAR-α). The most frequent translocation, t(15;17)(q22;q21), involves the promyelocytic leukemia (PML) gene, leading to the fused gene PML-RARα. Nevertheless, a few of these patients have variant translocations, either as simple or complex variant translocations involving chromosomes 15, 17 and one or several other variable chromosomes. We report a case of a 31-year-old woman with APL (M3 classical form) carrying an apparently balanced translocation t(17;20)(q21;q12) masking a cryptic t(15;17)(q22;q21). Peripheral blood examination showed hemoglobin 9.0 g/dL, white blood cell (WBC) count 2.2×10^9/L (ANC 0.88×10^9/L) and platelets 60×10^9/L. The blood film showed 12% of leukemic promyelocytes. Chromosomal analysis by conventional cytogenetics of the bone marrow showed 46, XX, t(17;20)(q21;q11.2) [16]/46,XX [4], with apparently normal chromosome 15. However, fluorescence in situ hibridization (FISH) analysis with the use of a PML-RARα dual-color probe (Vysis, Illinois, USA) showed a molecular insertion of 3'-RAR-α into PML at 15q22 to create a PML-RARα fusion gene. Molecular analysis by real-time QT-RT-PCR confirmed PML-RARα chimeric gene, detecting a bcr1 transcript. FISH analysis including painting probes of chromosomes 15, 17 and 20 (Cytocell Technologies, Cambridge, UK), revealed a balanced reciprocal translocation t(17;20)(q21;q12) as well as the presence of material from chomosome 17 inserted on chromosome 15. No evidences were found of material exchange between chromosomes 15 and 20. The patient was treated with anthracyclines and ATRA (PETHEMA LPA-99 protocol) and achieved complete remission (CR). Five years later, she remains in continuous CR. This case report illustrates the usefulness of combined cytogenetics, FISH and molecular biology to evidence the PML-RARα fusion gene in cases with APL without a classical

t(15;17).

This work was supported in part from a research grant from Generalitat Valenciana (Grupos 03/225).

P29

PRETREATMENT CHARACTERISTICS AND CLINICAL OUTCOME OF ACUTE PROMYELOCYTIC LEUKAEMIA PATIENTS ACCORDING TO THE PML-RARα ISOFORMS: UPDATED ANALYSIS OF THE PETHEMA TRIALS

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Three major isoforms of the specific PML-RARα fusion transcript have been described in APL according to the breakpoint within the PML gene: intron 6, bcr-1 (L, long form); exon 6, bcr-2 (V, variable form) and intron 3, bcr-3 (S, short form). The association between the PML-RARα isoforms and clinical outcome is still a controversial matter. The aim of the present study was to analyse the clinical and biological characteristics of APL patients and the potential impact on outcome according to the PML-RARα isoforms. Between November 1996 and December 2004, a total of 700 *de novo* genetically confirmed APL patients were enrolled in two consecutive PETHEMA trials (APL96 and APL99). Treatment consisted of all-trans retinoic acid (ATRA) and anthracycline monochemotherapy for induction, followed by three consolidation courses of anthracycline monochemotherapy with or without ATRA, and maintenance therapy with intermittent ATRA and low dose chemotherapy (methotrexate and 6-mercaptopurine). PML-RARα isoform was available in 544 cases (78%) who disclosed the following features: 289 M/255 F; median age 40 yrs. (range, 2-83); median WBC count 2.1×10^9/L (range, 0.3-460); median platelet count 21×10^9/L (range, 1-207); 435 M3 typical/106 M3 variant; relapse risk group according to Sanz score: 104 (19%) low-risk, 303 (56%) intermediate-risk and 137 (25%) high-risk. Two-hundred eighty-two patients had L-form (52%), 20 V-form (4%) and 242 S-form (44%). The S-form subgroup presented a significantly higher proportion of patients with WBC count > 10×10^9/L than patients with either L- or V-forms (35% vs 17% and 20%, respectively; $p<0.0001$), M3v features (25% vs 16% and 5%; $p=0.006$), CD34 expression (33% vs 7% and 7%; $p<0.0001$; n=374) and high-risk group (35% vs 17% and 20%; $p<0.0001$). No differences were found in additional cytogenetic aberrations between the three isoforms. There was a trend for a lower CR rate in patients with the V-form (75% vs 92% vs 89% for V-, L- and S-forms, respectively; $p=0.09$). Patients with the V-form also showed a lower 7-year disease-free survival (73%±11% vs 87%±2% vs 82%±3%; $p=0.02$) and a higher relapse-risk (27%±11% vs 11%±2% vs 17%±3%; $p=0.02$). In conclusion, despite S-form is associated with several negative prognostic features at presentation, this has no major impact on outcome when APL patients receive state-of-the-art treatments. By contrast, rare V-form type is related with a higher risk of relapse.

P30

LOW EXPRESSION OF THE DEOXYCYTIDINE KINASE GENE IN ACUTE PROMYELOCYTIC LEUKEMIA: A QUANTITATIVE REAL-TIME PCR ANALYSIS

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Background. The role of Ara-C in the treatment of acute promyelocytic leukemia (APL) is currently a matter of controversy. The cytotoxic activity of ara-C in leukemic blasts depends on activating enzymes, specifically deoxicitidine kinase (dCK) that is the key enzyme necessary to metabolize ara-C to its pharmacologically active form, ara-CTP (ara-C triphosphate) and inactivating enzymes such as the 5'-nucleotidases. Some authors have reported alterations in the expression of the dCK gene as one of the mechanisms responsible for clinical resistance to ara-C (Schirmer *et al.,* 1998, Veuger *et al,* 2001). A gene expression profiling analysis, carried out by our group, revealed a low level of expression of the dCK gene in 53 newly diagnosed patients with APL.

Aims. To elucidate the molecular and clinical role of dCK gene in APL, we analyzed dCK mRNA expression by real-time polymerase chain reaction (PCR) in 71 patients diagnosed of APL [33M/38F; median age: 42 yr (range: 3-90)]. In addition, known to have particular good response to treatment with high-dose ara-C, we analyzed 15 patients with AML with either t(8;21) (n=8) or inv(16) (n=7), [7M/8F; median age: 18 yr (range: 3-73)], as well as 67 patients diagnosed of AML without favourable karyotype [37M/30F; median age: 63 years (range: 28-94)] and $CD34^+$ selected cells from 16 healthy donors. Expression analysis was performed using ABI PRISM 7300 Sequence Detection Instrument and software (Applied Biosystems). Specific oligonucleotides and TaqMan probes for each selected gene were purchased from Applied Biosystems (Assay-on-Demand Gene Expression Products). All experiments were normalized to the housekeeping gene GAPDH as control for mRNA recovery and retrotranscriptase PCR efficiency.

Results. Preliminary results showed that dCK gene appeared significatively underexpressed in APL samples respect to all groups: AML with either t(8;21) or inv(16) ($p=0.006$), AML without favourable karyotype ($p<0.001$) and $CD34^+$ cell samples ($p<0.001$).

Conclusions. Our results show that dCK gene is underexpressed in APL, suggesting that it may contribute to the role that ara-C plays in the treatment of APL.

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P31

IMMUNOPHENOTYPIC CHARACTERIZATION OF ACUTE PROMYELOCITIC LEUKAEMIA: CORRELATION WITH BIOLOGICAL, CLINICAL AND PROGNOSTIC PARAMETERS

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Acute promyelocytic leukaemia (APL) is a rare subtype of acute myeloid leukaemia with distinct clinical, immunological, cytogenetic and molecular features. In the present study we analyzed the immunophenotypic characteristics of 45 consecutive APL patients out of 459 adult acute myeloid leukaemia (AML) patients, in order to define immunological diagnostic and prognostic parameters. APL diagnosis was based on morphological criteria and confirmed by the presence of the typical translocation t(15;17)(q22;q11) by cytogenetic analysis and of the PML-RARα fusion transcript by RT-PCR. Flow cytometric immunophenotyping was performed testing a panel of 26 directly conjugated monoclonal antibodies on bone marrow or peripheral blood specimens. All APL patients showed a mature myeloid immunophenotype, characterized by the presence of HLA-DR$^-$, CD13$^+$ and/or CD33$^+$ blast cells. CD2, CD11c, CD19 and CD56 were expressed in 20% (9/45), 6.7% (3/45), 8.9% (4/45), 13.3% (6/45) respectively. CD34 was positive in 2 CD2$^+$ APL patients. The most statistically significant markers associated with APL diagnosis in comparison to other AML subtypes were: CD2 (two tailed Fisher exact test: p=0.003), CD4^{dim} (p<.001), CD11b (p<0.0001), CD11c (p<0.0001), CD14 (p<0.001), CD34 (p<0.001), and TdT (p=0.03). Multivariate discriminant analysis was applied to define a 5-parameter discriminant function, based on CD11c, CD13, CD34, MPO, HLA-DR expression, that was able to correctly classify APL patients with a sensitivity >99% and a specificity of 94.7%. FLT3-ITD was detected by RT-PCR in 7 of 27 APL patients and was associated with aberrant immunophenotypic expressions, in particular with CD56: 5/7 FLT3-ITD$^+$ APL were CD56$^+$, 2/7 CD2$^+$, 1/7 CD19$^+$ and 1/7 CD22$^+$. CD2 were expressed more often in M3v than M3 and in bcr3 PML-RARα$^+$ APL; in our series no association was found between CD2 expression and clinical risk categories, disease-free survival (DFS) and overall survival (OS), even if a trend toward shorter remission duration and OS was present in CD2$^+$ APL. CD56 expression was correlated with presence of FLT3-ITD, lower DFS (p=0.002), extra medullar relapse (2 cases) and lower OS (p=0.02). We conclude that flow cytometric antigenic profile is associated with clinical and biological heterogeneity of APL and can have a prognostic value.

POSTER SESSION II
CLINICAL STUDIES

P32

SINGLE CENTER EXPERIENCE WITH THE AIDA PROTOCOL IN NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA OF ADULTS

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APL represents a particular subtype of Acute Myeloid Leukemia with specific biological and clinical features, among which are the characteristic chromosomal translocation t(15;17) detected in leukemic blasts and the frequent presence of clinical or laboratory disseminated intravascular coagulation. Anthracycline-based chemotherapy and All-Trans Retinoid Acid (ATRA) remain the cornerstone of APL treatment, even though their combination is associated with an appreciable mortality during the induction phase, as a result of bleeding complications and/or severe respiratory distress. Herein, we analysed retrospectively the outcome of 16 consecutive adult patients with APL diagnosed and treated in our Unit from December 1998 to October 2004. The analysis focuses more specifically on the parameters of treatment-related mortality, cause of death and disease-free survival post AIDA chemotherapy. All patients were suffering from the classical form of APL and were homogeneously treated as follows: induction phase consisted of ATRA orally and intravenous idarubicin (IDA) at conventional doses of 45 mg/m^2/d, D1 to complete remission and 12 mg/m^2/d, D2,4,6,8 (total of 4 infusions), respectively. Dose modifications for elderly individuals were not allowed. Those achieving complete remission were consolidated with 3 courses of chemotherapy without ATRA, where as non-remitters were taken off protocol and received other therapy. Patients in continuing hematological and molecular remission at the end of consolidation were finally administered maintenance therapy for 2 years with oral 6-mercaptopurine at 90 mg/m^2/d, oral methotrexate weekly at 15 mg/m^2 and ATRA for 15 days every 3 months. In all cases, the morphological diagnosis of APL was confirmed by chromosome and immunophenotypic analysis of blasts as well as molecular studies.

The median age of our patient cohort was 55 years (range 31-78) and the male/female ratio was 12/4. Three patients (3/16, 19%) were greater or equal to 65 years at APL diagnosis. Two cases (2/16, 12%) presented with a leukocyte count of greater or equal to 10×10^3/mm^3 while the median WBC at presentation was 6.5×10

P35

THE FIRST EXPERIENCE OF ACUTE PROMYELOCYTIC LEUKEMIA TREATMENT WITH ATRA (ALL TRANS RETINOIC ACID)

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Incidence data of acute myeloid leukemia (AML)in Ukraine is up to 800 new cases per annum.Among them there is low rate of M3 subtype of AML-acute promyelocytic leukemia (APL), approximately 22-24 cases per year.Before ATRA was integrated in treatment regimens the effectiveness of APL management was very poor. Since 1999 3 patients suffering from APL have been treated with ATRA (Vesanoid) in combination with chemotherapy in our institution. The diagnosis of APL was based on morphological criteria and immunophenotype. Cytogenetic analyses were performed using the standard technique of G banding. Fluorescent in situ hybridization (FISH) was used to confirm the diagnosis. All patients started to receive induction therapy with ATRA (45 mg/m^2) from day 1 of treatment and from day 5 chemotherapy course of idarubicin+cytarabine 3+7 (12 mg/m^2,100 mg/m^2 q12h) was added. Unfortunately one patient died from lung bleeding after 2 weeks of treatment. Two patients received complete remission (CR) after induction therapy, which was confirmed with FISH. Consolidation treatment included two courses of intermediate-doses Ara-C with etoposide,cyclophosphamide and vincristin. During this period ATRA was given continuously. In the first case the CR has been durating for 6 years already and in a second case the period of CR is 6 months and the patient continues to receive treatment regimens.

Conclusions. Use of ATRA during the period of induction therapy resulted in better achievement of CR in APL patients.It was proved that the most effective treatment with ATRA could be achieved by the long-term use,until CR is achieved,which can be confirmed by cytogenetic and molecular genetic investigation.

P36

TREATMENT OF NEW CASES OF ACUTE PROMYELOCYTIC LEUKEMIA WITH ARSENIC TRIOXIDE

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Introduction. Arsenic Trioxide is effective and it is approved for treatment of relapsed or refractory APL cases who are resistant to ATRA; but its effect on new cases of APL is not clear and it needs long term follow up to disclose the role of this drug in treatment of APL in combination with chemotherapy/ATRA or alone.

Materials and Methods. We studied 111 patients with APL (94 new cases and 17 relapsed cases) diagnosed by morphologic criteria and confirmed by cytogenetic analysis and/or RT-PCR for presence of PML-RARα fusion gene.

Arsenic Trioxide was infused at 0.15 mg/kg daily doses, until complete remission diagnosed by morphologic criteria or after 60 days. In patients with complete remission, after 28 days of rest, 0.15 mg/kg Arsenic Trioxide was infused daily for an additional 28 days as consolidation therapy. After complete remission, we studied minimal residual disease (MRD) by semi-sensitive RT- PCR on peripheral blood samples for one year,

Results. Complete remission observed in 95 patients (85.6%). The median time passed for achieving complete remission was 30 days. There was no significant difference in remission rate between new patients and the relapsed ones. During the induction phase, the most common cause of toxicity and mortality was APL differentiation syndrome (23 cases, 20.7%). Other adverse side effects were serosistis (7.2%) and hepatotoxicity (19.8%). With the median time of follow up of 16.5(1-57) months for patients in complete remission, one and two year disease free survival (DFS) was 88.3% and 63.7%respectively. 24 patients relapsed; 19 of them achieved second complete remission, again by Arsenic Trioxide. Median time of relapse was 17 months (4-33) and median time of second DFS after re-treatment with Arsenic Trioxide was 18 months. Third and fourth remissions were seen in some relapsed patients, again by Arsenic Trioxide. For patients in complete remission, one and three year survival was 95.5% and 87.6% respectively. Minimal residual disease was positive in 4 (8.3%) out of 48 cases during one year after remission induction; 3 of them relapsed clinically.

Conclusions. Arsenic Trioxide is effective as first line treatment for APL. Results of Arsenic Trioxide combination therapy with chemotherapy/ATRA needs further study. Also it seems that Arsenic Trioxide is applicable for relapsed patients and drug resistance is an unusual event.

Keywords. Arsenic Trioxide, APL.

P37

QUANTITATIVE ANALYSIS OF PML-RARα ON NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS TREATED WITH ARSENIC TRIOXSIDE AS FRONT-LINE THERAPY

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Introduction. The finding that arsenic trioxide (As_2O_3) induces apoptosis in APL cells, including APL cell that are resistant to RA, has generated a wave of excitement in both the laboratory and the clinic. Following these promising results specially in relapsed patients, the role of this agent is being presently investigated as front-line therapy for newly diagnosed patients.

Methods. From 111 patients with APL, 95 patients who achieved CR were sequentially evaluated during 4-60 months period of follow-up by conventional RT-PCR. A total of 30 patients (6 relapsed and 24 in continued long remission) were selected and monitored by quantitative real-time PCR (RQ-PCR) assay. Using *Hybridization Probes* technology, the expression of PML-RARα/G6PDH transcript ratio was analyzed in serial PB samples taken at different courses of disease and the results were compared with the clinical outcome.

Results. More than 90% of patients obtained molecular remission, as determined by conventional RT-PCR in 2-3 months after start of arsenic therapy. RQ-PCR confirmed these data, showing a rapid kinetic of reduction in PML-RARα/G6PDH ratio by more than one log after induction and more than 3-4 logs after consolidation therapy in more than 80% of patients. Serially monitoring of MRD showed that in all patients in stable remission the level of PML-RARα± ratio was always below the threshold in PB samples. In all relapsed cases with follow-up intervals of 1-6 months (median 3 months) a clinical relapse was predictable by

increasing NQ levels above the threshold.

Conclusions. Qualitative RT-PCR method had a limited value in predicting relapse early in majority of APL patients in CR. On the other hand, using a sensitive and quantitative method provided valuable information about effectiveness of arsenic as a front-line therapy in the management of newly diagnosed APL; and it also helps to predict treatment response and to identify individual patients that are associated with relapse. We were able to establish a threshold of transcript level in the post-consolidation period, above this level suggest that patients are in high risk of relapse, below that shows patients are in CR.

P38

ALL-*TRANS* RETINOIC ACID AND GENTUZUMAB OZOGAMICIN IN ELDERLY UNTREATED ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS NOT ELIGIBLE FOR CHEMOTHERAPY: A CASE REPORT

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All-trans retinoic acid (ATRA) combined to anthracycline-based chemotherapy are currently the reference treatment of patients with Acute Promyelocytic Leukemias (APL). Furthermore ATRA induces remissions in up to 90% with newly diagnosed APL and produces long-term remission in 60-80% of patients when given together with consolidation chemotherapy. Gentuzumab ozogamicin (GO) has shown efficacy in combination with ATRA as up-front therapy in patients with median age of 50 years and newly diagnosed acute promyelocytic leukaemia. This combination result in a remission rate of 85% and a DFS better than ATRA alone, with an acceptable toxicity profile. GO has been successfully used alone in relapsed or refractory reported cases, confirming its efficacy in APL. Here we report a treatment with Gentuzumab Ozogamicin (GO) following induction with ATRA alone in a patient 68 years old not eligible for the use of standard dose of anthracycline because of severe cardiac failure. In March 2004 a 68 years old man was referred to our institution because of severe leucopoenia (WBC: 0,65/mmc, Hb: 8,8 gr/dL, Plt: 189000/mmc) during the last month. He was chronically anticoagulated with warfarin because of mechanical cardiac valve from 1999 and his Left Ventricular Efflux Fraction (L-VEF) was clearly impaired (35%). Bone marrow examination revealed promyelocyte blasts infiltration. Cytogenetics showed the presence of translocation 15;17 confirmed by molecular examination for PML-RARα fusion gene transcript of the bcr-3 type. Diagnosis of acute promyelocytic leukaemia low risk according with the GIMEMA risk assesment was done. Protocol with retinoic acid alone at the dose of 45 mg/m^2 for 80 days was given as induction therapy. Complete hematological, cytogenetic and molecular remission was achieved and confirmed by bone marrow aspiration 60 days later. At day + 170 the patient was treated with consolidation treatment based on two monthly doses of 6 mg/m^2 of GO. Consolidation therapy was very well tolerated and the patient, at 14 months from diagnosis remains in complete haematological and molecular remission.

Actually he receives a maintenance program with ATRA 45 mg/m^2 for 15 days every three months. This treatment is planned for two years.

With this therapeutic strategy we conclude that it may be possible in older patients to minimize or eliminate standard dose chemotherapy in untreated APL at low or intermediate risk by combining ATRA+GO.

P39

LATE RELAPSE (>5 YEARS FROM DIAGNOSIS) IN ACUTE PROMYELOCYTIC LEUKEMIA (ACUTE PROMYELOCYTIC LEUKEMIA)

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Late relapses (> 5 years from dignosis) among APL patients have been seldom described: to evaluate their rate and clinical characteristics, 99 cases of APL newly diagnosed at our Institute from 1/88 to 12/97 were revised. Of them, 6 died during induction, 32 relapsed and 7 died in CR within 5 years from diagnosis. Thus, 53 patients were alive and in continuous 1st CR after 5 years from diagnosis: among these 53 long-lasting remitters, we observed 5 late APL relapses (9.4%) (2 males and 3 females, median age at relapse 27 years, 2 patients BCR1 and 3 BCR3, 2 patients intermediate and 3 high-risk score) after 60, 61, 71, 101 and 155 months from diagnosis. As induction treatment at diagnosis, 2 patients received Idarubicin alone and 3 AIDA protocol (ATRA+Idarubicin). An involvement of mastoid with headache and deafness occurred in 3/5 patients (60%), compared with 2/32 patients (6.3%) at early relapse (p<0.02).

The auricolar involvement was assessed by immunostaining (PG-M3) and molecular analyses in both cases in which biopsies were performed at relapse, whereas in the last case it was demontrated by CT scan. As to treatment of late relapse, 1 patient received ATRA alone followed by allogeneic transplantation and 4 patients GIMEMA 0191 protocol (ATRA for 30 days+Mitoxantrone and intermediate-dose Cytarabine for 6 days as induction and for 4 days as consolidation, followed by 2 years maintenance with pulse courses of ATRA): all patients achieved 2nd CR and are still alive: 4 of them are in 2nd molecular CR after 6, 33, 34 and 115 months respectively, while 1 relapsed after 15 months (at present in 3rd CR). In conclusion, a late relapse occurred in a sizeable portion of APL patients with long-lasting 1st CR: the high rate of ear involvement at relapse might be explained considering the ear as a *disease sanctuary*, where residual blast cells could eventually proliferate again after a long period. However, the prognosis of these patients remains favourable even without hematopoietic stem cell transplantation to consolidate the 2nd CR status.

P40

INCIDENCE OF SECONDARY MYELODYSPLASTIC SYNDROMES IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS IN LONG-LASTING MOLECULAR REMISSION: THE GIMEMA EXPERIENCE

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GIMEMA Acute Leukemia Working Party

The occurrence of a sMDS in patients potentially cured from a previous APL has been recently reported. To evaluate the real incidence and the clinical characteristics of sMDS post APL, the follow-up of 336 patients with newly diagnosed APL enrolled by 22 GIMEMA Centres in the LAP AIDA 0493 protocol has been revised. After the induction, 314 patients (93.4%) achieved complete remission (CR): of

them, 225 (71.6%) are still alive in 1st CR, 12 (3.8%) died in CR and 71 (22.7%) relapsed. The remaining 6 patients (1.9%) developed a sMDS while in 1st morphological CR, after a median time from CR achievement of 42 months (range 27±75). Among the 71 relapsed patients, 15 had no further data and 41/56 (73.2%) achieved a 2nd CR after salvage treatments: 2 of them (4.8%) developed a sMDS while in 2nd morphological CR, after 48 and 27 months from 1st CR, respectively. Furthermore, another patient showed a sMDS while in 3rd CR. As to clinical and biological characteristics of these 9 sMDS patients, there were 3 male and 6 female with a median age at sMDS diagnosis of 57 years (range 31-71): cytogenetic analysis failed in 2 patients, was normal in 1 patient and revealed abnormalities of chromosomes 5 or 7 in the remaining 6 patients, with a complex karyotype in 4 of them. One patient underwent allogeneic BMT soon after sMDS and is still alive after 57 months from BMT, 2 patients are in stable myelodysplastic phase after 25 and 63 months respectively. An evolution in Acute Myelogenous Leukemia (sAML) occurred in the remaining 6 patients, after a median sMDS duration of 7.6 months (range 2±45): 5 sAML patients received only supportive care±palliative chemotherapy, 1 patient underwent intensive chemotherapy but was resistant. All sAML patients died after a median time from AML evolution of 4 months (range 0.8±5). In conclusion, the incidence of sMDS post APL is very low in 1st CR, but seems to increase in patients achieving 2nd or subsequent CR: clinical characteristics and prognosis are similar to those observed in other sMDS. A longer follow-up will help us to define the late occurrence (> 5 years from CR) of this severe complication.

P41

SECONDARY ACUTE PROMYELOCYTIC LEUKEMIA THE EXPERIENCE OF A PORTUGUESE CENTER

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Therapy related acute myeloblastic leukemia (AML-t) and myelodysplastic syndrome are a category of the World Health Organization (WHO) classification of AML, and is usually linked to a worse prognosis. In that classification APL, with the t(15;17) or PML-RAR-α fusion gene, is a subcategory of AML with characteristic genetic abnormalities and has a favourable prognosis. About 5% of all APL cases are related to therapy (APL-t).1 Recent publications show that APL-t has a similar prognosis to *de novo* APL, when other prognostic factors, like age and performance status, are controlled.1 In this way AML-t prognosis appears to be greatly dependent on the genetic abnormalities present, and that the presence of relevant previous therapy may not be an independent prognostic factor.

To know the clinical characteristics of APL-t we reviewed the cases treated in our institution. The clinical files of the patients with APL-t, diagnosed between January 2002 and December 2004 were the source of information. Data related to initial malignancy and therapy, to APL characterization, to therapy response and survival were collected.

Tree of the 29 patients treated for APL where therapy related. Two were women; initial malignancy was breast ductal carcinoma in 2 cases and testicular germ cell tumour in 1 case; of the 2 breast cancer patients one received chemotherapy (vindesine, 5-FU, folinic acid and cyclophosphamide) and both received radiotherapy; the male patient received just chemotherapy (4 cycles of BEP- bleomycin, etoposide and cisplatin). The median time from diagnosis of the initial malignancy to diagnosis of APL was 38 months, range 24 to 54. Median age at diagnosis of APL was 38 years old, range 17 to 77.

Finally it can be said that APL-t, in spite of it secondary nature, can be treated successfully with modern intensive cytotoxic regimens.

P42

TREATMENT OF RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA BY ARSENIC-BASED STRATEGIES WITHOUT HEMATOPOIETIC STEM CELL TRANSPLANTATION IN HONG KONG: UPDATE AT EIGHT YEARS

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Background. For relapsed acute promyelocytic leukemia (APL) successfully treated by arsenic trioxide (As_2O_3), the optimal post-remission therapy reamins unclear. Hematopoietic stem cell transplantation (HSCT) is associated with high morbidity and mortality. Moreover, lasting remission is observed in many patients who are not candidates for HSCT, owing to advanced age or lack of donors, implying that HSCT is not mandatory for durable remission. We update our results of an As_2O_3 -based, non-HSCT regimen for patients with relapsed APL.

Materials and Methods. A total of 44 consecutive patients (20 men, 24 women, median age: 35 years, 12-72) with relapsed (relapse 1, R1=41, R2=3) APL were treated with an-As_2O_3 based, non-HSCT regimen. The time from last complete remission (CR) was 17 (6-243) months. Initial treatment was As_2O_3 (10 mg/day) either intravenously (n=16) or orally (n=28) until CR, followed by idarubicin consolidation (6 mg/m^2/day×9). Twenty-six patients received oral-As_2O_3 maintenance after CR. Post-As_2O_3 relapses were treated with oral As_2O_3 + all-trans retinoic acid (ATRA, 45 mg/m^2/day) until CR, followed by maintenance (two weeks of ATRA+As_2O_3 (AA) every 2 months for 2 years). Post-As_2O_3/ATRA relapses were treated with oral As_2O_3+ ATRA+ascorbic acid (1g/day, AAA) until CR, followed by consolidation/maintenance with the same regimen (2 weeks every 2 months for 2 years). Part of the induction and all of the maintenance therapies were given in the outpatient clinic.

Results. CR was achieved in 43/44 (98%) of patients after initial As_2O_3 treatment. Seventeen patients experience a further relapse, after a median of 17 (6-22) months after CR. There was an actuarial trend of reduced relapses in patients receiving As_2O_3-maintenance (p=0.086). Two relapsed patients died of cerebral leukemia before further treatment could be administered. Of the 15 relapsed patients treated with AA, 14 achieved CR again, 9 of whom have remained in remission (median follow-up: 41 months). Five patients with post-AA relapses were treated with AAA, with CR achieved in 4 cases. One patient developed a further post-AAA relapse and died. Two other patients died of unrelated causes while in remission after AAA therapy.

Conclusions. Our strategy resulted in an overall leukemia-free-survival of 78%. The results suggest that an oral and mainly outpatient As_2O_3-based, non-HSCT regimen is efficacious for relapsed APL. In terms of survival, costs, treatment side effects and patient tolerance, this strategy is comparable to, if not more favorable than, other treatment options based on high dose chemotherapy, graft-versus-leukemia effect, or monoclonal antibody. The minimal chemotherapy used in this regimen may also have theoretical advantages in reducing long-term sequelae in this potentially curable leukemia.

P43

ATRA ± ARACYTIN AND MITOXANTRONE IN THE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS IN 1ST RELAPSE: A SINGLE INSTITUTION EXPERIENCE ON 36 CASES

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From January 1991 to December 2003 we treated 36 APL patients in 1st relapse: 25 patients (Group A) received ATRA alone (45 mg/sqm) for 30 days as re-induction therapy, followed by a consolidation course of Ara-C (1 g/sqm×4 days, c.i. 6 h) + Mitoxantrone (6 mg/sqm×4 days); 11 patients (Group B) received as re-induction therapy ATRA (45 mg/sqm) from day 1 to 15 and Ara-C (1 g/sqm, c. i. 6 h) + Mitoxantrone (6 mg/sqm) from day 1 to 6, followed by the same consolidation course as Group A. We analysed the features at presentation as well as toxicity and results of both groups. In the Group A (12 males and 13 females, median age 35 years, 22 classic M3 and 3 M3 variant according to FAB classification, 12 low-risk, 7 intermediate and 6 high-risk according to risk relapse score) there were 13 patients in molecular relapse and 12 in morphological relapse at the time of treatment, with a median time to relapse of 1.2 years. During the ATRA re-induction, 18 patients (72%) had toxicities (nausea and headache); all patients achieved a complete morphological remission and 10 patients (40%) also a molecular remission. After the consolidation course, 12 patients underwent an autologous BMT, 6 patients an allogeneic BMT and 7 patients were treated with maintenance therapy (ATRA and MTX+6-MP). A 2nd relapse occurred in 14 patients (56%) (in 7/12 patients after autologous BMT and in all 7 patients after maintenance), with an overall median survival in this group of 4.9 years. In the Group B (7 males and 4 females, median age 40 years, 9 classic M3 and 2 M3 variant, 1 low-risk, 3 intermediate and 7 high-risk) there were 9 patients in molecular relapse and 2 in morphological relapse at the time of treatment, with a median time to relapse of 1.7 years. After the re-induction cycle (median time of neutropenia 16 days, median time of thrombocytopenia 13 days), 8 patients (73%) achieved a molecular remission and 3 were resistant. There were 3 cases of FUO but no toxicity related to ATRA. As to post-induction therapy, all 8 patients in molecular remission received the consolidation course, followed by PBSC transplantation in 1 patient, maintenance with pulsed ATRA in 4 patients and no further therapy in 3 patients. A 2nd relapse occurred in 4 patients (50%) (in 1/4 patients treated with pulsed ATRA and in all 3 patients with no further therapy), with an overall median survival of 3 years. In conclusion, a re-induction with ATRA±chemotherapy seems effective as to the achievement of 2nd molecular remission: however, post-remission strategies in both groups need to be optimized, and at present only allogeneic BMT seems to offer good chances of cure in these patients.

P44

TREATMENT OF MOLECULAR RELAPSES DETECTED IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS DURING MAINTENANCE THERAPY

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Nearly all of *de novo* APL patients (pts) undergo complete remission (CR) when treated with all trans retinoic acid (ATRA) plus chemotherapy. Nevertheless, a significant portion (20%) of this pts ultimately relapse during two first years of therapy. Minimal residual disease (MRD) monitoring of PML-RARA RNA using RT-PCR is widely recognized method for detecting of molecular relapses (MR) in APL pts during hematological remission. However, the MR definition and significance of therapy changing when MR is detected is still controversal.

We report the results of molecular monitoring and MR treatment in 40 APL pts [(17 men, 18 women, middle age 35 (18-72))] who underwent maintenance therapy after successful remission induction and consolidation. RT-PCR for PML-RARA were performed in all pts on fresh marrow aspirates before treatment and periodically (2-3 month) during all period of therapy (2 years after CR induction). RT-PCR was performed using recommendations of BIOMED-1 Concerted Action (1999). MR was defined as probable if chimeric transcript was detected once and was not find out by second investigation and as proved when PML-RARA was detected at least twice by consecutive investigations (in 2-4 weeks). All pts were devided in three groups. In first group 15 pts received maintenance by ATRA combined with chemotherapy. In 6 pts probable MR was detected. All of them received the same therapy and 5 are still in CR (6-43 mths), 1 pt relapsed in 1 mths after probable MR was identified. In 1 patiends proved MR was detected. The therapy was changed to Interferon alfa (IFN) + ATRA(5 days monthly) and now she is in CR for 42 mths. One pt in this group relapsed without any signs of MR. The median duration of 1st hematological remission for 13 pts in this group was 35 mths (13-62). The second group consisted of 19 pts. They received maintenance chemotherapy without ATRA. Four of them demonstrated proved MR. In one pt therapy was not changed and he relapsed in 1 months after MR detection. In 3 pts therapy was changed for AIDA in 1 and IFN +ATRA in 2 pts. All of them are in first CR (48-55 mths). In 5 pts probable MR was detected. In 2 pts therapy was not changed and 1 of them relapsed in 8 months after detection. The second is in CR. In 3 pts therapy was changed for ATRA-containing regimens (AIDA and IFN + ATRA). All of them are alive and in first CR. One pt relapsed inspite of negative PCR results. The median duration of 1st hematological remission for 15 pts in this group is 65 mths (4-84). The last group consisted of 6 pts and was treated by IFN+ATRA. In 2 pts probable MR was detected. In one pt treatment was not changed and he relapsed in 2,5 mths. Another pt was treated by AIDA regimen and still in CR. One pt demonstrated proved MR and his therapy was changed for AIDA. He is also in first CR. Two pts relapsed without previous detection of MR. 3 pts from this group in first CR for 43 mths (33-58). Summarizing our data, we have detected proved MR in 6 pts (15%). In 5 of them therapy was changed and they are still in 1st CR. 13 (32,5%) pts demonstrated PCR positivity only once. In 9 of them therapy was not changed and 3 pts relapsed. In 4 pts therapy was changed and they are in CR. 4 pts (10%) relapsed inspite of PCR negativity. According to our data, proved MR should be

considered for changing of maintenance therapy especially in patients not receiving ATRA for ATRA-containing regimens. Moreover, probable MR is also thought to have be treated by changed regimens. However, more investigations should be performed for definition of the best postremission treatment strategy in APL pts with once detected PML-RARα.

P45

RELAPSE AT CENTRAL NERVOUS SYSTEM IN ACUTE PROMYELOCYTIC LEUKEMIA AFTER TREATMENT WITH ALL-TRANS RETINOIC ACID AND ANTHRACYCLINE-BASED CHEMOTHERAPY: INCIDENCE, RISK FACTORS AND OUTCOME AFTER RELAPSE

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Background. An increasing number of extramedullary relapses, specially at central nervous system (CNS), in patients with acute promyelocytic leukemia (APL) treated with all-trans retinoic acid have been reported in recent years, rising the possibility of a higher risk of CNS progression in this setting. Nonetheless, the exact frequency of this event, their potential risk factors, and the optimal management are mostly unknown.

Objective. To analyze the incidence of CNS relapses and outcome after salvage therapy in an extensive series of APL patients undergoing frontline therapy with ATRA and anthracyclines.

Patients. Five hundred and ninety eight patients diagnosed of PML-RARα-positive APL who were treated according to PETHEMA protocols LPA-96 and LPA-99 during a 8-year period (6/96±12/04) were included in the analysis.

Results. Ten patients (5/5 male/female; age: 33.5, 10±71) presented a relapse with involvement of CNS after a median interval of 14 months (range: 6±50) since diagnosis, with this representing 17% of overall relapses. In six cases, CNS was the only anatomical site involved, whereas simultaneous bone marrow relapse was present in four additional patients. CNS involvement consisted of meningeal infiltration in all cases, with a concomitant intraspinal tumor in one patient. Actuarial risk of CNS relapse at 4-year was 2.1% (95% CI: 0.8±3.4). Hyperleukocytosis at diagnosis ($>10\times10^9$/L) was the only feature independently associated with an increased risk for CNS disease during evolution (p=0.003; RR 15.6; 95% CI: 3.3±73). Following diverse salvage strategies, 7 patients achieved second complete response (CR), two patients died during second-line therapy, and the remaining patient was refractory to intrathecal chemotherapy followed by radiotherapy. After a median follow-up of 16 months, 5 patients remained alive in second CR, this translating into a 2-year survival and leukemia-free survival after relapse of 48% (SE±16) and 69% (SE±19), respectively.

Conclusions. Although the occurrence of CNS disease in APL treated with ATRA-containing frontline therapy is infrequent, patients presenting with hyperleukocytosis at diagnosis have a higher risk of CNS involvement and should be considered for specific prophylaxis. Moreover, the optimal salvage therapy for this eventuality remains to be defined, although an approach similar to that employed for systemic relapses seems advisable.

P46

ISOLATED MENINGEAL RELAPSE OF ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL) is characterized by the fusion gene transcript PML-RAR-α and is now the most frequently curable acute leukemia. Current treatment includes all-trans-retinoic acid (ATRA) and anthracycline-based chemotherapy. About 90% of the patients achieve a complete remission after induction treatment. The overall incidence of relapse at three years is 7.5%. Infiltration of systemic central nervous system (CNS) at diagnosis is rare and lumbar puncture is routinally performed after induction therapy. There are rare reports of meningeal relapse. We describe here the case of a 40 years old female patient affected of acute promyelocytic leukaemia variant (AML M3 variant according to the FAB classification) located in the high risk group because of hyperleukocytosis. Bone marrow was hypercellular with an almost complete infiltration of atipical promyelocytes. Fluorescent in situ hybridisation (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR) confirmed the presence of the translocation t(15;17). Conventional cytogenetics also demonstrated t(15;17)(q22;q11) and in 11 of 20 analyzed metaphases also trisomy 8. Molecular analysis showed the bcr3 type of PML-RARα and additionally the FLT3 internal tandem duplication (FLT-ITD) was detected. Flow cytometric analysis of promyelocytes showed an aberrant phenotype ($CD45^{dim}$/$CD33^+$/$CD13^+$/$CD64^+$/HLA-DR^-/$CD56^-$). Thepatient was treated according to the GIMEMA protocol. Lumbar punction was performed after induction therapy and there was no CNS-infiltration and the patient received three intrathecal infusions of Methotrexate 12 mg and 6-Methyl-Prednisolone 40 mg as intracranial prophylaxis. The patient achieved molecular remission after induction therapy, remained in complete remission and started maintainance therapy with 6-Mercaptopurine and Methotrexate and ATRA. Only three months after initiation of maintainance therapy the patient presented with severe headache, visual disturbances and cervicalgia. On fundus oculi a bilateral papilledema with hemorrhagic effusion on the left eye was seen. Magnetic resonance imaging and computer tomography of the skull were normal. Lumbar puncture was performed and cerebrospinal fluid (CSF) demonstrated numerous atypical promyelocytes with the same flow cytometric antigen- expression as at diagnosis.FISH-analysis demonstrated positivity for t(15;17). Bone marrow aspirate revealed that the patient was still in complete molecular remission. Intrathecal therapy (TIT) with 12.5 mg Methrotrexate, 50 mg ARA-C and Depomedrol 40 mg every three days started immediately but after seven TIT atypical promyelocytes were still present at morfological examination of CSF. Therefore another induction cycle with Cytarabine, Mitoxantrone and ATRA has been started right now. FLT3-ITD, BCR 3-type and microgranular morphology have already been discussed as prognostic factors for relapse. It should be evaluated, if these parame-

ters and also additional chromosomal abnormalities could be integrated in the current scoring system and lead to perform a more aggressive front-line treatment and a more intensive or prolonged intrathecal prophilaxys program. Perhaps even in the absence of known adhesion molecules on promyelocytes, such as CD56 and CD11b, APL tends to relapse in the CNS. We are planning to perform an allogeneic bone marrow transplantation as soon as remission is obtained. We will update the abstract when further data are available.

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ACUTE RENAL FAILURE COMPLICATING ACUTE PROMEYLOCYTIC LEUKEMIA IN PREGNANCY

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Introduction. APL is uncommon in pregnancy and requires special consideration in the management for the survival of both mother and fetus. Disseminated intravascular coagulation (DIC) is well known cause of morbidity and mortality in patients with APL. For this reason, antifibrinolytic agent(tranexamic acid) with All-trans-retinoic acid(ATRA) were often given for treatment of patients. However, leukocytosis is common in patients with APL treated with ATRA, and these patients may develop RAS. We report herein, a case of RAS and acute renal failure in a pregnant Chinese woman.

Case study. A 33-year old Chinese woman was admitted because of unprovoked antepartum hemorrhage at 30 weeks gestation. Laboratory examination revealed hemoglobin 8 g/dL, Platelet 12×10^9/L and WBC 62×10^9/L with 91% abnormal promyelocytes. Coagulation profile showed PT=15 sec, APTT=48.9 sec, creatinine 77 μmol/L. ATRA(45 mg/m^2/day) and tranexamic acid were started immediately before delivery. Cesarean section was performed on the day of admission. Idarubicin(12 mg/m^2/day × 3 days) was started after delivery of baby. The baby was healthy and discharged subsequently. The patient developed acute renal failure on the next day with anuria, severe azotemia (creatinine 652 μmol /L, urea 32.8 mmol/L) requiring hemofiltration. Ultrasound examination of kidneys showed no structural abnormalities and both renal veins were patent. Autoimmune markers were all negative. Uric acid was 0.43 mmol/L. She also experienced respiratory distress and the CT thorax showed patchy infiltration in both lower lobes and bilateral pleural effusions. Echocardiogram showed ejection fraction of 60% and no pericardial effusion.The differential diagnosis included RAS, fluid overload due to acute renal failure and sepsis. Tranexamic acid was stopped and high dose dexamethasone was started. The respiratory condition gradually improved with resolution of lung infiltrates. The steroid was tapered down gradually and the lung infiltrations reappeared. Thus ATRA was stopped and the lung infiltrations resolved again with a course of steroid. The renal function also improved gradually and hemodialysis was discontinued 4 weeks later while bone marrow aspiration confirmed APL in remission.

Discussion. This case illustrates the possible risk of severe thrombosis causing renal failure when tranexamic acid was administered together with ATRA. This case also illustrates that emergency delivery at > 28 weeks of gestation is feasible in APL despite coagulopathy.

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ACUTE PROMYELOCYTIC LEUKEMIA IN A HIV INFECTED PATIENT: A CASE REPORT

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Human immunodeficiency virus (HIV) in associated with an increased incidence of haematological malignancies and solid tumors. The occurrence of acute myeloid leukaemia (AML) has been reported in HIV patients, with predominance of FAB M2, M4 and M5 type (1). Four cases of promyelocytic leukaemia (APL) in HIV infection have been reported up to the present. We describe the fifth case of APL.

A 46 years-old female was known to be HIV-1 positive since 2001. HAART Highly active anti retroviral therapy (HAART) was started when CD4$^+$ T-cell counts was 300/μL and plasma HIV-RNA was 89000 copies/mL. She begun HAART with nelfinavir and lamivudine. Then for the onset of lipodystrophic syndrome she stopped nelfinavir and started efavirenz. She went on with this therapy and she achieved a CD4$^+$ count > 500 cell/μL and HIV-RNA < 50 copies/μL. On June 2003, she stopped HAART as presented a gradual decrease of hemoglobin serum level and platelets count. She complained fatigue and one week before admission she had continuous -remittent fever with an acme of body temperature of 38°C. At the admission haemoglobin was 6.7 g/dL, total leucocytes count was 5090/microlitre, with a differential count of neutrophils 8.4%, 55% of promyelocytes. Platelets count was 1500/μL. Prothrombin time, activated partial thromboplastin time were normal limits, fibrinogen was 167 mg/dL and D-Dimer was 5550 ng/mL. Laboratory parameters were normal, except for LDH (796 UI/L). Urinalysis, EKG and chest-X-ray were normal. Physical examination revealed small laterocervical and inguinal lymphonodes. Bone marrow aspiration showed a massive infiltration of promyelocytes with multiple Auer bodies and strong positivity to peroxidase reaction. These cells expressed the following antigens: CD33 (80%), CD13 (78%), CD 71 (73%), CD117 (75%), CD34 and HLA-Dr was absent. PML/RAR± rearrangement (bcr 1) was observed by PCR analysis and cytogenetic analysis was not valuable for absence of mitoses. Diagnosis of APL was made.

The patient started induction therapy according to GIMEMA AIDA 2000 protocol. Including ATRA 45 mg/m^2. Prednisone (0.5 mg/Kg/die) was administered as prophylaxis of ATRA syndrome. As the toxic effect of HAART on bone marrow was excluded, she started at the same time of induction chemotherapy a new antiretroviral combination, including efavirenz, tenofovir dipivoxil and lamivudine. She presented a rapid decline of plasma HIV-1 RNA below 50 copies/mL After induction therapy, the clinical course was benign with mainly haemorrhagic complication requiring supportive care with platelets and red cells transfusions. By day 30 after induction chemotherapy she entered complete remission (CR). PCR analysis after induction therapy was not valuable. She was consolidated according to the low-risk group consolidation of GIMEMA AIDA 2000 protocol because of her HIV-infection. She started first consolidation cycle with idarubicine 5 mg/m^2/daily from day 1 to day 4 and ATRA 45 mg/ m^2/daily for 15 days. She underwent second course of consolidation one month later with mitoxantrone 10 mg/m^2/daily from day 1 to 5 and ATRA 45 mg/ m^2/daily for 15 days. Six weeks later she was consolidated

again with idarubicine 12 mg/m^2 on day 1 and ATRA 45 mg/m^2/daily for 15 days. Then she was in morphological and molecular CR. Consolidation courses were followed by oral mainteinance with ATRA 45 mg/m^2/daily for 15 days every three months, Methotrexate 15 mg/m^2/daily and 6-mercaptopurine 50 mg/m^2/daily. The patient is now in molecular CR on mainteinance therapy 19 months after diagnosis. The patient is still on HAART and at last examination HIV-RNA was < 50 copies/mL, CD4$^+$ count was 125 cells/μL,CD4/ CD8 ratio was 0.3.

In three of the four cases reported HAART was not stated, in the fourth case (from Kudva *et al.,)* HAART was discontinued for a short period during remission induction. No risk assessment is available in all of them. More over, in three of the four cases reported in literature ATRA was used alone to induce CR but just two patients achieved CR. In these two patients induction therapy was followed by consolidation therapy with anthracyclines and/or cytarabine. Another patient did not received consolidation and he was kept on mainteinance as he relapsed and died after 303 days. Last patient (5) was treated with *standard* induction therapy (details of therapy are not available) but she failed to achieve CR. Our patient well tolerated chemotherapy and concurrent HAART despite she is the oldest patient with concurrent HIV and APL. Our patient is at 15 months of CR and her probabilities of survival are 70-95% as in uncomplicated APL. In conclusion, CR was obtained with success in our patient according to standard treatment of APL with included ATRA and chemotherapy at diagnosis. HAART was continued and no AIDS-related complication were observed during chemotherapy to HIV$^+$ patients.

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FATAL ACUTE PANCHREATITIS SECONDARY TO ALL TRANS RETINOIC ACID TREATMENT IN A PATIENT WITH AN ACUTE PROMYELOCYTIC LEUKEMIA

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Introduction. Several adverse reactions have been described in association with the treatment with All Trans Retinoic Acid (ATRA), which is currently one of the cornerstones for the treatment for Acute Promyelocytic Leukemia. The ATRA Syndrome is the most common and potentially letal requiring preventive measures and agressive treatment if it is present. Other less common but potentially lifethreatening adverse effects are hypercalcemia, bone nechrosis and acute panchreatitis. The following report describes the clinical findings in a patient who developed a fatal acute pancreatitis treated in our Hospital.

Case report. The patient, a 51 years male with a mild mental deficiency, was admitted for oral bleeding, at physical examination he was pale and had erythematous, sweeling and bleeding gums with aspect of oral sepsis. Laboratory tests showed pancytopenia with an hematocrit of 29%, ANC 767 and 80.000 platelets × mm^3.Prothrombine was 67% and APTT 26 seconds. It was performed a bone marrow aspirate and biopsy showing 65% of promyelocytes. The immunophenotype by flow cytometry demostrated an abnormal population with intense expression of cMPO, CD13 and CD33 in absense of CD 34 and HLA-DR. Translocation 15,17 by polimerase chain reaction was positive.

It was started treatment with ATRA 90 mg/day and Daunorrubicin 60 mg/sm, treatment of coagulopathy with cryoprecipitate and platelet transfusions, antibiotics and removal of dental rests in bad conditions. He had a favorable response without evidences of infection and on day 9 since started with ATRA suddenly complained about intense upper abdominal pain, vomiting and later taquicardia, polipnea and hypotension. Laboratory tests revealed Amilasa 617U/L (Normal up to 110U/L), Lipase 1550 U/L (Normal up to 300U/L). Abdominal ultrasound showed aerobilia and panchreatic sweeling. On the same day, the patient was admitted at the Intensive Care Unit and treated as a severe panchreatitis but developed a refractory shock, metabolic acydosis Disseminated Intravascular Coagulation, multiple organic failure and death at day 10 from the start of treatment.

Conclusions. Acute panchreatitis is an infrequent complication of ATRA treatment. The reported case emphasized the rapid and progressively fatal course of this complication in a patient which otherwise had a favourable response to treatment.

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FAMILIAL ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia is a distinguished morphologically, clinical, pathophysiological and molecular acute myeloid leukemia. Familial acute promyelocytic leukemia (APL) was described unusually. We present a couple admitted for APL in the Department of Hematology Fundeni University Institute. First, the man 29 years old was diagnosed in October 1996 and dead with APL relapse in October 1997. The second, his wife 31 years old, was diagnosed in august 1998 and dead in February 1999 with APL relapse. Both received complete remission with all-trans retinoic acid therapy (in 43 days and 25 days respectively). After that both received standard chemotherapy (P.Fenaux protocol). Both relapse with leukemic cells in CNS and meningitis features. In both we described a particular aspects of the leukemic promyelocytes at cytological and ultrastructural examination: *mirror with handle* promyelocyte cells with peroxidase positive cytoplasm inclusions, with abnormal number of ribosome, cytoplasm vacuolization and rough endoplasmic reticule excessive developed. Some leukemic cells had viral-like inclusions.

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CLASSICAL ACUTE PROMYELOCITIC LEUKAEMIA EVOLVED IN MYELODYSPLASTIC SYNDROME

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We describe a patient with APL who developed MDS 40 months after entering complete remission (CR).

In July 2000, a 53-year-old woman was admitted to haematology unit for haemorrhagic syndrome. The blood count showed a hyperleucocytosis of 104×10^9/L with 86% of atypical promyelocytes. A diagnosis of myeloid leukaemia FAB subtype M3 variant was made by bone marrow analysis. No myelodysplastic changes were noticed on

this first bone marrow. Cytogenetic studies revealed t (15; 17) (q22; q21) in 7 out of 7 metaphases. RT-PCR studies of the PML-RARα fusion gene showed bcr-3 junction type. Cytofluorimetric analysis was compatible with the diagnosis of APL (CD13 85%, CD33 98%, HLA-DR 1%, CD45RA 91%, CD45RO 2%, CD34 18%, CD56 3%, CD9 46%). The patient presented a disseminated intravascular coagulation (DIC) with D-Dimers >1600 ng/mL (n.v. 278), PT 51.8 sec and mild hypofibrinogenemia: 139 mg/dL (n.v. 200-400). The patient reported a history of ulcerative recto colitis for which she received mesalazine during a period of more than 5 years, followed by colectomy in 1990 for scant response to medical treatment. The patient was included in the LAP AIDA protocol and received induction therapy consisting of idarubicin on days 2, 4, 6, 8, cytosine arabinoside (Ara-C) on days 1 and 2 , and daily all-trans retinoic acid (ATRA). An episode of cerebral hemorrhagy occurred during induction, with remission of clinical symptoms and radiological signs few weeks later when platelets count restored. The patient achieved CR after 1 month. Three cycles of consolidation therapy were applied consisting of idarubicin, Ara-C and ATRA; then the patient started maintenance treatment with ATRA, 6-MP and MTX from February 2001 until January 2003. On may 2003 while in CR, cytogenetic assay on bone marrow smear disclosed t(11;21)(q24;q11) on 17 out of 25 metaphases, no t(15;17) was detected; RT-PCR for PML-RARα rearrangement was also negative. Interphase FISH did not revealed any MLL gene rearrangements or translocations. Bone marrow aspirate showed diserytropoiesis with no typical APL cells. On October 2003 karyotype examination of bone marrow cells confirmed the t (11; 21) in 20 out of 24 metaphases and showed a new clone (4 metaphases) with 45, XX, del7. During a follow-up of 20 months patient showed mild neutropenia in blood count with median neutrophils count of 1.5×10^9/L, and a progressive anaemia (haemoglobin decreased from 12,3 to 9,4 g/dL). Bone marrow blasts count ranged from 9 to 5%. In April 2005 FISH revealed 94% of nuclei carrying monosomy of chromosome 7, while RT-PCR for PML-RARα rearrangement remained negative. No metaphases were detected in cytogenetic analysis. Patient is yet alive without any chemotherapy. Biological features rule out a recurrence of APL and are consistent with a diagnosis of therapy-related myelodysplastic syndrome (t-MDS). t-MDS in patients treated for APL is a rare event. The patient received mesalazine for her URC. Adverse effects from mesalazine are uncommon, including idiosyncratic worsening of the colitis symptoms, interstitial nephritis, pancreatitis, serious skin reactions, hepatitis and hepatic failure, and blood dyscrasias. Mesalazine is safe to use during pregnancy and for nursing mothers and it seems reduce the risk of developing colorectal carcinoma both inhibiting growth of colon cancer cells through a mitotic arrest and inducing apoptosis through partial activation of caspases. It could be excluded an implication of this drug in the development of late chromosomal abnormalities. The patient was exposed to topoisomerase II inhibitors anthracycline during induction and consolidation phase of treatment while she received the alkylating 6-MP at dosage of 75 mg daily during a period of 2 years as maintenance therapy. In our patient karyotypic analysis revealed a clone with t(11; 21), in a locus that not involved MLL gene, and another clone characterized by monosomy 7 that progressively expanded. The t (11:21) is not described in primary or secondary MDS and could represent a sign of chromosomal instability early after chemotherapy. Deletion of chromosome 7 is a cytogenetic change usually occurring after the use of alkylating agents. On the other hand, a very short latency period (9 months) elapsed between the end of maintenance treatment and the appearing of the monosomy 7 after exposition to alkylating agents. Chromosome 7q deletion is described as rare additional abnormality in association with t (15;17) at diagnosis, with no clear impact on prognosis of APL. In our patient the clone carrying the monosomy 7 is not correlated with the previous pathologic clone with t (15;17). Another important feature in this case is the relatively good behaviour of the t (11;21), and the progression to a poorer clinical outcome when the clone with monosomy 7 expanded. This case seems to show that a clone with monosomy 7 could develop early after exposition to chemotherapy, and could be responsible of ineffective myelopoiesis with overt myelodysplasia only when it acquires proliferative advantage.

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ACUTE PROMYELOCYTIC LEUKEMIA EXTRAMEDULLARY RELAPSED MIDDLE EAR

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Despite the excellent results achieved, about 30% of patients with acute promyelocytic leukaemia (APL) are not cured with modern therapy. Extramedullary involvement occurs infrequently in APL Between December 1992 and April 2005 we have evaluated 36 patients with primary APL, 9 relapsed (4 haematological; 4 molecular and 1 extramedullary). We describe here 1 patient who relapsed in the auditory apparatus, middle ear. She presented hypoacusia, earache. CT scan and MNR demonstrated ear involvement. This was diagnosed after front-line treatment that included retinoic acid (ATRA) and chemotherapy. She has not concomitant haematological relapse and the molecular control was negative in two opportunities after that. The diagnoses of the tumour was granulocytic sarcoma and the PCR on the biopsy was not possible because insufficient material. She was treated with Radiotherapy and ATRA. She was in haematological and molecular remission for 14 months, after that the haematological relapse occurred. Was suggested that ear disease localisation might represent a specific sanctuary in APL. We contribute this extramedullary relapsed case in middle ear; unique in 12 years work with this pathology in our institution since ATRA was introduced in treatment.

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HIGH RISK OF EARLY MOLECULAR RELAPSE IN ACUTE PROMYELOCYTIC LEUKEMIA SECONDARY TO DNA TOPOISOMERASE II INHIBITORS. REPORT OF TWO CASES

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Mitoxantrone is an anthracenedione derivative with potent cytostatic activity, which has been licensed for the treatment of worsening multiple sclerosis (MS). A rare complication of these treatment is the potential development of acute myeloblastic leukaemia/myelodisplastic syndrome (AML/MDS), which can be induced by drugs targeting DNA topoisomerase II, like mitoxantrone, anthracyclines and epipodophyllotoxins. In our institution we observed two such cases, which cause specific therapeutic problems, since mitoxantrone and anthracyclines are also the most active cytostatic drugs for the treatment of APL. A 57-year old man and 47-year old woman, affected by MS since 1998 and

1996, respectively, were first treated with interferon +/- corticosteroids and then with mitoxantrone 10 mg/mq every three months for 11 and 6 courses respectively (cumulative dose: 198 mg and 47 mg). Sixty-six and 60 months after the first course of mitoxantrone, a diagnosis of APL was made with the following characteristics: FAB M3 and M3v; typical immunophenotype, typical chromosomal translocation and molecular transcripts (bcr1/bcr2 and bcr1, respectively), intermediate and low risk according to the Gimema/Pethema scoring system. Both patients were treated with a modification of the current Gimema protocol (AIDA 2000), in order to limit the use of anthracyclines and to completely avoid the use of mitoxantrone, which had caused the emergence of APL. Both cases received induction treatment with ATRA alone (45 mg/mq/die for 30 days). In one case, two doses of idarubicin were added, due to marked leukocytosis. The treatment was well tolerated and morphological complete remission was achieved, with detectable pml/rar α, in both cases. The patients received three consolidation courses according to AIDA 2000 protocol for low/intermediate risk patients, with ATRA and idarubicin alone, which also substituted mitoxantrone at equivalent doses in the second course. Molecular remission was achieved after the first and third consolidation cycle, respectively, and patients started maintenance with 6-mercaptopurine, methotrexate and ATRA, according to AIDA 2000. Relapse occurred in both patients, four (molecular) and five months (molecular/hematologic) after the end of consolidation, at a significantly higher frequency compared to that observed in 68 consecutive patients with *de novo* APL treated at our Institution after the introduction of ATRA (100% vs 2,9%; $p<0.013$; Fisher's exact test). A second molecular remission, now persisting for 1-30 months, was obtained with ATRA followed by gemtuzumab/ozogamicin (GO) without side effects in one case and with ATO (0,15 mg/kg/die for almost 2 months) in the second ATRA-resistant patient, who experienced hepatotoxicity (WHO 3) and *APL differentiation syndrome*. Although a predisposition to the development of topoII therapy-related APL has not been demonstrated, a limitation in the use of potentially leukemogenic drugs in affected patients would be desirable. However, since that strategy seems to be associated, in our hands, to a higher frequency of molecular relapse, alternative strategies for the optimal management of this subset of patients should be explored.

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ANTI-LEUKEMIC AND ANTI-ANGIOGENESIS EFFICACY OF ARSENIC TRIOXIDE IN NEW CASES OF ACUTE PROMYELOCYTIC LEUKEMIA

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Arsenic trioxide is now considered the standard agent in treatment of refractory cases of acute promyelocytic leukemia (APL). This drug is also shown to have anti-angiogenesis effect against APL cells *in vitro*. In this study, we evaluated clinical efficacy and anti-angiogenesis effect of arsenic trioxide in 17 new cases of APL. Arsenic trioxide was given in a dosage of 0.15 mg/kg and remission rate, survival rate, toxicities and effect on vascular density of bone marrow was studied. Bone marrow vessels were examined using immunohistochemistry for von Willebrand Factor (vWF) and CD31 markers. Bone marrow vascular density was determined by calculating mean vessel number in three hot spot, high power microscopic fields. Bone marrow vascular density was reduced as identified by anti-vWF immunohistochemical staining (Mean before treatment=201.6/mm^3±20.4 (SEM), Mean after treatment=109.4±17.2(SEM), $p<0.001$), and anti-CD31 immunostaining (Mean before treatment = 199.17/mm^3±21.5 (SEM), Mean after treatment =99.5/mm^3±22.1 (SEM), $p<0.05$).

Treatment efficacy results showed 100% complete remission rate after median of 30 days and 72% survival probability after median 860 days of follow up. Main toxicities included hyper-leukocytosis , hepatic toxicity and APL differentiation syndrome. Our results imply that arsenic trioxide is an effective anti-leukemia and anti-angiogenesis agent in new cases of APL.