WORKSHOP III
MINIMAL RESIDUAL DISEASE

MRD MONITORING IN ACUTE PROMYELOCYTIC LEUKEMIA: UNRESOLVED ISSUES IN 2005

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Studies of MRD in APL over the last decade have resolved some issues related to its use as a means to predict clinical outcome but important issues remain to be clarified. In part this is related to the evolution and improvement in the treatment of APL. Four treatment-related developments have been particularly relevant to the state-of-the-art application of MRD in 2005. First, it was found by Fenaux, *et al.,* that concurrent treatment with all-*trans* retinoic acid (ATRA) and chemotherapy (CT) resulted in superior long-term outcome compared to the sequential administration of these two forms of treatment.[1] Second, Lo-Coco, *et al.,* provided evidence that re-treatment at the time of molecular relapse during first remission provided improved clinical outcome compared to a historical control group not re-treated until the time of hematological relapse (HR).[2] Third, Sanz, *et al.,* defined pretreatment criteria to classify patients for risk of disease recurrence (DR) following concurrent ATRA-CT and introduced risk-adapted consolidation therapy with dose-intensification and ATRA supplementation for intermediate (WBC <10K, platelets >40K) and high-risk cases (WBC >10K, platelets <40K) while maintaining standard therapy for low-risk patients (WBC <10K, platelets >40K).[3] In the latest Spanish trial (LPA99), this reduced the relapse rate in low- and intermediate-risk cases to <3%, which clearly could reduce the overall requirement for MRD monitoring. Fourth, the availability of alternative agents, particularly arsenic trioxide (ATO), has led to the formulation of novel induction regimens, which may add to the effectiveness of ATRA-CT[4] or which may reduce or avoid the potential adverse effects of CT.[5] Technological development is another major consideration regarding the current status of clinical prognostic application of MRD, and this is the focus of this report. Since its initial introduction in the early 1990's, double-nested RT-PCR (DN-PCR) has been the principal methodology for the detection of APL-specific PML-RARα mRNA in association with clinical trials.[6-11] Based on dilution experiments with positive sources of PML-RARα mRNA, DN-PCR as conventionally-applied has a detection sensitivity of 1 in 10^3 to 10^4, e.g., as documented for the proposed standard BIOMED-1 procedure.[12] In the context of clinical trials using ATRA + CT, several conclusions have been reached from consistent results in two or more studies. First, the finding of MRD after achieving complete remission (CR) but before completing 2 courses of consolidation therapy is not a reliable indicator of adverse clinical outcome and should not be the basis for modification of treatment in individual patients.[13] Second, the finding of confirmed MRD by DN-PCR immediately after the completion consolidation therapy (post consolidation therapy; PCT) is associated with a high incidence of HR in the absence of further treatment.[10,11,14] Third, a negative DN-PCR assay at PCT is not a highly-reliable indicator for continued CR, since DR can subsequently occur in up to 25% of patients [9-11] Fourth, MRD-negative patients at PCT require serial DN-PCR monitoring during the follow-up period (FUP), because conversion to MRD positivity is associated with a high risk of HR and because continued MRD negativity is with few exceptions associated with long-term CR and likely disease cure.[9-11]

Real-time quantitative RT-PCR (RQ-PCR) has been explored as an alternative to DN-PCR, with the principal documentation related to the first North American Cooperative Intergroup Trial in APL (Protocol INT0129)[15,16] and to a German Acute Myeloid Leukemia Cooperative Group (GAMLCG) study.[17] A successor North American Cooperative Intergroup trial (Protocol C9710) has also utilized RQ-PCR but the results from analysis of this trial are not yet available. Results of RQ-PCR and DN-PCR analysis of an exploratory Phase II trial with a small number of patients are herein presented to illustrate some points of comparison between RQ-PCR and conventional DN-PCR, as a basis to consider what role RQ-PCR may provide in the setting in which more-accessible, less-expensive DN-PCR has been so successfully applied. The Phase II trial was designed and executed at the M.D. Anderson Cancer Center (MDACC), E. Estey, PI, with the principal clinical objective of either eliminating (low-risk patients; WBC <10K) or minimizing (high-risk patients; WBC >10K) CT (gentuzumab ozogumicin; GO) through the combined use of ATRA and ATO.[5]

RQ-PCR has several theoretical advantages to DN-PCR. Several of these points are primarily technical related to ease of high through-put, semi-automated performance and decreased risk of false positive results due to contamination.[15,18] There are, however, two essential advantages compared to DN-PCR. First, there is a quantitative internal control to measure RNA integrity and transcription in each test sample. This provides greater assurance that negative assay results are not due to the generation by reverse transcriptase of insufficient cDNA template for PCR amplification than non-quantitative or semi-quantitative end-point amplification measurement of endogenous control genes by conventional DN-PCR. This advantage is particularly important near the detection limit of PCR assays, since PCR amplification is essentially stochastic, meaning that an increasing fraction of replicate PCR assays will become negative simply by chance as the detection limit is approached.[19,20] Thus, DN-PCR assays with *1 in 10^3 to 10^4 sensitivity*, which are typically run as a single determination, have a poorly-defined probability of producing false-negative results, which may be quite high at the 1 in 104 level, particularly if the test RNA is not of the same quality as the positive RNA dilution control. Although RQ-PCR assays also become stochastic, this occurs over a narrower sensitivity range due to the diminished variability of real-time assay measurements and permits a more accurate assessment of the probability of false negative results.[18] The second essential advantage of RQ-PCR is its quantitative nature per se, which provides an opening for either extension of or alternatives to DN-PCR applications. A principal practical extension of DN-PCR relates to the assessment of APL MRD at higher levels of sensitivity at PCT and in the FUP. This application follows previous observations that MRD remains detectable in long-term remission APL patients at levels below the detection sensitivity of conventional DN-PCR, either as the alternative fusion product of the t(15;17), RARα-PML,[21,22] or as PML-RARα quantified by an enhanced DN-PCR assay with sensitivity up to 1 in 10^6.[23,24] Thus, simply increasing the sensitivity of conventional, non-quantitative DN-PCR might decrease the positive predictive value of this relatively low-sensitivity assay by detecting clinically-irrelevant MRD.[13] On the other hand, if MRD at the 1 in 10^4 level is clinically-relevant, as concluded in most studies, and if false-negative DN-PCR assay results due to its stochastic nature contribute to false-negative results with respect to clinical outcome,

then RQ-PCR at a higher level of sensitivity to reduce or eliminate these stochastically-based false-negative assay results seems advantageous. More importantly, RQ-PCR can be used to explore the possibility that lower levels of MRD are clinically-relevant, particularly by assessing in serial analyses if dynamically-active MRD can be discriminated from relatively static, clinically-irrelevant MRD. To date, Protocol INT0129 is the only published clinical trial study using RQ-PCR that bears on this issue.[16] A pre-clinical analysis of the RQ-PCR assay used in INT0129 indicated that its sensitivity extended to the 1 in 10^5 to 10^6 range, based on dilution experiments in which PML-RARα copy numbers were normalized to copy numbers of the housekeeping gene glyceraldehyde-3'-phosphate dehydrogenase (GAPDH) to provide a normalized quotient (NQ) value.[15] As applied in the trial, the GAPDH copy number, a measure of sensitivity, varied up to 100-fold over a threshold level that was calculated to provide a minimum sensitivity of 1 in 10^4. Thus, the MRD sensitivity range in this study varied between 1 in 10^4 to 10^6 with a median near 10^5.[16] The principal finding was that an NQ value of 10-5 was associated with a 4-fold (at PCT) to 17-fold (during the FUP) increased risk of HR. Risks were also increased at lower NQ values, particularly at 10-6 associated with an 8-fold increase in the FUP, but the clinical risks of lower NQ values were difficult to assess because they were largely driven in the statistical analysis by the higher NQ values.[16] The incidence of DR in patients with 10^{-5} NQ was 67% and 77% at PCT and during the FUP, respectively, which is higher than a Medical Research Council (MRC) DN-PCR study after 3 courses of consolidation therapy (57%)[10] but lower than the results of two other studies (Italian and New York) in which all confirmed DN-PCR-positive patients experienced DR.[9,11,14] In trying to assess whether RQ-PCR provides a significant advantage over DN-PCR, the essential question is whether the larger fraction of RQ-PCR-positive patients at different cut-off levels with reduced clinical predictive values compared to the Italian and New York studies is effective in identifying patients at increased risk of DR, who are present in the DN-PCR-negative group from which the majority of overall DR cases are derived. This must take into account that continued DN-PCR monitoring during the FUP apparently identifies about 75% of patients destined for DR, if adequate BM samples are provided on schedule.[25]

This, then, is a complex analysis, and it would benefit from a direct comparison of RQ-PCR and DN-PCR in the same clinical trial. Recently, Lo-Coco, *et al.,* did report such a comparison and found only a 5% discrepancy in detecting MRD positivity between conventional DN-PCR and RQ-PCR (detected <10 copies of PML-RARα/10^4 cABL copies in 4 DN-PCR-negative samples), but they presented no opinion regarding any significance attributable to this apparently minor difference.[26] Recently, we have performed both RQ-PCR and DN-PCR assays on the same samples from *de novo* APL patients on the aforementioned MDACC Phase II clinical trial.[5] Triplicate RQ-PCR assays were performed using a constant amount of RNA (500 ng), which ensured 100% positivity in dilution controls for both the L-form and S-form of PML-RARα at 1 in 10^4 sensitivity and >50% positivity at 1 in 10^5 sensitivity. A total of 96 samples from 15 patients were tested by RQ-PCR of which 35 were positive in at least one of the triplicate assays. These RQ-PCR results broken down as a function of NQ value are compared to the results observed with the same samples using a slight modification of the BIOMED-1 DN-PCR protocol,12 which was 100% effective in detecting PML-RARα at a 1 in 10^3 dilution and 60-70% effective at a 1 in 10^4 dilution of positive control RNAs.

Table 1. RQ-PCR NQ range and detection sensitivity vs DN-PCR detection sensitivity..

	RQ-PCR Positive Samples (triplicates)				DN-PCR Positive Samples
NQ Range	Total+	3/3+	2/3+	1/3+	
10^{-3}-10^{-2}	14	14	-	-	5/5
10^{-4}-10^{-3}	7	7		-	4/4
10^{-5}-10^{-4}	7	7	-	-	7/7
10^{-6}-10^{-5}	7	7	-	-	/6
10^{-7}-10^{-6}	6	-	2	4	0/6
10^{-8}-10^{-7}	19	2	3	14	0/3
10^{-9}-10^{-8}	4	-	-	4	-

These results indicate that the RQ-PCR assay had about 10-fold higher sensitivity than the DN-PCR assay. It cannot be excluded that some of the RQ-PCR results are false-positives, as reported by others using a similar assay.[27] However, because of the stochastic nature of the assay, it seems likely that many of the single positives represent true detection. These data confirm the suspicion from the INT0129 trial that NQ >10^{-5} corresponds to PCR positivity by the DN-PCR assay, associated with a very high risk of relapse.16 It also supports the notion that lower NQ values, particularly the 10^{-6}-10^{-5} range, merit further exploration for association with outcome in the context of large clinical trials, as will be forthcoming from analysis of the C9710 trial. Two interrelated considerations seem of particular relevance to a decision of whether RQ-PCR with its high equipment, maintenance and assay run costs can be recommended as a replacement for or supplement to relatively inexpensive DN-PCR monitoring of MRD in APL. One is whether peripheral blood (PB) can be substituted for bone marrow (BM) as a monitoring source. The second is whether the dynamics of DR are such that HR can occur with some frequency from MRD levels undetectable by DN-PCR in less time than established BM monitoring intervals. Regarding the first point, based on an initial quantitative assessment of MRD in both BM and PB, using a high-sensitivity, competitive DN-PCR assay, Tobal, *et al.,* suggested that this substitution might be effective, although the normalized PML-RARα copy number was 1 to 2 orders of magnitude lower in PB at 5 of 7 points in which both sources were tested.[24] If this difference reflects a common pattern, it would be incompatible with successful conventional DN-PCR monitoring. This point is evident from the data plotted in Figure 1.

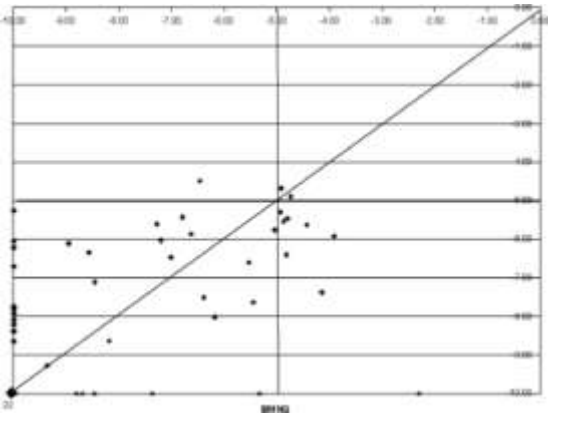

Figure 1. NQ values from 66 matched PB and BM samples obtained at PCT and during the FUP on protocol INT0129. Diagonal line plots theoretical equivalence. Large diamond represents 22 coincident negatives. Horizontal and vertical lines mark the 10^{-5} cut-off values for PB and BM, respectively.

Based on the data from Table 1 indicating that NQ 10-5 corresponds to the DN-PCR positivity threshold, only 3 positives would be detected using PB, while 10 would be detected using BM. On the other hand, at lower NQ values, PB was approximately as effective as BM (Table 2). Also, more PB samples[12] were positive with a matched negative BM sample than vice versa.[6]

Table 2. Number of positive BM and PB samples at different NQ cut-off levels (n = 66).

	NQ cut-off range		
Source	10^{-5}-10^{-4}	10^{-6}-10^{-5}	10^{-7}-10^{-6}
BM	10	14	20
PB	3	13	22
Both	11	16	32

These results are in accord with Tobal's suggestion that PB might be effective for MRD monitoring based on results using a higher sensitivity assay than conventional DN-PCR.[24] They also bear on the second consideration related to the dynamics of DR in which 25% or more of patients destined to experience HR will do so from a MRD level undetectable by DN-PCR within 3 to 6 months after a negative assay.[9,11,22,25] This indicates that APL cell replication dynamics are capable of producing the required 4 log or more increase in APL cell number during this time interval. It also suggests that some secondary transforming event occurs in these rapidly emergent DR cases that allows the transformed APL subclone to escape from host control mechanisms. The early detection of such subclones with acquired secondary lesions that enhance their malignant potential could be important for the institution of salvage therapy, particularly for immunologically-based therapeutics, such as GO, which may be most effective against a low burden of MRD. Clearly, RQ-PCR at frequent intervals, such as monthly, using conveniently and non-traumatically collected PB, could be effective in detecting sub-DN-PCR MRD and in monitoring for upward quantitative trend. In principal, this trend should discriminate patients with dynamically-active MRD from clinically-irrelevant low-levels of MRD in two successive testings, although the RQ-PCR monitoring experience during the INT0129 trial indicated that some reversible, benign upward trends might be encountered.[16] The INT0129 study also indicated that fluctuations in low-level MRD can span many months PCT, and, hence, it is anticipated that a much more complete assessment of the potential value of using RQ-PCR PB monitoring will be possible when the C9710 trial results become available, since hundreds of matched BM and PB samples at regular 4 month intervals were collected during the FUP. This assessment will also be more directly evaluable from another Phase 2 clinical trial conducted by a Johns Hopkins-led consortium (S. Gore, principal investigator) in which both DN-PCR and RQ-PCR are performed at 3-monthly intervals on BM with RQ-PCR additionally performed on PB monthly during the FUP with combined ATRA, ATO and CT limited to two courses. In summary, future recommendations regarding RQ-PCR monitoring of MRD need the input of ongoing protocol studies with investigational high-density monitoring and will require consideration of the therapeutic regimen and the assessment of individual patient risk. As has been suggested,[3,13] it does not seem reasonable or cost-effective to perform extensive, or perhaps any, testing in a patient who can be reliably predicted to have a ≤3% risk of relapse on an established treatment regimen. Nevertheless, because the clinical consequences of DR, even when detected early by DN-PCR, very significantly increases the risk to long-term health and survival, it seems likely that some stratified MRD monitoring scheme will be desirable. RQ-PCR seems particularly apt for this role, which could offset its greater short-term cost by detecting more patients destined for DR with ultimately much higher costs. A possible scheme, for example, would be to RQ-PCR test all patients immediately PCT and then to stratify the subsequent monitoring schedule based both on pretreatment prognostic criteria and on the RQ-PCR assay result. For example, a low-risk patient with completely negative PCT results might be spared any further MRD testing, while a high-risk patient with an NQ value between 10^{-7} to 10^{-5} range might mandate full-scale monitoring with monthly PB RQ-PCR supplemented by BM testing every 3 months. An alternative, more intensive monitoring scheme might be more appropriate for more experimental clinical protocols, particularly, accompanying salvage therapy in which a high long-term CR rate is less certain. Finally, because of its quantitative nature, RQ-PCR has been investigated for prognostic value by assessing the absolute level or changes in the level of MRD at earlier times in disease protocol studies. Two studies evaluated the level of PML-RARα prior to treatment with apparently different conclusions: the INT0129 study found no association between pretreatment NQ value and disease-free survival (DFS)[16], while the GAMLCG study found that patients with NQ values in the highest quartile had significantly decreased event-free survival (EFS).[17] In the latter study, however, 6 of the 8 *events* in 27 upper-quartile patients resulted in early deaths, suggesting that these two studies may be simply incomparable rather than so different. Two potentially significant differences between the studies were that INT0129 used GAPDH while the GAMLCG used cABL as the normalizing transcript and the GAMLCG used exclusively BM whereas 18/66 INT0129 samples were PB (there were no significant NQ differences between these sources, including 12 matched pretreatment PB and BM samples). Further evaluation of the possibility of using pretreatment NQ values as a prognostic indicator using all of the above parameters is currently under evaluation in the C9710 trial, since a molecular prognostic indicator that independently complements current blood count criteria remains of interest. Another suggested prognostic application of RQ-PCR is measurement of the rate of disease clearance. However, the presence or absence of MRD in APL, as detected by DN-PCR or quantified by RQ-PCR at the time of CR has uniformly been reported to have no prognostic significance.[9,10,16,17] The MRC study suggested that the rate of disappearance of MRD positivity by DN-PCR after successive consolidation therapy courses was an independent risk factor for DR.[10,25] However, considering the low incidence of refractory disease with continuing improvements in therapy, sequential monitoring prior to the termination of consolidation therapy seems unlikely to be rewarding except, perhaps, under special investigational circumstances.

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USE OF REAL-TIME PCR TO PREDICT RELAPSE IN ACUTE PROMYELOCYTIC LEUKEMIA: THE UK EXPERIENCE

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Since the cloning of the t(15;17)(q22;q21) chromosomal translocation in 1990, molecular analysis to detect an underlying PML-RARα fusion has become standard practice in the management of patients with acute promyelocytic leukaemia (APL), identifying those likely to benefit from targeted therapy in the form of all-*trans*retinoic acid (ATRA) and arsenic trioxide (ATO). In addition, minimal residual disease (MRD) monitoring using reverse transcriptase polymerase chain reaction (RT-PCR) has been shown to provide an independent prognostic factor, which has the potential to guide therapy in individual patients. Indeed, APL is the first subset of acute myeloid leukaemia (AML) in which MRD monitoring has become an accepted component of patient care. Although its role in the routine clinical management of APL is now being questioned in the light of the excellent outcomes now achieved with ATRA and anthracycline-based combination therapy, the experience gained through MRD detection in APL is likely to have an important bearing on the design of risk-adapted therapies for other subsets of acute myeloid leukaemia (AML) in the future.

Lessons from qualitative assessment of MRD in UK Medical Research Council (MRC) ATRA trial

Early studies involving MRD detection in APL used nested RT-PCR assays, which typically achieve sensitivities of approximately 1 in 10,000.[1-3] This approach was applied retrospectively to samples from 105 patients entered into the UK Medical Research Council (MRC) ATRA trial, which enrolled 239 patients between 1993 and 1997, where patients were randomised to receive a short course of ATRA (5 days prior to induction chemotherapy) as compared to a more extended course given simultaneously with induction therapy (ATRA given to complete remission (CR) or to a maximum of 60 days).[4] Use of allogeneic or autologous transplantation in first CR was abandoned during this study, since analysis of the MRC AML10 trial showed that for patients with AML with favourable karyotype (including those with t(15;17)) any benefit from transplant in terms of reduced relapse risk was offset by transplant-related mortality.[5,6] Gratifyingly, no deterioration in outcome has been observed in MRC studies for APL patients since routine use of transplant was discontinued. Molecular monitoring in the context of the MRC ATRA trial highlighted a major limitation of conventional RT-PCR approaches in that the majority of patients who ultimately relapsed tested PCR negative at the end of consolidation therapy, indicating that these assays lack sufficient sensitivity to detect residual disease. Nevertheless, conventional end-point PCR was shown to provide an independent prognostic factor in APL, with delayed clearance of disease-related transcripts (still detectable following 3 courses of chemotherapy) predicting a significantly higher risk of relapse (57% vs 25% at 5 years, p=0.004) associated with poorer overall survival (57% vs 89% at 5 years, p=0.02) 4. These data are in accordance with findings of a more recent study from the German AML Cooperative Group using *real-time* quantitative RT-PCR (RQ-PCR), in which patients who failed to achieve a 3-log reduction in PML-RARα transcript level within the first 3-4 months of therapy were found to have an increased risk of early relapse.[7] In addition in the MRC studies, conventional nested RT-PCR was shown to be of value in the context of transplantation in second CR.[8] These data, taken in conjunction with those from the GIMEMA group[9] are consistent with the current policy of using autologous transplantation as the preferred approach in *high risk* APL patients (defined as those with molecularly persistent disease, or in molecular or frank relapse) that have achieved molecular remission with re-induction therapy and have a PCR negative harvest, with allogeneic transplant being reserved for patients in whom harvesting proves problematic or who fail to achieve a molecular remission. MRD monitoring has also been shown to be of value in the post-transplant setting, guiding the need for additional therapy for patients with residual or recurrent disease to avoid relapse.[10]

Molecular monitoring using real-time quantitative RT-PCR (RQ-PCR) in APL patients treated in MRC trials

While MRD monitoring using conventional nested RT-PCR has provided valuable prognostic information, as discussed above, its clinical utility has been somewhat compromised by failure to detect residual disease in a significant proportion of APL patients who ultimately relapse.[3,4] This may reflect suboptimal scheduling of MRD assessments in the light of assay sensitivities achievable in clinical samples and the kinetics of disease relapses; in addition *false negative* results may be due to variation in RNA quality/ quantity and efficiency of the reverse transcription (RT) step. In this regard, quantitative PCR approaches using hydrolysis (*Taqman*) or hybridisation probe technology (reviewed[11]) afford a number of advantages in comparison to conventional *end-point* assays, with the potential to enhance the predictive value of MRD assessment. In particular, quantitation of fusion gene and endogenous control gene transcripts enables more reliable determination of kinetics of molecular remission achievement or relapse, as well as identification of poor quality samples that could potentially give rise to *false-negative* results. Standardised assays for the PML-RARα fusion and appropriate endogenous control genes have been established by the Europe Against Cancer (EAC) Group.[12] In a retrospective analysis of samples from 47 patients treated in the MRC ATRA trial, we found that RQ-PCR using the optimised EAC PML-RARα primer/probe sets increased rates of MRD detection in comparison to the conventional nested RT-PCR assay and provided an independent predictor of relapse risk and overall survival.[13] Other studies relating to MRD detection using RQ-PCR methodology in APL are encouraging; in the relatively large series (n=123) relating to the randomised US Intergroup ATRA trial, PML-RARα transcript level at the end of consolidation was found to be of prognostic value.[14] This study also revealed that, despite the higher sensitivity of RQ-PCR, a significant number of patients who ultimately relapsed tested PCR negative at the end of consolidation. This underlines the importance of serial monitoring to increase the predictive value of this approach. A challenge in the modern management of APL is how to improve further on the excellent outcomes now achieved with ATRA and anthracycline-based therapy. Nevertheless, it is worth noting that despite the success of this approach, approximately 10-20% of patients still relapse following first-line therapy. While it is well recognised that presentation with a leucocyte count >10×10^9/L is the most important prognostic variable in APL, predicting higher rates of induction death and relapse, analysis of 318 patients from the MRC AML10 and 12 trials treated with extended courses of ATRA and intensive chemotherapy has revealed that over half of relapses occur in patients with low presenting leucocyte counts (<10×10^9/L) and could not otherwise have been predicted on the basis of pre-treatment characteristics (R. Hills, K. Wheatley, unpublished data). In order to improve cure rates still further it is critical to identify subsets of patients at increased risk of relapse who could benefit from

additional therapy. Previous studies from the GIMEMA group have shown that molecular surveillance can successfully predict the majority of relapses in APL.[15] Moreover, preliminary evidence suggests that there may be a benefit for treating relapsing patients pre-emptively at the time of subclinical disease rather than in frank relapse.[16] While this was a non-randomised historical comparison, which did not adjust for time-shift bias, such that the conclusion should be treated with caution, it seems highly plausible that early intervention for impending relapse would improve outcome, particularly given the problems of morbidity and mortality due to haemorrhage when treating patients with clinically overt disease. This has been taken into account in the design of the current MRC AML15 trial, in which APL patients are prospectively monitored by RQ-PCR to identify those with *high risk* disease, who receive additional therapy in the form of ATO and transplantation whilst in first morphological remission (Figure 1). A key aim of this study is to determine whether rigorous molecular monitoring as a means of targeting additional therapy only to those patients with poor risk disease can reduce rates of frank relapse. The study has now accrued over 160 cases of APL and is set to recruit a large cohort of patients (>300). Such large data sets are absolutely essential in order to develop optimal schedules for MRD assessment and reliably establish the value of measurement of MRD as a means of determining treatment approach. Resolving these issues in the context of APL will carry important implications for development of risk-directed strategies based on MRD detection in other subsets of AML.

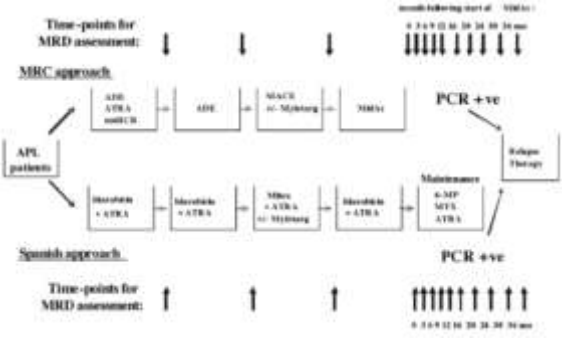

Figure 1. Flow diagram for patients with acute promyelocytic leukaemia in MRC AML15 trial and time-points for MRD assessmen.t.

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C11

ANALYSIS OF TIE-2 EXPRESSION IN ACUTE PROMYELOCYTIC LEUKEMIA: EVIDENCE FOR A SUBTYPE DISPLAYING ENDOTHELIAL FEATURES

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TEK, or Tie-2, is a receptor tyrosine kinase that is known as a functioning molecule of vascular endothelial cells. Gene targeting approaches have shown that Tie-2 and its ligands, the angiopoietins, are essential for vascular development and maintenance. Recent studies clearly indicate that Tie-2 expression is required for bone marrow hematopoiesis and, in particular, Tie-2/Angiopoietin-1 signaling regulates the quiescence and survival of hematopoietic stem cells. Furthermore, recent studies have suggested that dysregulation of VEGF and Angiopoietin signaling pathways may play an important role in the pathogenesis and clinical features of hematologic malignancies. To explore a possible role for this tyrosine kinase receptor in the development of leukemia, we explored its expression and potential function in acute promyelocytic leukemia (APL) and others AMLs. To better understand how this receptor is involved in leukemogenesis we examined the relationship with the angiogenic receptors VEGF-R1, VEGF-R2, VEGF-R3.

Tie-2 receptor expression has been explored in APL patients at mRNA and protein level by flow cytometry, RT-PCR and Western Blotting.

According to the level of Tie-2 expression we have classified the APL into three groups: Tie-2 negative (Tie-2^-, about 10%), Tie-2 moderately positive (Tie-2^+, about 45%) and Tie-2 highly positive (Tie-2^{++}, about 45%). Tie-2^{++} AML, display some specific features consisting in an usually high expression of membrane myelo-monocytic markers (i.e., CD11b), a high Flt3 and Ac133 expression and a concomitant elevated expression of the three membrane receptors for vascular endothelial growth factor (VEGF-R1, VEGF-R2 and VEGF-R3).The addition of exogenous angipoietin-1 or angiopoietin-2 to Tie-2^+ APL blasts promoted their survival, but not their proliferation.

In parallel, studies on normal hemopoietic progenitor cells induced to selectively differentiate along the erythroid, granulocytic, monocytic or megakaryocytic lineages, provided evidence that Tie-2 is selectively expressed in monocytic and megakaryocytic cells, both at the level of immature precursors and mature elements.

Based on these observations we propose that Tie-2^{++} APL may represent an AML subtype derived from the malignant transformation of a hemopoietic/endothelial precursor cell displaying monocytic and endothelial features.

In line with this hypothesis recent studies have led to the identification of a subpopulation of monocytes Tie-2^+ capable of differentiating *in vitro* and *in vivo* to endothelial cells.

C12

SPONTANEOUS APOPTOSIS LEVELS PREDICT RELAPSE RATE AND OUTCOME IN ACUTE PROMYELOCYTIC LEUKEMIA

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Multidrug resistance (MDR) and apoptosis are key prognostic tools in the heterogeneous response of AML patients to treatment. MDR-associated phenotype overexpression was frequently reported as a main factor of failure of treatment in AML (Del Poeta, 1996). Moreover, higher levels of anti-apoptosis related proteins (bcl-2, bcl-xL, Mcl-1) were found in AML patients presenting a poor outcome (Del Poeta, 2003). Low or absent MDR expression was previously described in APL (Paietta, 1994), while there are only few data on the prognostic role of apoptotic and anti-apoptotic protein levels. The today very good response of APL pts both to all-transretinoic acid (ATRA) and arsenic trioxide, notoriously targeted to increase apoptosis in myeloid blast cells, raised our interest to evaluate the real weight of apoptotic pathways on APL prognosis. Therefore, from 1990 to 2004, 37 patients, affected by *de novo* APL, median age 38 years(range 18-74), treated with intensive chemotherapy regimens (24 pts with LAP 0493 or AIDA 2000 protocols based on ATRA plus idarubicin and 13 pts with LAP 0389 protocol based on idarubicin or idarubicin plus cytosine arabinoside), were tested at our Institution. The principal aims of our study were: 1) to evaluate and correlate bax/bcl-2 ratio, representing a measure of spontaneous apoptosis, with the MDR-1 levels, determined by the P-glycoprotein phenotype (PGP) and 2) to demonstrate the clinical significance of MDR and spontaneous apoptosis. PGP, bcl-2 and bax proteins were determined by multicolor flow cytometry. MDR-1 was evaluated as percentage with the monoclonal antibody MRK-16 and the threshold was set at >20%. Bax/bcl-2 ratio was obtained dividing mean fluorescence intensity (MFI) of bax by MFI of bcl-2. The threshold was set at the median value >0.5. Bax/bcl-2 ratio was determined in 25 pts whose 24 were treated with ATRA based regimens. Sixteen patients were bax/bcl-2 ratio positive (16/25; 64%) and 16/37 (43%) pts were PGP positive. The global complete remission (CR) rate was 76% (79% for ATRA based protocols and 69% for LAP 0389 pts). No significant correlations were found between a higher PGP expression and response, relapse or outcome. There was no correlation between higher PGP expression and lower bax/bcl-2 ratio, since 8/14 PGP+APL pts presented higher bax/bcl-2 ratio levels. On the other hand, there was a significant correlation between bax/bcl-2 ratio <0.5 and the relapse rate (6/7; p=0.01). Moreover,higher bax/bcl-2 ratio levels determined at diagnosis were significantly correlated both with a longer overall survival (OS) (67% vs 33% at 6 years; p=0.04) and a longer disease free survival (DFS) (68% vs 29% at 7 years; p=0.01). In conclusion, higher bax/bcl-2 ratio levels and lower PGP expression are more frequent in APL pts in comparison with the other AML subgroups explaining their favorable prognosis. Nevertheless, in our experience, lower bax/bcl-2 ratio levels were significantly correlated with a higher tendency to relapse and bax/bcl-2 ratio levels higher than 0.5 predicted a better outcome with regard both to OS and DFS in APL. Our preliminary considerations, although it is necessary to confirm them on a larger number of pts, confer an important prognostic role to the mitochondrial apoptotic proteins, such

as bax and bcl-2, in APL. That has to be taken in account when further therapeutic strategies are to be planned in order to resolve the key problem of long-term relapses.

C13

CD34 AND CD2 EXPRESSION IN ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS ARE ASSOCIATED WITH BIOLOGICAL CHARACTERISTICS

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Compared to other acute myeloid leukemias, acute promyelocytic leukemia (APL) displays a distinctive immunophenotypical profile including CD33, CD13 and CD9 antigen expression and negative staining for HLA-DR. We investigated CD34 and CD2 expression on APL blast cells to verify the possibility of distinguishing a subset with some biological characteristics. We analyzed CD34 and CD2 expression in 125 newly diagnosed APL patients. Median age was 42 yrs (range 15±88 yrs). One-hundred and thirteen (90%) cases were classified as M3 and 12 (10%) as M3 variant (M3v). The parameters considered were WBC and PLT counts, hemoglobin levels, percentage of peripheral blood blasts, CD15, CD56 and HLA-DR expression, and the PML-RARα isoform, to assess their relationship with CD34 and CD2. Cut-off values for defining antigen positivity were >10% for CD34, and >20% for the other markers.

Two patient groups could be identified: $CD34^+$ $CD2^+$ (23 cases), $CD34^-$ $CD2^-$ (71 cases); the remaining 31 patients could not be considered because they had heterogeneous expression of CD34 and CD2. All the six cases of M3v considered were $CD34^+$ $CD2^+$ (p=0.0001). The median WBC count was 8.5×10^9/L and 2.2×10^9/L for the $CD34^+$ $CD2^+$ and $CD34^-$ $CD2^-$ groups, respectively (p=0.0007). The median PLT count was lower in CD34+ CD2+ than in $CD34^-$ $CD2^-$ cases (20×10^9/L vs 27×10^9/L, p=0.04). The median percentage of peripheral blood blast cells was higher in $CD34^+$ $CD2^+$ than $CD34^-$ CD2- patients (89% vs 27%, p=0.0001). There was no significant difference between the two groups in terms of age, sex, CD15, and CD56 expression whereas the HLA-DR antigen was more evident in the $CD34^+CD2^+$ group (p=0.03). The BCR3 PML-RARα rearrangement type was more frequent in the $CD34^+CD2^+$ group (p=0.001). When M3v cases were excluded, the $CD34^+$ $CD2^+$ and $CD34^-$ $CD2^-$ groups still had statistically different values for WBC, PLT count and percentage of peripheral blood blasts. Moreover, CD15 expression was associated with the $CD34^-$ $CD2^-$ group (p=0.04). Our results show that in APL the$CD34^+$ $CD2^+$ phenotype is associated with higher WBC, lower PLT counts, a higher percentage of peripheral blood blasts and the BCR3 isoform.

These data suggest that immunophenotypical analysis could distinguish an APL subset with different biological characteristics. Further studies in homogeneously treated APL patients are needed to assess the prognostic impact of this immunophenotypical pattern.

WORKSHOP IV
THERAPY OF ACUTE PROMYELOCYTIC LEUKEMIA RELAPSE

C14

COCKTAIL THERAPY INCREASED THE SURVIVAL RATE OF ACUTE PROMYELOCYTIC LEUKEMIA

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Background. Arsenic trioxide provides significant benefits in newly diagnosed and relapsed acute promyelocytic leukemia (APL) respectively. However, the high relapsed rate is still threatened the life of APL patients. Which regimen should be used to overcome or reduce the relapse in consolidated treatment is a key problem at present. We performed a pilot study about that.

Objective. To compare the effectiveness and security of cocktail therapy with arsenic trioxide therapy in APL consolidated treatment.

Methods. Sixty-five APL patients, who once received arsenic trioxide treatment and obtained complete remission, were enrolled in this study. Patients were divided into two groups according to the different consolidated regimens. After reinforced treated with DA (daunomycin and cytarabine) or HOAP (harringtonine, vincristin, cytarabine and prednisone) for two course, Group A involved twenty cases received arsenic trioxide consolidated, Group B included forty-five cases treated with the cocktail therapy, alternatively treated with arsenic trioxide, all trans-retinoic acid and chemotherapy (DA or HOAP). The relapse rate, the survival rate and the central nervous system infiltration rate were compared in 3 years followed up.

Results. The relapsed rate of Group A was 55%, which was higher than that of Group B(17.8%).The re-remission rate after the first relapse in Group A was 22% , which was lower than that of Group B(42.8%). The central nervous system infiltration rate of Group A was 28%, which was higher than that of Group B(6%).The average survival time of Group A was 10.5±4.2months, which was shorter than that of Group B (22.5±5.5 months).The three-year survival rate of Group A was 15%, which was less than that of Group B (65.8%).

Conclusions. Cocktail therapy alternatively treated with arsenic trioxide, all trans-retinoic acid and chemotherapy will be the reasonable regimen for APL consolidated treatment . Which provided benefited on inhibiting relapse and central nervous system infiltration of APL.

C15

HIGH-DOSE CYTARABINE AND MITOXANTRONE IN CONSOLIDATION THERAPY FOR YOUNGER PATIENTS AND MAINTENANCE THERAPY WITH LOW-DOSE ORAL IDARUBICIN AND ALL-TRANS RETINOIC ACID FOR OLDER PATIENTS WITH NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA

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The objective of our study was to evaluate i) high-dose cytarabine in consolidation therapy in younger patients (age 16-60 years) and to evaluate ii) per oral low-dose idarubicin in combination with ATRA for maintenance therapy in older patients (>60 years) with newly diagnosed acute promyelocytic leukemia (APL). Younger patients received induction therapy according to the AIDA protocol (all-trans retinoic acid (ATRA) 45 mg/m p.o. days 1-28, idarubicin 12 mg/m i.v. days 2,4,6,8) followed by one cycle of ICE (idarubicin 12 mg/m i.v. days 1,3,5, cytarabine 100 mg/m i.v. cont. days 1-7, etoposide 100 mg/m i.v. days 1-3) and two cycles of HAM (cytarabine 3 g/m q12 h, days 1 to 3; mitoxantrone 10 mg/m, days 2, 3). Older patients received an age-adjusted AIDA protocol (ATRA 45 mg/m p.o. day 1-6, ATRA 15 mg/m p.o. days 7-35, idarubicin 12 mg/m i.v. days 2,4,6) followed by one cycle of age-adjusted A-HAM (cytarabine 500 mg/m q12 h i.v. days 1-3, mitoxantrone 10 mg/m i.v. days 2,3, ATRA 15 mg/m p.o. days 3-28) and six monthly courses of outpatient maintenance therapy (5 mg idarubicin p.o. days 1, 4, 7, 10, and 13; ATRA 15 mg/m p.o. days 1 through 28). From 1995 to 2004, 96 younger and 11 older patients were enrolled. Eighty-five (89%) and 8 (73%) patients achieved a complete remission, 10 (10%) and 3 (27%) patients died from early/hypoplastic death (ED/HD) and 1 (1%) and 0 patients were refractory to induction therapy in the younger and the older age-groups, respectively. One patient with refractory APL to AIDA achieved a CR after combination therapy of ATO and ATRA. In the group of younger patients 86 patients received at least one cycle of HAM. In the group of older patients 8 patients received one cycle of age-adjusted A-HAM followed by six monthly maintenance cycles. Median follow-up for the whole population was 48 months. Overall (OS), event-free (EFS) and relapse free (RFS) survival after 4 years were 84% and 55%, 76% and 55%, 85% and 75% for the younger and the older age-groups, respectively. OS (p=0.03) and EFS (p=0,03) were significantly influenced by presenting white blood cell counts (cut point 10/µL) with an OS of 95% and 75% after 4 years for patients with <10.0/µL (n=50) and patients with >10.0/µL (n=57), respectively, whereas there was no difference in RFS (p=0.11). In univariate analysis FLT3-ITD mutations showed no impact on OS, EFS and RFS. In multivariable analyses for OS statistically significant unfavorable variables were WBC (>10/µL) (p=0.01) and additional cytogenetical aberrations (p=0.05), whereas for RFS no variable entered the model. In conclusion high-dose cytarabine in consolidation therapy without subsequent maintenance therapy for younger patients with newly diagnosed APL is an effective treatment approach. For older patients maintenance therapy with six monthly course of low-dose per oral idarubicin in combination with ATRA is effective and well tolerated.

C16

FAILURE-FREE SURVIVAL ANALYSIS OF THE ALLG APML3 TRIAL I NCORPORATING ATRA, INTENSIVE IDARUBICIN, AND TRIPLE MAINTENANCE COMBINED WITH MOLECULAR MONITORING IN PREVIOUSLY UNTREATED ACUTE PROMYELOCYTIC LEUKEMIA

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The impressive response to induction with ATRA and idarubicin (IDA) in untreated APL reported by GIMEMA and PETHEMA prompted the Australasian Leukaemia and Lymphoma Group (ALLG) to evaluate substitution of a second cycle of IDA in place of conventional consolidation chemotherapy.

Treatment of 100 eligible patients [age >=18 yrs, t(15;17) and/or PML-RARα positive] from 25 institutions in Australia and New Zealand was initiated with ATRA 45mg/m^2/d and was continued until complete remission (CR). Prednisone 50 mg/d was added for white cell counts (WCC) above 10×10^9/L and/or signs of ATRA syndrome. IDA#1 (12 mg/m^2) was given on days 2, 4, 6, and 8, and a second identical cycle (IDA#2) was administered after hemopoietic recovery. All patients in hematological and cytogenetic CR after IDA#2 received 3 cycles of intermittent ATRA, 45 mg/m^2/d for 14 days every 28 days. After the first 31 patients, the protocol was amended to include 2 years of maintenance (ATRA 45 mg/m^2/d for 14 days every 3 months, oral methotrexate 15 mg/m^2/week, and 6-mercaptopurine 90 mg/m^2/d).

Medians (ranges) for age, WCC and platelets were 40 yrs (19-73), 2.4×10^9/L (0.4-109) and 25×10^9/L (4-180) respectively. PML breakpoint analysis identified 51 as bcr-1, 11 as bcr-2 (confirmed by sequencing), and 34 as bcr-3. Four patients lacked initial molecular samples but were t(15;17) positive. Thirty-nine of 89 patients (44%) expressed mutant FLT3 transcripts (internal tandem duplications and/or codon 835/836 mutations). A highly significant association of FLT3 mutations with bcr-3 breakpoints was noted (p<0.000001). The level of PML-RARα transcripts (quantitated by DNAzyme-mediated real-time RT-PCR) was also correlated with PML breakpoint (bcr-3 > bcr-2 > bcr-1; p<0.00001), and with FLT3 mutations (p<0.0001). The proportion of patients still PML-RARα positive after IDA#1, IDA#2 and intermittent ATRA was 69%, 7% and 0% respectively. Eight patients (8%) died within 30 days, 2 patients (2%) were withdrawn with resistant leukemia, and the remainder (90%) achieved CR. With an estimated potential median followup of 3.4 years, actuarial overall survival (OS) is 85% at 3 years. At the same time point, estimated disease-free survival is 66%, and remission duration is 68%.

Failure-free survival (FFS) was defined as time from commencement of ATRA therapy to either (i) relapse (hematological, cytogenetic or molecular), (ii) death in remission, or (iii) off protocol because of failure to achieve remission, excessive toxicity, refusal to continue, or death. Estimated FFS for the entire group is 54% at 3 years.

In univariate Cox proportional hazards regression analysis, the presence of FLT3 mutations was the single most important pre-treatment predictor of inferior FFS (p=0.011) and OS (p=0.019). Other factors associated with an increased probability of failure include higher WCC (p=0.025), lower platelet count (p=0.037), and bcr-3 break-

points (p=0.049). In multivariate analysis, the best models for predicting FFS were a 2-factor model incorporating FLT3 mutations and log(platelets) (p=0.005), or a 3-factor model with FLT3 mutations, log(platelets), and log(WCC) (p=0.009). FFS and remission duration were significantly improved following the addition of maintenance to the APML3 protocol (p=0.0069 and p=0.0044, respectively). In conclusion, the APML3 protocol achieved hematological and durable molecular remission in the majority of patients, but the presence of FLT3 mutations was associated with a significant reduction in FFS and OS. These data support the development of novel protocols which incorporate FLT3 inhibitors in the management of APL.

C17

ELDERLY PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA: LONG-TERM RESULTS OF THE GIMEMA LAP AIDA 0493 AMENDED PROTOCOL

Latagliata R, Breccia M, Fazi P, Vignetti M, Cupri A, Sborgia M, Vincelli D, Candoni A, Salvi F, Rupoli S, Martinelli G, Kropp MG, Di Rienzo N, Zampaglione V, Venditti A, Melillo L, Cimino G, Petti MC, Avvisati G, Lo Coco F, Mandelli F
for the GIMEMA Acute Leukemia Working Party
GIMEMA Acute Leukemia Working Party

In order to reduce toxicity in elderly patients with newly diagnosed APL, since March 1997 the Italian Cooperative group GIMEMA evaluated an amended AIDA protocol for patients aged > 60 years, consisting of the same induction with ATRA and Idarubicin but only 1st consolidation course (Idarubicin + Cytarabine), followed by 2 years maintenance with ATRA alone. Up to now, 56 patients (25 males and 31 females, median age 66.2 years, 46 with PS 0-1 and 10 with PS 2) are fully evaluable. At onset, according to GIMEMA-PETHEMA risk score, 18 were low-risk (32.5%), 31 intermediate risk (55%) and 7 high risk (12.5%). After induction treatment, 54 patients (96.4%) achieved CR and 2 (3.6%) died from haemorrhage (1) and infection (1). ATRA syndrome was documented in 5 patients (9.3%). 13/56 patients (23.2%) showed during induction other toxicities (WHO 3 - 4) not related to ATRA. After CR achievement, 2 patients died in CR from haemorrhage (1) and infection (1) and 52 received the consolidation course: on the whole, during consolidation 4 patients (7.6%) had a toxicity WHO 3-4 and 2 of them (3.8%) died from haemorrhage (1) and infection (1). The remaining 50 patients started maintenance treatment: up to now, 12 patients (22.2%) relapsed, after a median time from morphological CR of 19 months (range 7-86). Overall survival (OS) was 76.1% and 73.3% at 3 and 5 years, respectively. Disease free survival (DFS) was 64.5% and 61.3% at 3 and 5 years, respectively. At the univariate analysis, PS=2 (p=0.0019), WBC count > 3×10^9/L (p=0.018) and male gender (p=0.03) had a bad prognostic impact on DFS, while only PS=2 (p=0.05) did it on OS. Age, PLTS count, WBC count > 10×10^3/L, and risk score did not affect both OS and DFS. At the multivariate analysis on DFS, only PS=2 retained prognostic significance (HR=3.8). In conclusion, the amended GIMEMA protocol has shown to be effective and safe in elderly APL patients, as the rate of death in CR was reduced with the same relapse rate when compared with previous results in not amended GIMEMA LAP AIDA 0493.

C18

LONG-TERM OUTCOME OF RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA FOLLOWING TREATMENT WITH ALL-TRANS RETINOIC ACID AND ANTHRACYCLINE-BASED CHEMOTHERAPY. ANALYSIS OF MOLECULAR AND HEMATOLOGICAL RELAPSES OF PATIENTS INCLUDED IN PETHEMA PROTOCOLS LPA96 AND LPA99

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Background. The combination of ATRA and anthracycline-based chemotherapy results in cure of more than 70% of patients with newly diagnosed acute promyelocytic leukemia (APL). Nonetheless, it remains a subset of patients who will relapse after this highly efficient front-line therapy and for whom the optimal salvage strategy is currently not well defined.

Patients and methods. Forty-four patients (59% male; age: 36, 3-71) diagnosed with PML-RARα-positive APL and treated according to PETHEMA protocols LPA96 and LPA99 who presented with a molecular failure (MOLrel, n=14) or overt hematological relapse (HEMrel, n=30) during the period 1996-2003 were included in this study. Salvage therapy consisted of ATRA and chemotherapy with high-dose ara-C (HiDAC) and mitoxantrone in most cases (77%), followed by stem-cell transplantation (SCT) in 25 patients (autologous, 15; allogeneic, 9; syngeneic, 1).

Results and Conclusions. Median time to relapse since achievement of complete response (CR) was 15 months (6-49). Among molecular failures, 3 patients were treated due to molecular persistence after consolidation therapy while 11 corresponded to molecular relapses. Twelve out of 14 patients (86%) with MOLrel attained second molecular CR, which persisted at last up-date in 10 cases (5-year leukemia-free survival -LFS-, 80%±13%). With regard to patients treated due to hematological relapse, 24 (80%) achieved second CR, without detectable PML-RARα in 16 (53%). However, 16 patients have experienced second relapse after a median interval of 9 months (3-16), with this translating into a 5-year LFS of 22%±9%. Analysis of prognostic factors identified age (<=40 vs >40, 5-yr survival: 48% vs 15%, p=0.01) and disease status at salvage therapy (MOLrel vs HEMrel, 5-yr survival: 60% vs 23%, p=0.03) as the only predictive variables of survival after relapse. Moreover, treatment for MOLrel (5-yr LFS: 80% vs 22%, p<0.01) and achievement of a PCR-negative status prior to SCT (5-yr LFS: 46% vs 0, p=0.02) correlated with a longer response duration after second-line therapy. In conclusion, outcome of patients presenting with a hematological relapse after front-line therapy with ATRA and anthracyclines and treated with a salvage therapy based on HiDAC and ATRA followed by SCT is poor. Therefore, design of alternative second-line strategies for this subgroup of patients is warranted.

C19

EARLY DEATH IN ACUTE PROMYELOCYTIC LEUKEMIA: ANALYSIS OF THE PETHEMA MULTICENTER LPA 96 AND LPA 99 TRIALS USING ATRA AND IDARUBICIN

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The combination of ATRA with anthracycline-based chemotherapy in the induction regimens has improved the antileukemic efficacy in acute promyelocytic leukaemia (APL), achieving an 85 to 90% complete remission (CR) rate, with virtual absence of leukemic resistance. No such progress has been made in the reduction of the early death (ED) rate during the induction therapy, in spite of an improved control of coagulopathy, and remains as a major cause of treatment failure.

Patients and Methods. We prospectively analyzed the incidence, clinical characteristics and predictive factors for ED in patients with *de novo* APL enrolled into the multicentric PETHEMA LPA96 and LPA99 trials. Induction treatment consisted of ATRA and idarubicin (AIDA induction). In the LPA99 trial we added prophylaxis with tranexamic acid (100 mg/kg/d) and prednisone (0.5 mg/kg/d) to prevent hemorrhagic death and retinoic acid syndrome. ED was defined as any death occurred during the induction treatment and until the achievement of a complete remission.

Results. Six hundred forty nine patients with genetically confirmed APL were included in the study, but 21 patients (3.2%) were registered as early deaths. The main cause of pre-therapy death was hemorrhage at central nervous system (CNS) (62%). Six hundred twenty eight patients were evaluable for induction therapy. There were 175 in the LPA96 and 453 in the LPA99 trials. There were no differences in the clinical or biological characteristics of patients in both trials. Overall, 54 patients (8.6%) died during the induction therapy. There were 16/175 (9.1%) deaths in the LPA96 trial and 38/453 (8.4%) in the LPA99 trial. The main causes of death were hemorrhages, infections and retinoic acid syndrome in 31 (57.4%), 17 (31.5%) and 5 (9.3%) patients, respectively. There were no differences in the causes of death in the two trials. The majority of patients who died due to bleeding, did so during the first week of therapy (14/31; 45.2%), and the most frequent localization of lethal bleeding was CNS (19/31, 61.3%). Hemorrhage and infection mortality were different in patients younger and older than 60 years (72 and 16% vs 37.5% and 56%, respectively; p=0.01). Platelet levels and coagulation parameters at diagnosis of APL did not influenced the risk of ED. In the multivariate analysis, age >69 years (p<0.0001), WBC >10×10^9/L (p<0.0001) and serum creatinine >1.4 mg/dL (p=0.016) at presentation were independent risk factors for ED. Age >69 years (p=0.05), WBC >10×10^9/L (p<0.0001) and serum creatinine >1.4 mg/dL (p<0.0001) were risk factors for ED due to hemorrhage, and age >59 years (p<0.0001) was the only risk factor for ED due to infection.

Conclusions. ED in APL patients treated with AIDA induction is 8.6% and hemorrhage is the main cause of death. Prophylaxis with tranexamic acid and prednisone lacks of benefit.

C20

CLINICAL FEATURES AND OUTCOME OF PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA PRESENTING CD56 ANTIGEN EXPRESSION REATED WITH THE PETHEMA LPA99 TRIAL

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Background. The relationship between CD56 expression by leukemic cells and poor outcome in patients with acute promyelocytic leukaemia (APL) has been reported on small and heterogeneous series. The improvement in prognosis of APL reported with some state-of the-art treatments may have modified this suggested relationship.

Aim. to analyse the prognostic relevance of CD56 expression in a large series of patients homogeneously treated with a state-of the-art protocol with ATRA and anthracycline alone for induction and consolidation therapy.

Patients and Methods. Between November 1999 and December 2004, a total of 526 consecutive *de novo* genetically confirmed APL patients were enrolled in the PETHEMA LPA99 multicenter trial. Treatment consisted of all-trans retinoic acid (ATRA) and idarubicine alone for induction followed by three consolidation courses of anthracycline monochemotherapy with ATRA (except for low-risk patients according to Sanz score), and maintenance therapy with intermittent ATRA and low dose chemotherapy with methotrexate and 6-mercaptopurine. Clinico-biological features and clinical outcome were analyzed in 334 patients (63%) for whom data from CD56 expression at diagnosis were available.

Results. Forty-one patients (12%) showed expression of CD56 in greater than 20% blasts at diagnosis and were considered as $CD56^+$. The $CD56^+$ subgroup presented a significantly higher proportion of patients with age >60 years (32% vs 17%, p=0.02), PML/RARα fusion transcript S-isoform (70% vs 40%, p=0.002), and with expression >20% of other surface antigens such as CD7 (21% vs 4%, p=0.001), HLA-DR (18% vs 4%, p=0.004) and CD15 (53% vs 30%, p=0.006). There was no significant difference on complete remission (CR) rate according to CD56 expression (82% vs 90%; p=0.3), and no effect was observed on 5-year leukemia-free survival (LFS) (88% vs 91%; p=0.6). Only one extramedullary relapse occurred in the $CD56^+$ patients. Multivariate analysis confirmed that CD56 expression had not prognostic value on CR rate and LFS.

Conclusions. This study shows that CD56 expression has no major impact on CR rate and LFS when APL patients receive a state-of-the-art treatment. Induction and consolidation strategies should not be modified according to the CD56 expression in APL patients.

C21

UNDER-REPRESENTATION OF BCR1 SUBTYPE OF PML-RARα FUSION GENE IN ACUTE PROMYELOCYTIC LEUKAEMIA IN INDIAN PATIENTS

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Acute promyelocytic leukemia (APL) is characterized by the t(15;17) leading to the formation of PML-RARα fusion gene. PML-RARα is believed to be critical for leukemogenesis and provides suitable targets for molecular diagnosis. The presence of a PML-RARα rearrangement in cases of morphologic APL remains the best predictor of favorable response to ATRA, which confers improved survival when combined with chemotherapy. Establishing the presence t(15; 17) at the molecular or cytogenetic level is critical for planning the therapy for APL patients. A total of 36 patients of APL (median age 30.4 years range 11-62 years, M:F 1.1:1) were analyzed. The WBC count ranged between 1.6×10^9/L to 132×10^9/L (median 21.8 $\times10^9$/L).This is higher than that reported in the West. RNA from bone marrow or peripheral blood of 36 patients at presentation was extracted and reversely transcribed and studied for the frequency of t(15:17) by nested RT-PCR and real time PCR (RQ-RT/PCR). Molecular evidence for t(15;17) rearrangement was identified in 35/36 patients morphologically diagnosed as M3 by nested RT-PCR; whereas RQ-RT/PCR succeeded in detecting PML-RARα rearrangements in all the cases. In 13/36 patients (36%), a 3' (bcr1/bcr2) PML breakpoint was identified, whereas 23 (64%) patients had a 5' bcr3 breakpoint. The prevalence of bcr3 (short isoform) was found to be significantly higher than that of bcr 1 (long isoform) (64% vs 36% p=0.03). Haematological remission following induction with ATRA and Daunorubicin , was achieved in 78% patients. This is lower than that reported in the West where it is seen in 95% patients. After two courses of consolidation, molecular remission was observed in 71% patients. It is thus concluded that APL in Indian patients is more often associated with high TLC and bcr3 transcript and this may be responsible for their poorer outcome.

C22

SEQUENTIAL VALPROIC ACID/ALL-TRANS RETINOIC ACID TREATMENT REPROGRAMS DIFFERENTIATION IN REFRACTORY AND HIGH-RISK ACUTE MYELOID LEUKEMIA

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Epigenetic alterations of chromatin due to aberrant histone deacetylase (HDAC) activity and transcriptional silencing of all-trans retinoic acid (ATRA)-pathway are two events linked to the pathogenesis of acute myeloid leukemia (AML) that can be targeted by specific treatments. A pilot study was carried out in eight refractory or high-risk AML patients not eligible for intensive therapy to asses the biological and therapeutic activities of the HDAC inhibitor valproic acid (VPA) used to remodel chromatin, followed by the addition of ATRA, to activate gene transcription and differentiation in leukemic cells. Hyper-acetylation of histones H3 and H4 was detectable at therapeutic VPA serum levels (>50 μg/mL) in blood mononuclear cells from 7/8 patients. This correlated with myelo-monocytic differentiation of the leukemic clone, as revealed by morphologic, cytochemical, immunophenotypic and gene expression analyses.

Differentiation of the leukemic clone was also proven by FISH analysis showing the cytogenetic lesion +8 or 7q- in differentiating cells. Hematological improvement, according to nestablished criteria for myelodysplastic syndromes, was observed in two cases. Stable disease and disease progression were observed in five and one case, respectively. In conclusion, VPAATRA treatment is well tolerated and induces phenotypic changes of AML blasts through chromatin remodelling. Further studies are needed to evaluate whether VPA-ATRA treatment by reprogramming differentiation of the leukemic clone might improve the response to chemotherapeutic agents in leukemia patients.